Mistra
Environmental
NanoSafety
Phase II

ANNUAL REPORT 2022

COVER ILLUSTRATION: FRIDA NILSSON

Content

Message from Management

Our Mistra Environmental Nansafety programme is nearing its end, in September 2023. Therefore, much scientific and administrative efforts these past 12 months have been put into communicating key research results to stakeholders and on building collaborations, projects and partnership beyond the programme itself. One such collaboration, which we are especially proud of, is the thematic collaboration initiative, NanoSafe4All. This initiative gathers researchers and industry to develop solutions to real life problems connected to nanosafety. The end goal is to establish a National Nanosafety Center.

Another focus has been on planning and providing input into the upcoming conference EuroNanoForum in June 2023. Some of our researchers will present research on nanosafety and the regulatory EU landscape. To this end, our programme was granted a prolongation unto September 2023. This extension has allowed us to not only plan the conference, but to further consolidate networks, and scientific collaboration within our programme. We are also in the midst of planning our own final conference, also in June. At this event, we will highlight how our research have made significant scientific impacts, and contributed to societal and environmental impacts beyond academia.

We are proud over the fact that we have gathered such a strong consortium over almost nine years of activity. People have formed strong scientific and personal bonds. For the remaining programme time period, we intend to identify ways to continue our collaboration, to ensure that environmental nanosafety research continues to grow and develop.

More research and industry collaborations are greatly needed, with much work left to do in terms of developing methods to describe nanoparticles throughout their lifecycle. The regulatory gap between EU and industry, and companies' hesitance to use nanomaterials, are also factors that still need to be addressed in the future. As researchers and company representatives, we hope we can continue to contribute to the field in new, and different constellations – all made possible by the call for a Mistra Environmental Nanosafety programme back in 2013.

PHOTO: UNSPLASH

About the 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 the most interesting in phase one, which ended in 2018.

Work packages:

- **WP1** – Scaling up: Assessing structural change and fate of nanoparticles when entering natural ecosystems
- **WP2** – Chemical weathering and biocorona formation on nanoparticles
- **WP3** – Transformed nanoparticles effect on aquatic organisms, food chains and cell models
- **WP4** – Proactive risk assessment, regulation and creation of stakeholder learning alliances
- **WP5** – Safe handling nanomaterial after product end-of-life
- **WP6** – Management, communication, economy and stakeholder relations

The programme gathers researchers from Lund University, Chalmers University of Technology, University of Gothenburg, Karolinska Institute, KTH - Royal Institute of Technology, and the Technical University of Denmark. Two main industry partners are part of the programme: SYSAV and Tetra Pak, as well as a number of associated partners.

ILLUSTRATION: FRIDA NILSSON

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

- **WP6** 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.

Selected Highlights 2022/23

Several events and activities were organised, targeted at different key stake holders. These highlights illustrate important scientific developments, and communications and policy efforts, during the time period March 2022–2023.

Meeting state authorities

During autumn 2022, the programme administration contacted several Swedish state authorities with a view to meet relevant personnel to present key research results. They include Naturvårdsverket, Havs- och vattenmyndigheten, Arbetsmiljöverket och Kemikalieinspektionen. We initiated dialogue with Havs- och vattenmyndigheten, and received an invitation from Kemikalieinspektionen to present for its General Director. On 12th December, Mistra Environmental Nanosafety researcher, Steffen Foss Hansen, presented research related to the EU chemical legislation (REACH), which came into effect in 2020. He also highlighted how the different work packages within the Mistra Nanosafety programme are currently testing nanoparticles and their effects in relation to wetlands, aquatic species, waste handling, and human health.

Presenting for the European Chemical Agency Nanomaterial Working Group

In October, Mistra Environmental Nanosafety researchers, Steffen Foss Hansen, Maria Bille Nielsen, Rickard Arvidsson, together with Lars Skjolding and Anders Baun presented key research results for the European Chemical Agency Nanomaterial Working Group, in Helsinki, Finland. They gave a presentation on the topic: Environmental exposure estimation for nanomaterials, on nano-specific methods currently available, and/or on PNEC derivation. The presentation opportunity illustrated how Mistra Environmental Nanosafety research is considered of interest, and valuable to organisations regulating the nanomaterial policy landscape.

Creating Trust in Nanotechnology, a Tetra Pak and NanoSafe4All event

As a response to many companies being hesitant to use nanomaterials in innovation, the Mistra Environmental Nanosafety programme, and the NanoSafe4All collaboration initiative, together with Tetra Pak, organised a webinar on, trust, communication and collaboration for safe innovation in nanotechnology, on 1st February. It featured presentations from researchers, partners and other organisations on nanosafety research, communication, available testing methods for nanoparticles, and more. With more than 80 registrants from across societal sectors, it highlighted the importance of continuing discussions on how to build trust and transparency in nanotechnology.

Ground breaking nanomaterial research on ecocorona formation

Knowledge about the basic mechanisms of interaction of natural organic matter with plastic nanoparticles is vital to understand the corona formation on the particles because it can have significant effect on the ecotoxicity. Researchers within the Mistra Environmental Nonosafety programme have performed a study on the molecular dynamics of plastic particles. They developed an atomistic model of carboxylated polystyrene nanoparticle with differently charged densities depending on the pH of solution. Interactions of organic molecules called Temple-Northeastern-Birmingham (TNB) which are representative of natural organic matter with negatively charged polystyrene nanoparticle were simulated at an atomistic level. This is the first study in which such interactions have been simulated at atomistic level of polystyrene nanoparticles. The results show that the interaction of TNB and negatively charged polystyrene at pH 7 is mediated by the Na^+ ions. As the surface charge of polystyrene increases more counterions i.e., Na^+ accumulated near the surface which in turn bring more water to the interface.

From the study, the researchers can predict that in low salt media there is less chance of ecocorona formation on the polystyrene nanoparticles compared to the high salt case. This can be important for determining how plastic particles behave in sweet and salt water. It may also be important for the interpretation of polystyrene nanoparticle behaviour in wetlands as explored in another study.

The study:

- Rama, P, Gallego-Urrea, J.; Abbas, Z. 2022. **Interfacial Interactions of Humic Acids with Polystyrene Nano-plastics in Aqueous/Ionic Environments: A Molecular dynamics exploration.** *Environmental Science Nano* (accepted)

Interdisciplinary science to further understanding of the fate of metal and metal oxide nanoparticles

Researchers within the Mistra Environmental Nanosafety programme have conducted two interdisciplinary studies, combining material and surface science, chemistry, biology, and ecotoxicology, to evaluate the fate of different metal and metal oxide nanoparticles (NPs) when dispersed into freshwater. The studies aimed to assess changes over time and the role of interactions with natural organic matter (NOM) on the physico-chemical and surface characteristics, mobility, stability, transformation/dissolution patterns, and ecotoxic potency towards a piscine cell line at in vitro conditions. The results show that NOM interacted with metallic NPs in varying degrees over time, forming a biocorona via its carboxylate groups.

The studies highlight how an interdisciplinary approach is crucial in assessing the potential risks associated with the dispersion and environmental exposure of engineered and non-intentionally formed metallic nanoparticles (NPs). They demonstrate that interactions with natural organic matter and other chemical constituents in freshwater can affect particle properties such as stability, mobility, and transformation/dissolution patterns. These effects are highly specific to the type of NP and duration of exposure, and can also impact the toxic potency of the dispersed NPs.

The results provide better understanding of the environmental fate and impact of engineered and non-intentionally formed NPs. They can also be used to develop predictive models and regulatory frameworks for managing potential risks associated with NPs.

The studies:

- A. Khort, M. Brookman-Amissah, J. Hedberg, T. Chang, N. Mei, A. Lundberg, J. Sturve, E. Blomberg, I. Odnevall. 2022. **Influence of natural organic matter on the transformation/dissolution of metal and metal oxide nanoparticles and their ecotoxic potency on a piscine cell line at simulated freshwater conditions – a short-term laboratory study.** Nanoimpact, 25.
- A. Khort, T. Chang, A. Saeed, E. Blomberg, M. Billie Nielsen, S. Foss Hansen, I. Odnevall. 2023. **Effects of interactions between natural organic matter and aquatic organism degradation products on the transformation and dissolution of cobalt and nickel-based nanoparticles in synthetic freshwater,** Journal of Hazardous Materials, 445, 5130586

PHOTO: LUND UNIVERSITY

Communication and outreach

As the programme is nearing its end, many internal and external communication activities were organised to consolidate research results, and highlight key results to a wider public.

Example of activities include two programme meetings, one in Copenhagen in spring, and one in Lund during autumn. These meetings gathered all the programme participants, and included presentations from different researchers. Monthly steering groups meetings also continued, with a focus on discussions of how to maximise the output from the programme.

The administration also put a lot of effort into planning two upcoming conferences: the Mistra Environmental Nanosafety final conference, and EuroNanoForum, which is of strategic importance in terms of reaching international target groups, and as a platform where researchers and partners within the Mistra Environmental Nanosafety programme can make important contacts.

The mentorship programme concluded. It was well received and much appreciated by both the mentees and the mentors who participated. Nine PhD students (with one student from the Mistra STEPS programme) entered the programme, and eight finished it. These types of efforts are an important aspect of the Mistra Environmental Nanosafety programme overall since they aim to equip students and mentees with necessary skills to enter into researcher and industry collaborations, and to grow their careers and networks.

Examples of other external activities include the aforementioned dialogue with state authorities to present our research; and the planning of the event, Creating Trust in Nanotechnology, and the Citizen Science project, When less is More. During the year, researchers within the programme have been visiting high schools in Skåne to develop a survey which maps common household goods containing nanomaterials. The project has proved challenging in terms of engaging schools to take on the survey. In the coming months, the project will be reviewed, with a plan to make the learning materials available for teachers and students across Sweden to increase participation in the survey.

PHOTO: ISTOCK

Scientific conferences and seminars during the time period

SweNanoSafe online workshop on micro- and nanoplastics together with the Swedish Environmental Protection Agency, 27 January, 2022
Bengt Fadeel, KI, *"Potential human health risks of micro- and nanoplastics"*.

SETAC Europe 32nd Annual Meeting, Copenhagen, 15–19 May, 2022
Clausen L.P.W., Nielsen M.B., 2022., Oturai, N., Syberg, k., Hansen S.F, DTU *"How Environmental Regulation Can Drive Innovation – Examination of Three EU Environment Regulations"*.

École nationale supérieure de chimie de Paris, France, 25 May, 2022
Inger Odnevall, KTH *"Corrosion and surface-chemistry studies of metallic surfaces connected to health and environmental aspects – a selection of ongoing interdisciplinary activities"*.

Nano Week 2022, , Limassol, Cypern by Nanosafety Cluster, 2–24 June, 2022
Bengt Fadeel, KI, *"Surface modification mitigates silica nanoparticle-triggered cytotoxicity and cytokine release"*.

NanoSafety course at Lund University (also online) June 2022: *"NanoSafety – after the products' end-of-life"*.

Materials for a Sustainable World, KTH Materials Dialogue, 14 June, 2022
Inger Odnevall, KTH, *"The surface chemistry of metallic materials governs their potency for adverse health and environmental effects"*.

European Chemical Agency Nanomaterial Working Group, in Helsinki, Finland, 2022
Steffen Foss Hansen, Maria Bille Nielsen, DTU, Rickard Arvidsson, Chalmers, and Lars Skjolding Anders Baun presented key research results.

Mistra Environmental Nanosafety programme online seminar, 25 October, 2022
Franca Stabile, LU, Prasad Rama, GU, Jasreen Kaur, KI. Moderator: Prof. Bengt Fadeel, KI, and Prof. Joachim Sturve, GU. *"Young Investigators Forum: Focus on (eco) toxicological effect of micro- and nanoplastic particles"*.

The strategic TEC workshops at MAX IV. Twice during 2022
Jenny Rissler, LU, *"Environmental Samples at Balder"* and *"X-ray absorption spectroscopy measurements on heavy metals in ash particles at BALDER"*.

NanoLunds annual meeting, 11 October, 2022
Jenny Rissler, LU, *"Sustainable and safe – from research to waste"*

Invited presentation at Naturvårdsverket (EPA) EPA, 12 December, 2022
Tommy Cedervall, LU, "with the aim to deliver a basis for stakeholder decision support"

Nano – When less is more! A combined outreach and citizen science initiative It has been tested on 6th grade and high-school students in Sweden, Lund May 2022, and Klippan, January 2023
Tommy Cedervall, Mikael Ekvall, Martin Lundquist, LU, *"The project entails development of teaching material and a citizen science part where the students help mapping consumer products containing nanomaterials in their homes."*

Avfall i nytt fokus Conference, Gothenburg, 12–13 March, 2023
Jenny Rissler, LU, *"Kan avancerade analysmetoder vara nyckeln till återvinning?"*

Publications

Gupta G, Fadeel B. 2022. **Copper is the new show-stopper.** Toxicol Sci. 2022 Aug 25;189(1):3-4.

Fadeel B. 2022. **Nanomaterial characterization: understanding nano-bio interactions.** [review]. 2022, Biochem Biophys Res Commun.

Fadeel B. 2022. **Understanding the immunological interactions of engineered nanomaterials: role of the bio-corona. [review].** Wiley Interdiscip Rev Nanomed Nanobiotechnol. Nov;14(6):e1798.

Ekvall, M.T., Gimskog, I., Hua, J., Kelpsiene, E., Lundqvist, M., and Cedervall, T. 2022 **Size fractionation of high-density polyethylene breakdown nanoplastics reveals different toxic response in Daphnia magna.** Sci Rep 12, 3109.

Arvidsson, R., G. Peters, S. F. Hansen, and A. Baun. 2022. **Prospective environmental risk screening of seven advanced materials based on production volumes and aquatic ecotoxicity.** NanoImpact 25: 100393.

Furberg, A., R. Arvidsson, and S. Molander. 2022. **A practice-based framework for defining functional units in comparative life cycle assessments of materials.** Journal of Industrial Ecology 26(3): 718-730.

Hansen, S. F., R. Arvidsson, M. B. Nielsen, O. F. H. Hansen, L. P. W. Clausen, A. Baun, and A. Boldrin. 2022. **Nanotechnology meets circular economy. Nature Nanotechnology** 17(7): 682-685.

Monikh, F.A., Hansen, S.F., Vijver, M.G., Kentin, E., Nielsen, M.B. Baun, A., Syberg, K., Lynch, I., Valsami-Jones, E., Peijnenburg, W.J.G.M. 2022. **Can Current Regulations Account for Intentionally Produced Nanoplastics?** Environmental Science & Technology 56(7): 3836–3839.

Palmås, K. and & N. Surber. 2022. **Legitimation crisis in contemporary technoscientific capitalism'**, Journal of Cultural Economy, 15:3, 373-379.

Shanley, D., J. Cohen, S. Stack and N. Surber. 2022. **Looking beyond the 'horizon' of RRI: moving from discomforts to commitments as early career researchers,** Journal of Responsible Innovation, 9:1, 124-132,.

Gupta G, Cappellini F, Farcal L, Gornati R, Bernardini G, Fadeel B. 2022. **Copper oxide nanoparticles trigger macrophage cell death with misfolding of Cu/Zn superoxide dismutase 1 (SOD1). Part Fibre Toxicol.** 2022 May 10;19(1):33.

A. Khort, M. Brookman-Amissah, J. Hedberg, T. Chang, N. Mei, A. Lundberg, J. Sturve, E. Blomberg, I. Odnevall. 2022. **Influence of natural organic matter on the transformation/dissolution of metal and metal oxide nanoparticles and their ecotoxic potency on a piscine cell line at simulated freshwater conditions – a short-term laboratory study.** Nanoimpact, 25.

A. Khort, T. Chang, A. Saeed, E. Blomberg, M. Billie Nielsen, S. Foss Hansen, I. Odnevall. 2023. **Effects of interactions between natural organic matter and aquatic organism degradation products on the transformation and dissolution of cobalt and nickel-based nanoparticles in synthetic freshwater,** Journal of Hazardous Materials, 445, 5130586

Karin Lovén, Christina Isaxon, Erik Ahlberg, Marie Bermeo, Maria E. Messing , Monica Kåredal, Maria Hedmer, Jenny Rissler, 2023. **Size-resolved characterization of particles >10 nm emitted to air during metal recycling**, Environment International.

Mumberg, Tabea, Steffen Foss Hansen, Anders Baun, and Rickard Arvidsson. 2023. **Assessment of Graphene-Based Materials against the Substances of Very High Concern Criteria.** Scientific Report E2023:001. Division of Environmental Systems Analysis: Chalmers University of Technology, Department of Technology Management and Economics.

Progress Report for the Mistra Environmental Nanosafety Work packages

WP1 – SCALING UP ASSESSING STRUCTURAL CHANGE AND FATE OF NANOPARTICLES WHEN ENTERING NATURAL ECOSYSTEMS

The overarching goal of WP1 is to scale up effects from nanoparticles (NP´s) from laboratory to natural conditions and to assess their fate and effects by using large-scale experiments and modelling. Regarding modelling we had three main milestones, including:

1) Complete our previous work on molecular dynamics (MD) simulations of carboxylated polystyrene nanoparticles interaction with natural organic matter. The reason behind choosing the carboxylated polystyrene particles for MD simulations was that same particles were used in experimental studies of meso-cosmos at Lund university and in this way simulations data could be used to understand the experimental findings. Status: *The work has been completed and an article is in review, with the title: "Interfacial Interactions of Natural Organic Matter: with Polystyrene Nano-plastics in Aqueous Environments: Rama, Gallego-Urrea and Abbas. Environmental Science Nano, 2022.(In review).*

2) The second milestone was to simulate the interaction of amine functionalized polystyrene particles with the protein present in *Daphnia*. This work is performed in close collaboration with WP3 where they have investigated the toxicity of positively charged polystyrene particles on *Daphnia*. Status: *The simulations work has been completed and we are in the process of writing a manuscript.*

3) The third milestone was to start experimental work on interaction of Svanne river fulvic acid (SRFA) with carboxylated polystyrene and their aggregation and modelling the interaction of SRFA with carboxylated polystyrene by MD simulations. Status: *This work is in progress, and we have performed experimental work on the aggregation as well as modelling.*

Regarding the experimental part of WP1, we had two major milestones to reach out for and both are now completed according to plan.

4) Hence, the fourth milestone of WP1 was to construct replicated wetlands with natural plants and animals and expose a complete ecosystem to nanoparticles. In order to study the *fate* of nanoparticles we used gold-doped polystyrene nanoparticles, and, thanks to the gold doping, we were able to track where the particles ended up. Status: *The experiment is successfully finished, all data analyzed and the manuscript "Fate and biological uptake of nanoplastics in freshwater ecosystems" by (Stábile, Hansson, Gallego-Urrea, Nwachukwu, Soorasena, Rivas-Comerlati, Ekvall) is to be submitted in April 2023.*

5) The fifth milestone was to, in addition to the fate also assess the *effects* of NP´s as they enter natural ecosystems. Status: *In a somewhat different experimental design (stepwise higher concentrations of NP´s in a replicated regression design) we performed a long term study to assess biological effects of polystyrene NP´s. The study was successfully performed, most of the data are analyzed and a manuscript is in preparation and will be submitted in June 2023 ("Effects of polystyrene nanoparticles on aquatic ecosystems" Ekvall, Stábile and Hansson). In addition, we performed a spin-off study using environmental DNA (eDNA) to assess any biodiversity changes among decomposers (bacteria and fungi), i.e. addressing a major ecosystem service. This main part of this additional study is analyzed, although some of the genetic data had to be re-sequenced due to too low amounts of DNA.*

In summary, 2022 has been a very productive year within WP1 and most of the milestones are reached with only minor deviations from the plan.

WP2 – CHEMICAL WEATHERING AND BIOCORONA FORMATION ON NANOPARTICLES

WP2 is focused on generating a mechanistic understanding of the transformation/dissolution behavior of engineered and non-intentionally formed metal and metal oxide nanoparticles (NPs) if dispersed into freshwater environments. Specifically, this interdisciplinary research seeks to examine changes in NP characteristics upon interaction with biomolecules such as natural organic matter forming a biocorona, and how this change their physico-chemical properties, surface characteristics and composition, extent of metal dissolution, colloidal stability, mobility which determines their environmental fate. The ultimate goal is to assess if these changes in environmental biotransformation/dissolution characteristics of metallic NPs can explain and/or be linked to potential adverse toxic effects on aquatic organisms under in-vitro conditions (WP3). Interdisciplinary fundamental studies are conducted under simulated in vitro conditions, both on assemblies of particles (powder) and on single NPs, using a wide range of spectroscopic, diffraction, electrochemical, and chemical tools. In addition, the research aims of this WP is to generate novel and improved scientific knowledge to enhance the regulatory (REACH) guidance documents for testing and regulating nanomaterials (WP4).

The main research activities in 2022/23 have focused on finalising and publishing research projects that answer whether the adsorption of natural organic matter (NOM) or excreted biomolecules from Daphnia (surface weathering) promotes or mitigates the environmental transformation/dissolution of various metal and metal oxide NPs in freshwater (FW). Additionally, these studies investigate whether information on their environmental transformation potential and polarizing capacity can be utilized for grouping, read across, modeling, and risk assessment. The studies have also addressed the effects of biotransformation/dissolution of metallic NPs on fish cell lines and zooplankton. The results are summarized in two published papers (Khort et al., 2022 and 2023), and another paper is currently submitted (Kelpsiene et al., 2023).

Other research activities during 2022/23, in collaboration with WP1, WP3, and WP5, have focused on the characterisation of aerosol particles generated during waste treatment of electronic and electrical equipment (WEEE) and their ecotoxic potency to Daphnia magna and fish cell lines. A manuscript is currently under preparation. Interdisciplinary studies are ongoing on 2D-materials (MXenes) in collaboration with DTU and Karolinska Institutet (WP3, WP4), studying the characteristics, transformation/dissolution pattern, and stability of MXenes in freshwater with and without NOM, and in biological media of different chemistry (e.g., chloride content, and interactions with PAH or PGG). These studies aim to understand the relation of the physico-chemical and biotransformation characteristics and toxic effects on zebra fish and how to integrate such findings into the regulatory framework for 2D materials.

Other ongoing activities include trophic transfer studies of WC-Co and Co NPs from worms to fish and crayfish in collaboration with WP3. These results will be published during 2023.

Studies in WP2 have also been conducted on correlations between particle size, shape, grain size, and microstructure of single metal NPs in FW with and without NOM interactions, and NP dissolution. These studies have been completed, and several manuscripts are currently being prepared. Short-term transformation/dissolution studies of single particle experiments and assemblies of Ni NPs in FW with and without NOM have also been completed, and a manuscript is currently prepared. Additionally, the possibility of using single-particle plasmonic nanospectroscopy to study the interaction of nanoscopic polymers (PMMA thin films as model systems) with FW with and without NOM has been explored, although slightly delayed due to parental leave of the Post Doc working in the project.

WP3 – TRANSFORMED NANOPARTICLE EFFECT ON AQUATIC ORGANISMS, FOOD CHAINS AND CELL MODELS

WP3 (KI) concluded a comprehensive study using a panel of amorphous silica particles provided by our partners at Nouryon, and we could show that the amorphous silica particles elicited pro-inflammatory effects while silane modification of the particle surface served to mitigate these effects. In this study, KI applied advanced analytical methods to elucidate the cellular impact of silica nanoparticles, including nanoSIMS (nanoscale secondary ion mass spectrometry), a technique based on the simultanous collection of multiple secondary ions from sample surfaces to create elemental maps at high lateral resolution (50 nm) and with high sensitivity (ppm in element imaging). Figure X shows nanoSIMS composite images of $^{12}C^{14}N^-$ and $^{28}Si^-$ ion maps from THP-1 cells exposed to bare *versus* silane modified silica nanoparticles (2.5 µg/mL). We submitted our manuscript last year (2022), and it was recently returned to us for revision.

Additionally, WP3 submitted a manuscript (joint effort between LU and KI) last year in which we could show that polystyrene (PS) nanoparticles, a surrogate model of "nanoplastics" found in the environment, can be detected in the gut of daphnids by using Raman confocal microscopy-spectroscopy. We also studied the impact of the same PS nanoparticles using an *in vitro* model of the human gastro-intestinal tract. Barrier integrity was determined using the TEER method.

KI has also investigated the potential impact of two-dimensional (2D) materials using zebrafish embryos as a model. Specifically, we have explored $Ti_3C_2T_x$, a member of the family of transition metal carbides and nitrides known as MXenes. We first determined the stability of MXene suspensions in various media using DLS and found that the material agglomerated in E3 medium (commonly used in zebrafish studies) while a stable dispersion could be achieved in EPA VS medium. Then, we exposed zebrafish embryos (5 dpf) to $Ti_3C_2T_x$ MXenes (in EPA VS medium) and found no evidence of adverse effects. The internalization of the MXenes in zebrafish was confirmed using a combination of Raman confocal microscopy, transmission electron microscopy (TEM), and inductively coupled plasma mass spectrometry (ICP-MS) (for Ti).

Remaining tasks in WP3: This year, KI will establish 3D cell spheroids of human liver cells in order to study the impact of nanoparticles in a more realistic *in vitro* setting. We will thus investigate cell viability as well as aryl hydrocarbon (Ah) receptor activation and CYP450 induction. The AhR is a key sensor of xenobiotic substances. Additionally, we will finalize the ongoing work on MXenes using our zebrafish model, and plan to publish this with DTU and KTH.

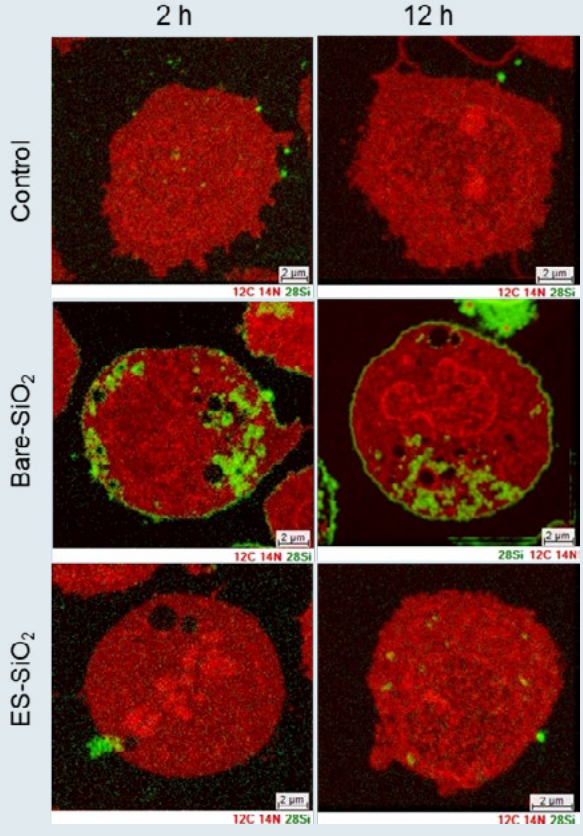

Figure X. Label-free nanoSIMS analysis of THP-1 cells exposed to bare and silane modified silica nanoparticles for 2 h and 12 h. The particles are non-fluorescent; the green signal corresponds to $^{28}Si^-$.

WP4 – PROACTIVE RISK ASSESSMENT, REGULATION AND CREATION OF STAKEHOLDER LEARNING ALLIANCES

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

In 2022, much of the work within WP4 has revolved around the International Chemical Secretariate (ChemSec). They cater the so-called SIN List of chemicals which they believe should be substituted now, which in turn is based on the criteria for substances of very high concern in the REACH legislation. One of our studies has been about assessing whether the 2D nanomaterial graphene and other graphene-based compounds fulfill these criteria for substances of very high concern. By scanning the available scientific literature, information about the carcinogenicity, mutagenicity, reproductive toxicity, bioaccumulation, persistence, and ecotoxicity of graphene was obtained. The outcome showed very limited information about the carcinogenicity, bioaccumulation, and persistence of graphene and similar compounds. Regarding mutagenicity, several studies showed genotoxic effects, and some even mutagenicity. A limited number of studies also showed reproductive toxicity. The main conclusion of the study is therefore that the mutagenicity and reproductive toxicity of graphene and graphene-based materials need to be investigated more thoroughly in further dedicated studies. This work is presented in a published report.

Another study we conducted analysed the recent debate about whether the nanomaterial carbon nanotubes qualify to the substances of very high concern. ChemSec has recently included carbon nanotubes on the SIN List, but several nanosafety researchers then reacted to this inclusion and wrote a critique in the journal Nature Nanotechnology, which was subsequently responded to be ChemSec. This debate was analysed in the present work. A number of arguments were first identified. For example, ChemSec argues that carbon nanotubes are hazardous and that regulating them as a group is the only feasible way of ensuring safety. The nanosafety researchers, on the other hand, argue that it is unscientific to regulate carbon nanotubes as a group, since this group consists of many different compounds with varying hazardous properties. They also suggest that carbon nanotubes can be made safe by future innovations and developments. From these arguments from both sides, three core values were identified. First, both sides care about environmental protection and environmental safety, arguing that their respective approaches are the best to safeguard that. Second and third, the nanosafety researchers also emphasize good science and technological progress in their arguments. The analysis thus shows that this seemingly technocratic debate about the safety and regulation of carbon nanotubes actually rests upon three underlying ethical values. This work is presented in a recently accepted paper in the journal *NanoEthics*.

Work has also been performed investigating how nanomaterials might assist in moving towards a circular economy and this work has been published in Nature Nanotechnology. Specifically, we have investigated the state of the art within reuse, recycling, refurbishing, etc. of nanomaterials and nanoproducts. We find that there are several aspects that nanomaterials developers should take into consideration e.g. toxicity and persistency of nanomaterials in the products, during the use and waste phase.

A scientific analysis of existing regulation when it comes to nanomaterials has been performed and is currently under peer-review. In the paper, we analyse existing legislation with regard to their applicability with a specific focus on definitions, information requirements, monitoring requirements and enforcement. We find that most of the recommendations developed more than 20 years ago on the management of nanomaterials have now been fulfilled, but that guidance with regards to the formation of coronas still has to be addressed.

Finally, we have also worked on analysis of how the results of WP1-3 of the Mistra Environmental Nanosafety programme might be used by European and Swedish regulators and this work was presented to the nanomaterials expert group of the European Chemicals Agency as well as the Swedish Chemical Agency (KEMI).

WP5 – SAFE HANDLING OF NANOMATERIALS AFTER PRODUCT END-OF LIFE

The knowledge of risks related to waste management and recycling of products containing nanomaterials is still poor, knowledge that is urgently needed in order to develop appropriate strategies reducing risks. WP5 focuses on understanding risks related to end-of-life management for products containing nanomaterials and to develop appropriate strategies for safe handling and minimize human and/or environmental exposure. Risks include both immediate exposure of humans and a long-term exposure of the ecosystem.

In 2022 the exposure/emission measurements performed earlier at facilities recycling electronic waste and metal scrap were further analyzed and the results summarized in a manuscript submitted in November to Environment International and accepted for publication in March 2023. The main conclusions from the study are that for all processes studied, the vast number of particles emitted to air were nanoparticles (<100nm), even though these are mechanical recycling processes. We also show that the chemical composition varies with particle size. The large fraction of nanosized particles that were emitted, and the fact that the composition of these is different from the larger particles, raises questions that needs to be further addressed including toxicological implications, both for humans and for the environment.

In November 2021 we revisited one of the facilities to collect the PM1 fraction (dominated by the nano particles) of airborne particles using a high-volume sampler. The purpose of the collection of particles was to collect particulates for more detailed characterization, but most importantly to collect material to perform eco toxicity testing. The eco-tox tests was performed during 2022 and we are now in the process of summarizing the results.

We also continued the work with emissions related to demolition and recycling of nanoparticle reinforced concrete: with the development of standardized emission testing and with evaluating the results from a first experiment using the set-up for studying the emissions from three types of CNT reinforced concrete. The results will be summarized in a publication that will be finalized in 2023.

WP6 – MANAGEMENT, COMMUNICATION, ECONOMY AND STAKEHOLDER RELATIONS

The main focus for WP6 during the year has been on communicating key research results to stakeholders and on building collaborations, projects and partnership beyond the programme itself. Some activities include meetings with state authorities such as Kemikalieinspektionen to present our research; the planning and organising of the event, Creating Trust in Nanotechnology, with more than 80 registrants, and the Citizen Science project, When less is More, aimed at schools across Skåne.

The administration also put a lot of effort into planning two upcoming conferences: the Mistra Environmental Nanosafety final conference, and EuroNanoForum, which is of strategic importance in terms of reaching international target groups, and as a platform where researchers and partners within the Mistra Environmental Nanosafety programme can make important contacts.

The mentorship programme was also concluded. It was well received and much appreciated by both the mentees and the mentors who participated. Nine PhD students (with one student from the Mistra STEPS programme) entered the programme, and eight finished it.

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