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NANOTOX 2024

23 – 25 September 2024 – Venice, Italy

BOOK OF ABSTRACTS

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DAY 3 - 25 SEPTEMBER 2024

Day 1
23 September 2024

Session 1A

14:30-16:00

HAZARD ASSESSMENT

Novel mechanistic insights in NMs and advanced materials' toxicity

Chairs: Sabina Halappanavar & Tobias Stöger

Alberto Bianco

CNRS, I2CT, CNRS, Strasbourg, France

14:30-14:50
Immune responses and biodegradability of graphitic carbon materials

Carbon materials are endowed of unique properties and advantages, and they exhibit a broad range of applications. However, their potential risks to human body and the environment have raised several concerns. Graphene and single-walled carbon nanotubes (SWCNTs) are the most developed carbon materials, and they have already been implemented in a wide variety of industrial sectors, opening the door to possible occupational and consumer exposure. Macrophages hold a main role in immune activation against nanomaterials, and human primary macrophages, extracted from human blood donors, are a suitable model to obtain adequate information about human health effects of nanomaterials in general. This presentation will focus on the comparison of different types of industrially-employed graphene and carbon nanotubes, in order to understand how their physicochemical characteristics could affect the cellular behavior [1]. The phagocytic profiles of macrophages have been correlated to the cytotoxic effects. Based on cell activation and cytokine secretion, we also hypothesized how the immune system can degrade these carbon-based materials. In addition, in the environmental context, three peroxidases, namely horseradish peroxidase, Eucodis peroxidase 13 and manganese peroxidase, were used to investigate the enzymatic degradation of graphene and SWCNTs. Morphology and structural defects were compared before and after the treatment using transmission electron microscopy and Raman spectroscopy.

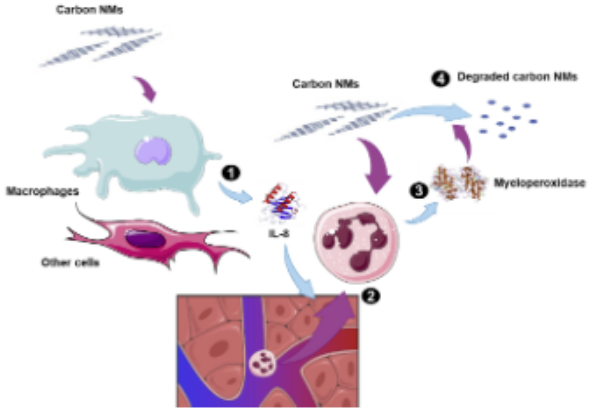

Figure: Possible mechanism describing the role of IL-8 mediating carbon material degradation. 1) Carbon materials produces the liberation of IL-8 by macrophages and other cells. 2) IL-8 attracts neutrophils to the tissues where carbon materials accumulate. 3) Neutrophils secrete myeloperoxidase activated by carbon materials. 4) Myeloperoxidase is a potent peroxidase enzyme with the capacity to degrade these materials.

[1]. Artiga Á., Lin H., Bianco A. Interaction of industrial graphene and carbon nanotubes with human primary macrophages: assessment of nanotoxicity and immune responses. Carbon 2024, under revision.

Han Lianyong*, Carola Voss, Meshal Ansari, Maximilian Strunz, Verena Haefner, Carol Ballester-Lopez, Ilias Angelidis, Christoph H. Mayr, Trine Berthing, Thomas Conlon , Qiongliang Liu, Hongyu Ren, Qiaoxia Zhou, Otmar Schmid, Ali Önder Yildirim, Markus Rehberg, Ulla Vogel, Janine Gote-Schniering, Fabian J. Thesis, Herbert B. Schiller, Tobias Stoeger

*Helmholtz Center Munich, German Research Center for Environmental Health, Munich, Germany

14:50-15:04

Pathogenic dynamics of lung macrophages during injury and regeneration caused by fiber-shaped nanomaterials

Due to their fiber-shape and asbestos homology, some engineered carbon nanotubes (CNTs) cause persistent lung inflammation and injury, thereby contributing to the development of chronic lung diseases (CLDs), like fibrosis, emphysema and even cancer. Upon inhalation, lung macrophages represent the first targets for particle-cell interactions. Combining flow cytometry and single-cell RNA sequencing (scRNAseq) in mouse lungs exposed to different carbonaceous nanomaterials, we found that instillation of CNTs and particularly needle-like MWCNT (Mitsui-7) caused persistent depletion of alveolar macrophages (AMs). The permanent disappearance was validated by intravital microscopy via monitoring lung resident phagocytes. Therefore, deep investigations of material-dependent injury mechanism with their effect on cell-specific inflammatory response pattern, setting the environment for tissue repair and regeneration at single-cell resolution shall help to better understand the potential link between respiratory toxicology and CLDs.
To characterize the mode of injuries, we profiled 12 programmed cell death (PCD) pathways using our scRNAseq data. Surprisingly, pyroptosis, the inflammasome-dependent pro-inflammatory PCD was not detected in macrophages of CNT-exposed lungs, and no IL-1β release into airspace was observed. Instead, necroptosis revealed to be the major PCD pathway in AMs. Necroptosis is mediated by kinases RIPK1 and -3, and Ripk1 is persistently upregulated in CNT-exposed AMs. Integrating published scRNAseq of mouse lung fibrosis and cigarette-smoke induced COPD model also revealed a prominent activation of necroptosis in AMs. These investigations support the contribution of necroptosis to lung inflammation and CLD development.
Interestingly, at d6 after MWCNT exposure, remaining AMs exhibit a pro-inflammatory signature, characterized by high Il1a, Ccl3, -24, Fn1 and Spp1 mRNA levels. Elevated IL1a, an important alarmin released from necrotic cells, were also detected in the airspace, IL1a release from MWCNT-damaged primary AMs could be reproduced in vitro. Simultaneously with AM depletion, transitional macrophages (transMacs), characterized by high Arg1 and Spp1 expression accumulated in the lungs. RNA velocity suggested that transMacs stem from interstitial macrophages and monocytes, particularly with a high Ccl2 and Ccl7 signature. However, even at later time points, transMacs failed to acquire signature (Chil3, Lpl) and phenotype (SiglecFhigh, CD11blow) of mature AMs. Compared to monocytes and interstitial macrophages, transMacs downregulate Ccr2 and Cx3cr1 expression, but retain comparable Itgam (CD11b) levels. Comparing transMacs to different CLDs highlight their profibrotic signature, similar to macrophages of the bleomycin-induced lung fibrosis model. Finally, our study demonstrates the dynamics of lung macrophage during lung inflammation and injury caused by fiber-shaped materials, and underscores their roles in related CLD conditions.

Fiorenza Rancan*, Sabrina Hadam, Xiao Guo, Annika Vogt

*Charité - Universitätsmedizin Berlin, Department of Dermatology, Venerology and Allergy, Germany

15:04-15:18

Ex vivo skin models for the assessment of dermal nanotoxicity

Different skin in vitro and in silico models have been developed as alternative to animal models for testing the toxicity of topically applied drugs, chemicals, nano-formulations, or medical devices. Three-dimensional systems like reconstructed skin cultured at the air-liquid interface offer the possibility of mimicking several features of the skin barrier and to test effects on keratinocytes and fibroblasts. Recently models with other

types of cells like melanocytes and endothelial cells have been proposed. However, these models do not comprise neither skin appendages like hair follicles, sebaceous and sweat glands nor skin immune active cells (e.g., dendritic cells, macrophages, mast cells, T and B cells. Regarding nanomaterials, the pilosebaceous unit has been found to be a niche where nanoparticles can accumulate and translocate to the viable epidermis and dermis, thanks to the peculiar structure of the epithelia barrier around the hair follicles along with the high local concentration of accumulated materials. In the viable skin, there are many immune cells like Langerhans cells in the epidermis, dendritic cells, macrophages, mast cells, and B-cells in the dermis that can take-up material by phagocytosis and thus can specifically interact with nanoparticles. Ex vivo human skin has been used since decades to test skin penetration of drugs. Only in recent years skin tissue culture has been used to develop models for specific pharmacological and toxicological testing. In our group, different ex vivo models have been developed to simulate healthy and inflammatory skin as well as chronic wounds (Figure 1). In these set-ups, skin is cultured in trans-well inserts and pre-treated to simulate barrier disruption or chronic inflammation. These models are used to study corrosion and irritation events by histological analysis and detection of necrosis (LDH release), apoptosis (caspase 3/7 activity), inflammatory and anti-inflammatory cytokines. Furthermore, the models were used to detect, in healthy and inflamed skin, the uptake of nanoparticles by different skin cell populations, the up-regulation of dendritic cell activation markers (CD 86, HLA-DR) and their migration out of skin. The results show that human full-thickness skin is a three-dimensional complex environment with extracellular enzymatic activity, inflammatory mediators, and several active immune active cells that is specifically useful to test the fate and interaction of nanoparticles with healthy and diseased skin.

Guo Chang*, Martin Leonar, Alison Buckley, Sarah Robertson, James Warren, Tim Gant Eugenia Valsami-Jones, Rachel Smith

*UK Health Security Agency, Toxicology department, Harwell Science Park, United Kingdom

15:18-15:32

Understanding how CeO2 nanoparticles modulate bleomycin-induced inflammatory and fibrotic events in both in vivo and in vitro models

Cerium oxide nanoparticles (CeO2NPs) from some diesel fuel additives and other applications have been detected in ambient air. Concerns have been raised over their potential human health impact in situations of inadvertent exposure. Oxidative mechanisms have been suggested as a common feature for pulmonary injury in response to airborne particulate matter, including engineered nanomaterials. To understand in depth how CeO2NPs may influence oxidative stress induced pulmonary inflammation and fibrotic events, we used both in vivo and in vitro bleomycin-induced lung injury models. Male Sprague-Dawley rats were intratracheally instilled with bleomycin or saline (control) followed by nose-only inhalation exposure to nano-sized CeO2NP aerosols (mass concentration 1.8 mg/m3) or water (controls) for 3 hours per day for 4 days per week for one or two weeks. At 3 days post exposure, animals were sacrificed and bronchoalveolar lavage (BAL) fluid, lung histopathology and global mRNA expression analysed. Bleomycin exposure resulted in an increase in total BAL cells, fibrotic staining and significant induction of inflammatory and oxidative stress on mRNA sequencing analysis. Modifications of these responses by one-week exposure to CeO2NPs included attenuation of fibrotic staining and gene expression markers of lung function, inflammation and epithelial-mesenchymal transition (EMT). CeO2NP alone resulted in increased inflammatory responses but did not appear to cause fibrotic changes. Interpretation of these responses at a cellular level was further explored using 3D human small airway epithelium cultures (SmallAirTM) in an aerosol exposure air-liquid-interface system. This also indicated that some bleomycin-induced cellular responses could be attenuated by exposure to CeO2NP aerosols.

Hongyu Ren*, Tobias Soteger, Markus Rehberge, Lianyong Han

*Helmholtz Munich - German Research Center for Environmental Health, Munich, Germany

15:32-15:46

Linking the molecular mechanisms of alveolar macrophage cell death induced by inhaled nanoparticles with the subsequent pathological outcome

Background: Carbon Nanomaterial (CNM) can be released into the environment as combustion by-products and engineered materials. Recent research has shown that inhalation of CNMs differing in shape, such as spherical carbon nanoparticles (CNPs) or fiber-shaped nanotubes (CNTs) triggers different response pattern ranging from acute and resolving to persistent and chronic lung inflammation. Our recent in vivo (mouse) and in vitro data showed that multi-walled nanotubes (MWCNTs) specifically and persistently kill alveolar macrophages (AM), however the immunological consequences are not clear. Here we hypothesize that the AM death pathway determines the fate of lung inflammation, as acute and resolving or chronic.
Results: Exposure of primary alveolar macrophages (AMs) to MWCNTs (Mitsui-7) led to significant decrease of cell viability in a dose-dependent manner (IC50: 10±5 µg/ml). By contrast, CNPs and DWCNTs showed relatively low toxicity (IC50: >100 ug/ml) to AMs. Galectin-3 imuno-fluorescence analysis demonstrated significant induction of lysosomal membrane permeabilization only after MWCNT treatment. Yet ,CNPs, DWNCTs and MWCNTs can inhibit lysosomal acidification, as indicated by decreased acridine orange staining. Interestingly, ROS production in AMs triggered by CNP(IC50: 100ug/ml) and DWCNTs(IC50: 100ug/ml) exceeds that by MWCNTs(IC50: 10±5 µg/ml). Multiplex Cytokine detection demonstrated with high dose(32ug/ml) MWCNTs treated AMs release high level of CCL-2, 4, CXCL-1, TNF-a, IL-6 and IL-1b while CNPs revealed inert in triggering any cytokine release.
Conclusion: Mechanical lysosomal membrane damage by MWCNT seems to play a major role while lysosomal acidification which is essential for autophagy related degradation seems dispensable in AM cell death and the ubsequent inflammatory outcome. Future studies shall investigate the down-stream cellular events of lysosomal membrane damage and its relation to inflammatory cytokine release.

Jonathan Shannahan*, Saeed Alqahtani, Akshada Shinde, Arjun Pitchai, Christina Ferreira

15:46-16:00

Pulmonary Susceptibility to Nanoparticle-Induced Inflammation due to Dysregulation of Lipid Signaling in a Metabolic Syndrome Mouse Model

Underlying health conditions enhance toxicity and inflammatory responses following inhalation exposures to particulate matter such as nanoparticles. Individuals suffering from metabolic diseases represent a prevalent and growing susceptible subpopulation of global concern. A common feature of metabolic and other diseases is lipid dysregulation which may mediate susceptibility to inhalation exposures such as nanoparticles. Specifically, lipids mediate inflammation and are involved in both pro-inflammatory and resolution processes. To investigate lipid contributions to nanoparticle-induced susceptibility via enhanced pulmonary inflammation we have performed multiple studies utilizing healthy and metabolic syndrome mouse models exposed to 20 nm silver nanoparticles via oropharyngeal aspiration. Recently, we demonstrated the metabolic syndrome mouse model exhibits exacerbated inflammatory responses following pulmonary exposure to nanoparticles compared to a healthy mouse model. This enhanced response was associated with a more robust acute inflammatory response as well as a sustained inflammatory response. It is likely this increased inflammation may predispose individuals suffering from metabolic syndrome to toxicity and disease. This exacerbated nanoparticle-induced pulmonary inflammation was associated with acute dysregulation of pro-inflammatory mediators including lipids at 4 hours post-exposure. Additionally, lipid metabolism enzymes were determined to be uniquely altered suggesting disruption in the production of specific lipid mediators that may be contributing to inflammation. At 24 hours we identified a disruption in specific resolution mediator signaling

both at the ligand and receptor level within metabolic syndrome mice. Metabolic syndrome mice did not completely resolve inflammation by 28 days post-exposure while the healthy mouse model return to homeostasis earlier. These nanoparticle-induced alterations in the metabolic syndrome mouse model, suggested lipids could be targeted for therapeutic benefit and addressing nanoparticle-induced inflammation. To elucidate lipid contributions, healthy and metabolic syndrome mice were treated with statins during a portion of disease development prior to nanoparticle exposures or with specific lipid resolution mediators following exposure. Treatment with either statin reduced inflammation observed in metabolic syndrome mice without altering the response observed in the healthy model. This modulation of inflammation was associated with inhibition of lipid alterations in metabolic syndrome mice. Lipid resolution mediator treatments following exposure demonstrated distinct benefits of specific lipid treatments to modulate inflammation in both healthy and metabolic syndrome models. Overall, our studies suggest a dysregulation of lipids involved in inflammation contribute to metabolic syndrome-associated susceptibility to nanoparticle inhalation. This mechanism may be leveraged for treatment strategies specific for this sensitive population.

Session 1B

14:30-16:00

GROUPING AND READ-ACROSS, HYPOTHESES AND IATA EDITING/DEVELOPMENT

AOP/MoA-based grouping (multi-omics)

Chairs: Andrea Haase & Agnes Oomen

Roland Grafström*, Pekka Kohonen, Vesa Hongisto, Penny Nymark, Susan Dekkers, Andrea Haase, Nina Jeliazkova, Otmar Schmid, Tobias Stöger, Blanca Suarez-Merino, Ulla Birgitte Vogel

*Karolinska Institutet, IMM Institute of Environmental Medicine, Sweden

14:30-14:55

The HARMLESS Artificial Intelligence High-Throughput Screening Approach (AI-HTS) to Materials Safety Evaluation

Artificial Intelligence (AI) is set to revolutionize hazard and risk assessments by leveraging "Big Data" and advanced modeling techniques. However, the diverse and growing field of materials presents a challenge due to the scarcity of extensive datasets necessary for AI. The EU-funded HARMLESS project introduces a tiered new approach methodology (NAM) to navigate this complexity. We employ a two-tiered approach, starting with a "high-throughput screening (HTS)" to define dose-response relationships across 72 diverse materials. This is followed by "high-throughput transcriptomics" for a deeper analysis of toxic modes of action (MoA). n the first tier, we apply the cell models THP-1, BEAS2B, A549, and HepG2 within Misvik's Tox5 scoring framework to assess changes across five endpoints: cell numbers, apoptosis, mitochondrial integrity (ATP content), DNA damage, and oxidative stress at varied concentration levels over 6, 24, and 72 hours. The second tier introduces an AI-driven Predictive Toxicogenomics Space (PTGS) model to further define toxic MoAs and identify adverse outcome pathways (AOPs). This tier builds on the first by evaluating differently expressed genes and PTGS scores, thereafter, focusing on key events within 32 lung AOPs and 33 liver AOPs. Our results showcase a range of toxic potencies, cell line sensitivities, and AOP activations, enabling us to group materials by toxic MoA. Through comprehensive analysis, including benchmark dosing (BMD), point-of-departure (PoD) ranking, and grouping based on toxic active concentrations, we address uncertainties and map response ranges. Our approach includes variance and p-value analysis, confidence interval definition, outlier detection, and robust summary statistics across different modeling methods, endpoints, times, and cell lines.

The detailed examination within the PTGS framework, alongside multiple assessment concepts and AOP anchoring, ensures extensive coverage of cellular toxicity mechanisms. The HARMLESS AI-HTS method not only aims to produce valuable "big data" for its objectives but also explores the potential of machine learning to streamline testing protocols. This initiative represents a significant step forward in efficiently assessing material safety, showcasing the power of AI in environmental health sciences.
Project HARMLESS received funding from the European Union's HORIZON 2020 Research & Innovation Programme under Grant Agreement no. 953183.

Veronica Dumit*, Yuk-Chien Liu, Aileen Bahl, Pekka Kohonen, Roland Grafström, Penny Nymark, Christine Müller-Graf, Andrea Haase, Mario Pink

*German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Germany

14:55-15:10
Assessing nanofiber pathogenicity through an omics-based meta-analysis

This study addresses the complexities of assessing nanofiber toxicity within the framework of the Fiber Pathogenicity Paradigm (FPP), which establishes a link between fiber structure and durability and their potential to cause diseases, as demonstrated by asbestos. The unique challenge with nanofibers arises from their nanoscale diameter, which results in reduced rigidity, increased tangling, and changes in typical fiber features. This is further complicated by the lack of validated methods for measuring rigidity of nanofibers. Through a comprehensive meta-analysis of 89 transcriptomics and 37 proteomics studies, our research seeks to deepen the understanding of carbon material toxicity and introduce an innovative approach for evaluating morphology-driven toxic effects. Carbon materials were classified into four distinct categories: non-fibrous, high aspect ratio materials with shorter length, tangled, and rigid fibers, using Mitsui-7 as a reference for pathogenic fibers. The meta-analysis discerned specific cellular responses for each category, allowing for the clear differentiation of rigid fibers from other carbon material forms. Subsequently, a random forest model was then developed to predict material morphology, which revealed the unexpected pathogenic potential of NM-400 due to its secondary structures, despite previously being considered non-pathogenic due to its short length. By correlating toxicological effects with material morphology, particularly for fibers, this work underscores the importance of morphological factors in toxicological assessments. This study aids in identifying materials of concern and lays the groundwork for the development of New Approach Methodologies (NAMs) for testing strategies and contributes to the advancement of safe and sustainable nanomaterial design.

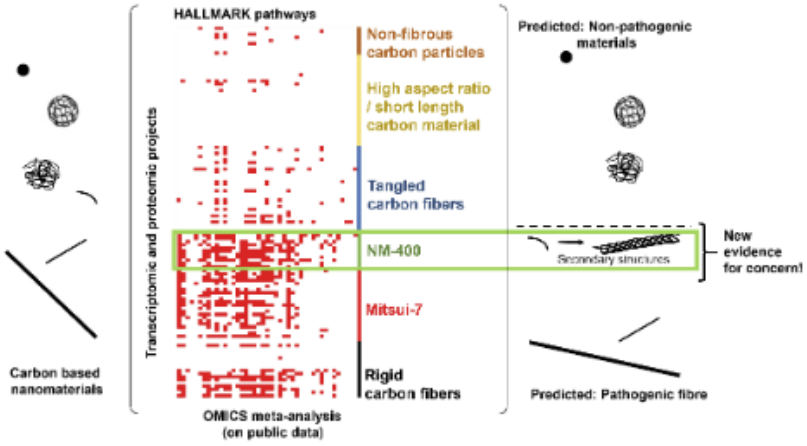

Figure: Meta-analysis of omics studies to discern varying effects among non-fibrous, short/high-ratio, tangled, and rigid carbon fibers, shedding light on toxicity mechanisms and linking fiber-like harm to material structures.

Acknowledgements: This work was supported by the EU H2020 project HARMLESS (GA 953183) and by the BfR Sonderforschungsprojekt 1322-777.

Pekka Kohonen*, Penny Nymark, Vesa Hongisto, Muhammad Irfan Khan, Roland Grafström

*Karolinska Institutet, IMM Institute of Environmental Medicine, Sweden

15:10-15:25
NAM-focused materials safety assessment utilizing predictive toxicogenomics space (PTGS)-driven retrieval of adverse outcome pathways in transcriptomics data

The Predictive Toxicogenomics Space (PTGS) modeling methodology was constructed based on big data analysis, bioinformatics, and early artificial intelligence (AI) concepts for predicting risk of liver injury from drugs (Kohonen et al, Nat. Commun., 2017). In this study, we expanded the applicability of PTGS to assessment of materials toxicity in the lung. Specifically, we took a PTGS-driven tiered informatics approach to anchor gene expression profiling results into the adverse outcome pathway framework by analyzing gene ontologies (GOs) in key events (KEs) for lung AOPs described in the AOP Wiki. Assessment of dose or time-dependent omics data in six publications describing toxicity of 10 materials in human lung cell models generated a fit-for purpose analysis protocol. The materials included asbestos, crystalline and amorphous silicas, and variants of MWCNT, CuO and TiO2. The first tier included assessment of LOELs for cytotoxicity, differential gene expression and PTGS component mode-of-action (MoA) analyses. These results set the stage for investigating activated AOPs by either a High-SEnsitivity Approach (HSEA) for ultra-high AOP coverage versus a High-SPecificity Approach (HSPA) for AOP selection from correlating the PTGS MoA patterns versus KEs and AOPs under stringent thresholds. All assessed materials activated the PTGS algorithm set to sensitively detect toxicity mechanisms, and accordingly generated dose-dependent PTGS-LOEL data. The materials further variably activated between few to many of 12 of 13 assessed PTGS components. PTGS-component MoA scores for the materials detected activation of 29 lung AOPs to variable KE activation depths with the HSEA, including for 11 AOPs activation of all described KEs. Differently, the PTGS-driven HSPA markedly increased the specificity of the AOP selection to levels from no active AOPs for several materials, to variably from one to 19 AOPs for up to five of the tested materials. Interpretation of the derived MoA patterns indicated that several materials activated AOPs and KEs coupled to inflammation, fibrosis, and cancer. Finally, the MoA patterns from either PTGS components, AOPs or KEs indicated similarity among several materials as they variably clustered in two to four groups. We applied a standardized analysis which considers data completeness and quality considerations and eliminates arbitrary differential gene expression cut-offs where all gene expression levels are considered (FAIR principle applied = reliable + transparent). Overall, we conclude from these studies that PTGS can serve as a giant AOP-applicable toxicity biomarker that captures/describes dose response and toxic MoA of diverse materials.

Alex Zabeo*, Matteo Carisi, Fabio Rosada, Lisa Pizzol, Danail Hristozov

*Greendecision, Italy

15:25-15:40
Hierarchical clustering-based grouping using OWA based similarity matrix

This presentation explores a grouping approach that leverages hierarchical clustering for group formation. It introduces the concept of an Ordered Weighted Averaging (OWA) based similarity matrix, a novel method for quantifying the resemblance between data points. By incorporating the OWA operator, the similarity matrix can capture complex relationships within the data, leading to more nuanced groupings.
The presented approach was tested against a literature review-based dataset related to immobilization of Daphnia magna exposed to five different NFs of Nano Copper Oxide. The results of applying the methodology in the case study demonstrated that two main groups are obtained which are aligned to nanomaterial's properties. The presentation will discuss the benefits of this approach for data analysis and provide insights into its applications.

Miguel A. Bañares*, Victor Alcolea-Rodriguez, Veronica I. Dumit, Rico Ledwith, Raquel Portela, Andrea Haase

*CSIC, Instituto de Catalisis, Spain

15:40-15:55
Engineered nanomaterial surface reactivity impact on cellular homeostasis and cytotoxicity

Here we explore the cellular mechanisms of engineered nanomaterials (ENMs) focusing on the role of reactivity for cellular toxicity and the interplay with autophagy as a possible cytoprotective mechanism with the overarching aim to establish a suitable screening strategy to facilitate ENM categorization.
Reactivity is a crucial parameter for characterizing ENM toxicity as it can lead to different adverse outcomes. Different assays can be applied to test for reactivity, e.g., measuring the formation of reactive oxygen species (ROS), oxidative capacity, thiols consumption, or by assessing the number and nature of surface-active sites. These assays also allow for insights into the underlying physicochemical properties. Here we evaluated the effects of 10 different ENMs in A549 and dTHP-1 cells by utilizing different reactivity assays by measuring ROS generation and determining the surface-active sites using methanol chemisorption and temperature-programmed surface reaction along with basic cell viability tests (using water-soluble tetrazolium (WST-1) and Lactate Dehydrogenase (LDH) assays). We also performed proteomics investigations to get information about the underlying MoA below cytotoxic concentrations. Non-reactive ENMs like SiO2 NM-200 showed no significant impact on cell viability. Conversely, highly reactive ENMs such as CuO and ZnO (NM-110 and NM-111) markedly affected cell homeostasis in both cell types. Interestingly, some ENMs such as TiO2 generated ROS but did not have negative effects on cell viability. Proteomics data prompted us to autophagy as a possible cytoprotective mechanism. We could confirm that moderately reactive ENMs like TiO2 (NM-101 and NM-105) and CeO2 (NM-211 and NM-212) induced autophagosome formation, evidencing autophagy as a defensive mechanism. We therefore suggest implementing autophagy assays into screening strategies. They appear useful for the categorization of ENMs and can support a more accurate prediction of the cellular toxicity of ENM.

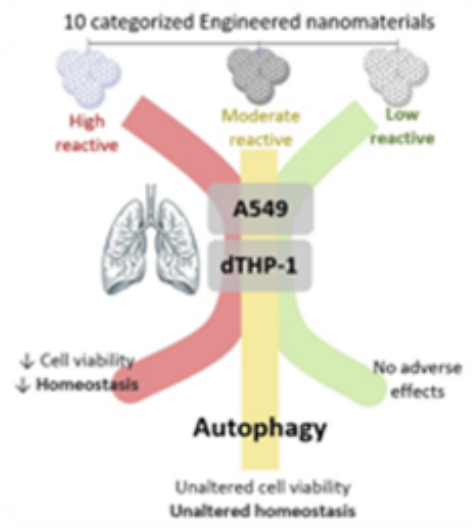

Figure: Relevance of nanomaterials degree reactivity level on homeostatis and cell viability.

Acknowledgments: This work was supported by the EU H2020 project NanoInformaTIX (GA 814426), by the BfR Sonderforschungsprojekt 1322-734, and by La Caixa Foundation Health Research project HR21-00813. We acknowledge the provision of reference-engineered nanomaterials by the JRC (Joint Research Centre) and Dr. Juan Riego Sintes.

Session 1C

14:30-16:00

RISK ASSESSMENT AND MANAGEMENT

Exposure assessment

Chairs: Susan Dekkers & Carlos Fito

Wouter Fransman*, Ruby Vermoolen, Hasnae Ben Jeddi, Henk Goede, Remy Franken, Neeraj Shandilya, Eugene van Someren

*TNO, Risk Assessment of Products in Development, Netherlands

14:30-14:50
Nano Exposure Quantifier for Safe-by-Design of Advanced Materials

Exposure to manufactured nanomaterials (MNs) is a growing concern for occupational health and safety. Reliable methods for assessing and predicting MN exposure are essential to mitigate associated risks. This study presents the development of the Nano Exposure Quantifier (NEQ), a mechanistic model designed to assess airborne MN exposure in the workplace. By utilizing a standardized dataset of 131 MN measurements from existing exposure studies, the model demonstrates its effectiveness in estimating MN exposure levels for particles smaller than 10 µm. The NEQ provides estimates in terms of particle number concentration accompanied by a 95% confidence interval (CI), enabling a comprehensive assessment of MN exposure. The SbD concept applied in the integrated NEQ-SbD tool introduces a comparison between a baseline exposure assessment and an (improved) SbD exposure assessment. The NEQ-SbD is an easy-to-use online tool that guides users to prevent or mitigate exposure to nanomaterials at the worksite where nanomaterials are manipulated or handled during a wide range of activities. This allows the tool user with an informed decision to assess airborne exposure and to select, compare and identify appropriate risk management measures (RMM). The SbD module was developed using various information sources that support the SbD process, including the (1) RMM effectiveness based on analyses of an Exposure Control Efficacy Library (ECEL), (2) RMM performance using a Computational Fluid Dynamics (CFD) model, (3) e-cards based on a qualitative analysis of RMM information sources and (4) guidance for SbD strategy using an exposure directionality assessment. The main purpose of the SbD module lies in guiding the user to the most sensitive (exposure) parameters and allowing side-by-side comparison of potentially suitable RMMs. This NEQ-SbD module is illustrated using a worked example for the transfer of nano powders, showing the possibility of identifying SbD solutions for both safe-by-process and safe-by-material design purposes. The NEQ-SbD tool is a valuable tool for the SbD of nanomaterials and as a decision-making tool to support SbD risk management strategies that lead to minimizing health risks associated with occupational exposures.

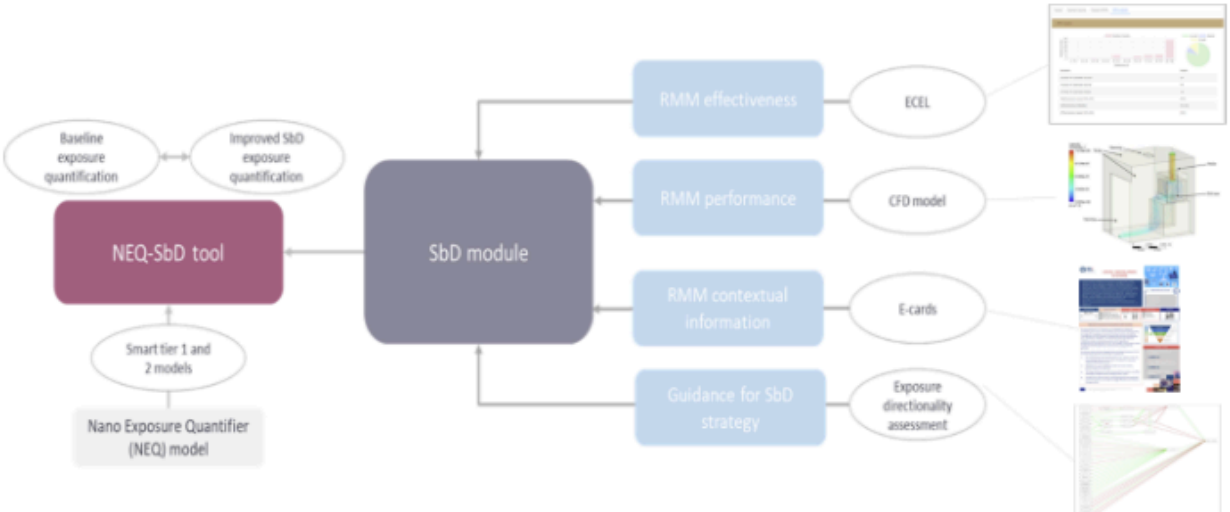

Figure: Workflow of the NEQ-SbD online tool

Carla F. Martins*, Ana Rita S. Alberto, João Laranejira, Jorge Salvador, Verónica Vela Vela, Elena Barbero, Céline Lett, Ivonne Trebs, Thomas Schleeh, Patricia Farias, Olavo Cardozo - Andreas Stingl

*ISQ - Instituto de Soldadura e Qualidade, Portugal

14:50-15:04

Real-time monitoring of airborne nanoparticles supporting the implementation of SSbD strategies during the production of MCNMs and HARNs

Facing growing concerns over environmental and health implications of nanotechnological advancements, DIAGONAL project includes safety and sustainable considerations at the heart of the innovation processes. The project is focused on incorporating environmental, health, and safety considerations into nanotechnology innovations, aligning with the Chemicals Strategy for Sustainability and the Green Deal. The project grounds in the Safe Innovation Approach (SIA) and Safe and Sustainable-by-Design (SSbD) principles, in line with European Commission guidelines that emphasize minimizing health and environmental impacts through sustainable practices. The project specifically targets gaps in risk assessment, management, and governance of nano-enabled products, especially multi-component nanomaterials (MCNMs) and high aspect ratio nanomaterials (HARNs).

In DIAGONAL, experimental and modelling studies are conducted to understand the interactions and transformations of nanomaterials, aiming to create safer industrial applications particularly for small and medium-sized enterprises (SMEs). This aim to support industry confidence in adopting sustainable nanomaterials. A significant aspect of DIAGONAL is its focus on occupational safety during nanomaterials production. and on real-time monitoring during the production of nanomaterials at industrial demonstrators. Portable real-time measurement equipment to semi-quantify airborne nanoparticles and air sampling was employed, following recommended approaches such as those described in EN 17058:2018, ISO/TR 12885, and ISO/TS 12901-2. This strategy helps optimize workplace exposure controls and enhances worker safety during the manufacture of MCNMs and HARNs.

Phornano, one DIAGONAL's industrial test cases, developed the VERDEQUANT green nanotechnology which aimed sustainable manufacturing of zinc oxide
(ZnO) nanopowders. Real-time monitoring tools were used to assess safety during production, analysing airborne nanoparticles' size, surface area, and distribution, confirming low levels of particle release and minimal inhalation exposure risks. Also, air sampling was performed, and off-line analysis were carried out (SEM and EDX) to identify and characterize the airborne particles. In the SEM (Fig. 1) images it was possible to identify the presence of ZnO particles, although, their concentrations were found to be small according with the online monitoring readings.

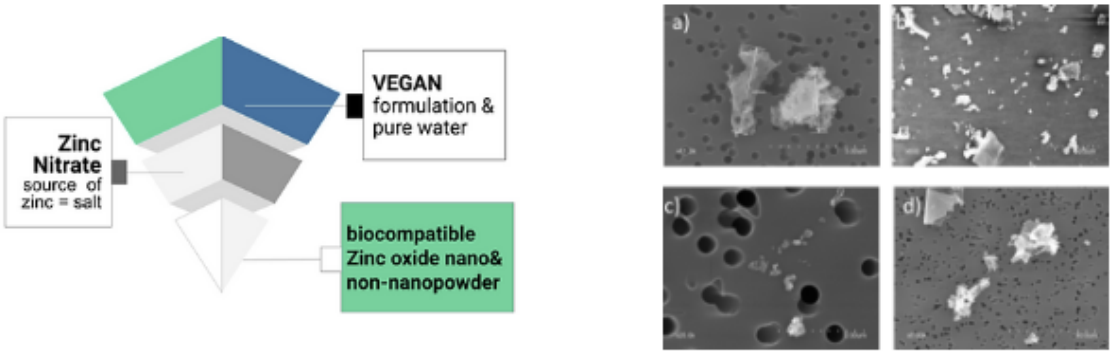

Figure 1 - VERDEQUANT processes using a vegan formulation fully replacing fossil-based reagents (left) and SEM images from air sampling during the production process (right).

Overall, DIAGONAL exemplifies the integration of safety and sustainability considerations from the earliest stages of nanotechnology development, setting a benchmark for responsible practices. This project not only fosters a safer, more sustainable future for nanotechnology but also aids in guiding nanosafety governance bodies by sharing its findings and developments.

Gunther Van Kerckhove*, Rudolf Bieri, Kevin Sparwasser, Zoltán Szakács, Amaia Soto Beobide, George. A. Voyiatzis, Konstantinos S. Andrikopoulos, Detlef Schuler, Christophe Bressot

*OCSiAl Europe Sarl, Luxembourg

15:04-15:18
Risk assessment along the life cycle of TUBALL™ nanocomposites

We report about the assessment of a nanocomposite containing SWCNT, represented by brand TUBALL™, in support of the Chemicals Strategy for Sustainability and the Safe-and-Sustainable-by-Design framework. We highlight challenges in implementing regulatory needs to assess safety and offer alternative solutions, in particular to TUBALL™ we like to achieve. This SWCNT forms strong agglomerates prohibiting dosing in (eco-) toxicological studies. Separation is typically performed by sonication or ball milling. Sonication was not successful and ball milling has the disadvantage of disintegrating single fibres / primary particles, thereby altering their morphology, and risking that findings would not actually be representative for the fibres. Thus, not every study assessing hazards can be performed in a technically correct way. Assessing EHS-risks in the use of TUBALL™ we initially tested for hazards, followed by exposure assessments. Production of SWCNT is performed in an enclosed and well monitored process. Occupational worker exposure during manufacturing is monitored using an aerosol-based material specific approach, commercialized by STAT PEEL Ltd. 'Exposure during use' was quantified for example with indoor treadwear experiments, performed at Calspan, US. These investigations represented the life cycle of tires showing no free SWCNT was released to the environment. To assess environmental risks at the end-of-life cycles, represented by landfilling or incineration, ecotoxicity studies were performed using wear debris from thermoset, thermoplastic and elastomer samples containing TUBALL™ additives. No toxicity was found from the TUBALL™ itself. Moreover, when incinerated these consumer goods showed no release of any free SWCNTs during or after the process. Taken together, our studies show that following an (occupational) exposure-based approach, costly and time consuming "in vivo" inhalation toxicity studies, may be waived, which are currently "technically not feasible" to TUBALL™. In addition, since relevant exposure does not take place along the life cycle. It highlights that focusing on single fibres / primary particles (as requested by regulation) is not always technically feasible, and not representative of the material found in consumer products. TUBALL™ has a specific morphology, and shows a unique behavior during testing, preventing some standardized test models to be applied. We don't see the necessity to invest massive resources in trying to investigate inhalation exposure of SWCNT - single fibres / primary particles if there will be no relevant exposure possible.

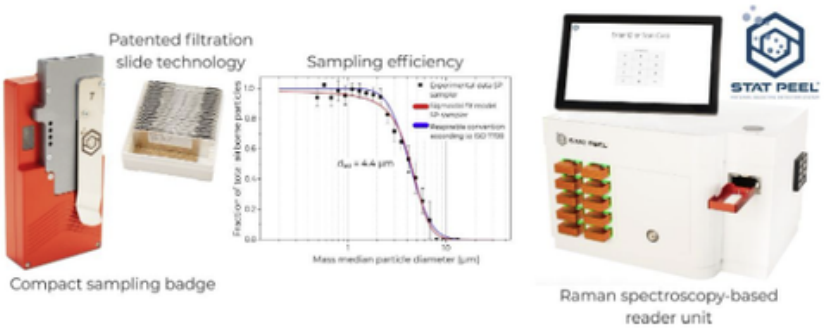

Figure 1. STAT PEEL Identifier system.

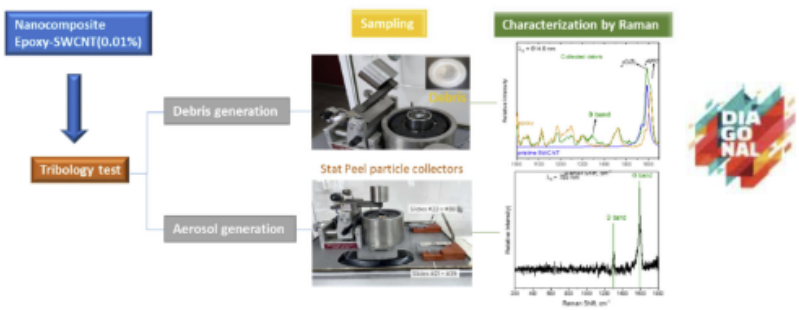

Figure 2. schematic representation of experimental setup - study case from DIAGONAL.

Camilla Abrahamsson*, Christina Isaxon, Neeraj Shandilya

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15:18-15:32
Airborne nanoparticles emitted during industrial bag emptying of fumed silica

Nano-silica (NS) is currently one of the most used engineered nanomaterials (ENMs) globally, with successful application as additives into common construction materials such as cement, concrete, coatings, and paint to improve their properties1. Although amorphous silica is considerably less toxic than in the crystalline form, the potential health hazards of amorphous silica in the nanoform are still debated, especially for smaller particle sizes2. Since nano-silica is commonly produced and handled as a powder, the potential for aerosolization and inhalation in occupational settings needs to be investigated in industry-relevant settings and processes. In this work, particle emissions from bag dumping into a reactor were characterized in a chemical factory setting. For each shift, a single worker emptied eight 150 kg bags in succession, where each bag took 45 minutes to empty. In Figure 1, averaged number particle size distributions are displayed for the first and final bag in the near-field (NF, 1 m from the mission source) and far-field (FF, 20 m from the emission source).
The pre-activity background displayed a unimodal size distribution with a peak at 70 nm, both in NF and FF. During the emptying of bag 1, a slight increase could be seen for the smaller particle sizes. For the final bag, the size distribution had shifted into a bimodal distribution, with peaks at 20 nm and 70 nm. The 20 nm peak is most likely activity-related, gradually built up during the shift and could also be observed in the FF, indicating spatial spread of the emissions across the facility. This suggests that current dust mitigation measures are inadequate for emissions in this size range. Considering the high deposition in the distal lung for particles in this size range3 as well as the increased toxicity of nano-silica for smaller particles2, these emissions need to be closely monitored and controlled until their toxicity has been thoroughly evaluated.

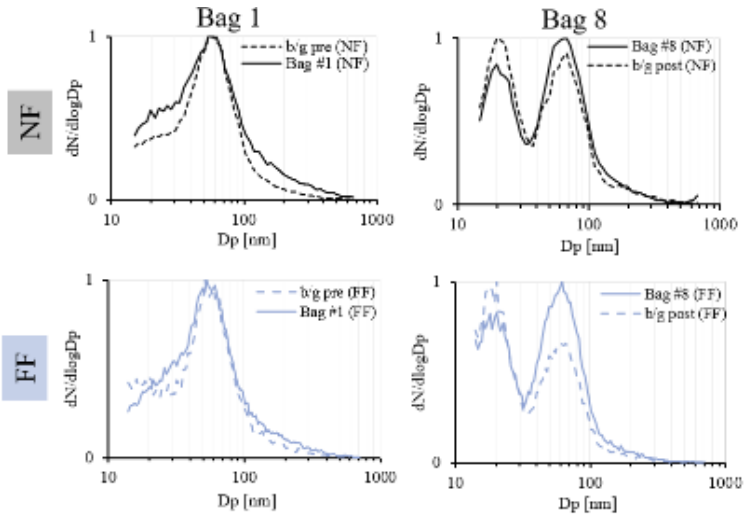

Figure 1: Normalized number particle size distributions in the near field (NF) and far-field (FF). The size distribution for each bag is averaged across the time for emptying each bag and compared to the background before the start of the activity (b/g pre) and after finished activity (b/g post).

This work was funded by the NMBP-16 project HARMLESS (GA no 953183) and AFA Insurance (dnr 20010).

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https://doi.org/10.1186/s12989-017-0190-8

Eun Gyung (Emily) Lee*, Ryan Gill - Aliakbar Afshari, Walter McKinney, Vamsi K. Kodali, Jakook Gu, Gary Casuccio, Kristin Bunker, Traci Lersch, Keith Rickabaugh, Aaron Erdely

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15:32-15:46

Characterization of aerosolized particles during mechanical manipulation of multi-walled carbon nanotubes containing concrete

Inhalation exposures to carbon nanotubes are known to cause adverse pulmonary effects. The manipulation of multi-walled carbon nanotubes (MWCNT)-reinforced concrete (e.g., cutting and grinding) in construction might release free MWCNT. This study was conducted to characterize aerosolized particles during cutting of MWCNT containing concrete blocks (0% [control], low%, and high%). A fully automated concrete-cutting chamber was built to simulate an actual cutting process of concrete. Number-based particle size distributions and particle number concentrations were measured using direct-reading instruments. Filter-based samples were used to determine 1) concentrations of respirable dust, silica, and elemental carbon (EC, selected as a surrogate for MWCNT), 2) mass-based particle size distributions using an impactor, and 3) particle morphologies and protruded MWCNT particles using automated and manual electron microscopy techniques. The average particle number concentration was the highest for the control (163,821 particles/cm3), followed by the high% (144,029 particles/cm3) and low% (131,689 particles/cm3). Regardless of the block type, the number-based particle size distributions revealed one main mode at 0.72 µm indicating no shift in the size distribution from addition(s) of MWCNT. Nevertheless, the mass median diameters shifted to the right for the MWCNT-reinforced concretes (4.4 µm, 5.1 µm, and 5.0 µm for the control, low%, and high% blocks, respectively), indicating a predominant respirable fraction. The respirable dust (silica) concentrations were 316 (98.2) mg/m3, 419 (36.2) mg/m3, and 412 (121) mg/m3 for the control, low%, and high% blocks, respectively, revealing statistically significant differences between low% and control/high% blocks. The EC was below the limit of detection for all samples. Respirable particles frequently appeared agglomerated, comprising paste and aggregate minerals. The quartz and feldspar aggregates were predominantly angular with flat sides and smooth surfaces, while the paste particles were irregularly shaped with coarse surfaces. The relative concentration of particle elemental composition by number showed that low% and high% MWCNT concrete consisted primarily of Ca-Si (> 15%) in the paste and Si-O (> 30%), Si-Al-K (> 23%), and Si-Al(CaNa) (> 14%) in the aggregates. Similar results were seen for the particle elemental composition by weight. Additionally, examination of over 100 particles/sample type confirmed no presence of protruded MWCNT. This study suggests that more respirable dust and silica concentrations can be generated depending on the MWCNT weight percent in concrete than the control. Although no protruding MWCNTs were observed, a toxicity study should be conducted to determine any potential adverse health effects caused by MWCNTs wrapped with other minerals (e.g., silica).

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15:46-16:00

Form-specific prospective environmental risk assessment of graphene-based materials in European Freshwaters

As the application of graphene-based materials (GBMs) in diverse fields increases, the necessity for environmental risk assessments also grows. In a previous study we performed a material flow analysis of GBM, identifying release pathways and estimated concentrations in environmental compartments. In that study,GBM was treated as a group of materials although the importance of considering the different forms of GBMs for environmental risk assessments has been recognized.

Pristine graphene (pG), graphene oxide (GO), and reduced graphene oxide (rGO) are the most important forms of GBM which all have different properties and behavior. To bridge this gap, we propose a form-specific environmental risk assessment method. This innovative approach combines predicted environmental concentrations (PECs) obtained through a dynamic probabilistic material flow analysis with form-specific predicted no-effect concentrations (PNECs) obtained by Probabilistic Species Sensitivity Distributions. The results are form-specific risk characterization ratios (RCRs) for pG, GO and rGO, aiming for a more accurate understanding of GBMs' environmental impacts.

The PEC values in European freshwaters for the year 2030 were predicted to be 0.67 ng/L (Q25 and Q75 of 0.49-0.81 ng/L) for pG, 0.32 ng/L (0.25-0.39 ng/L) for GO, and 0.32 ng/L (0.25-0.39 ng/L) for rGO. These concentrations indicate comparable levels, suggesting similar extents of exposure in surface water. The PNECs were determined to be 34 µg/L for rGO, followed by pG with 22 µg/L (13-31 µg/L), and GO with 14 µg/L (11-17 µg/L). The RCR for various forms were much below below 1 (mean of each form between 10-4 and 10-6) signalling a very low environmental risk of all forms (Figure 1). Despite the potential for a significant future increase in demand and therefore environmental release for these materials, our analysis indicates that their environmental impact remains minimal.

Our results enhance the understanding of the potential risks associated with the production and use of GBM and underscore the significance of considering the distinct forms of GBM in environmental risk evaluations. This comprehensive understanding of GBM's environmental dynamics can inform effective management strategies aimed at minimizing environmental impacts.

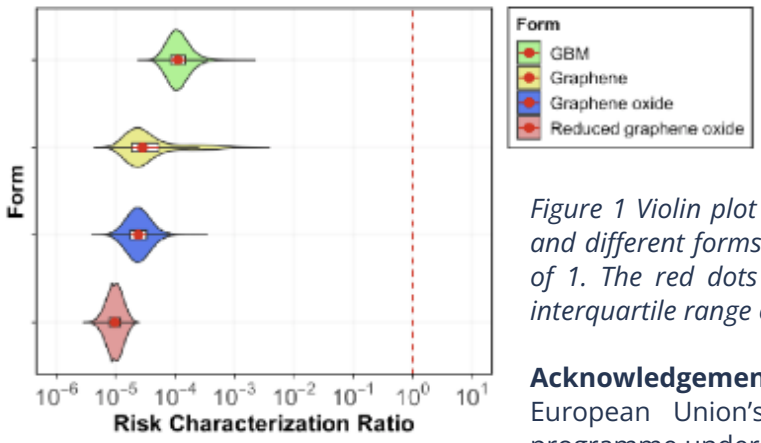

Figure 1 Violin plot of the risk characterization ratio (RCR) of GBM and different forms of GBM. The red vertical line indicates the RCR of 1. The red dots show the means and the red lines show the interquartile range of the distributions.

Acknowledgements: This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952924 (SUNSHINE)

Session 2A

16:30–18:00

HAZARD ASSESSMENT

Novel mechanistic insights in NMs and advanced materials' toxicity

Chairs: Harald Krug & Blanca Suarez

Karen Smeets*, Nelly Saenen, Margo Witters, Frank Van Belleghem, Jan Mast, Eveline Verleysen, Alex Remels

*Hasselt University, Centre for Environmental Sciences, Belgium

16:30-16:45

The role of mitochondria in nano- and micro-particle-specific toxicity

Although we increasingly encounter nano- and microparticles (NPs-MPs) in everyday applications such as face masks, personal care products, and foodstuff, our understanding of how their physicochemical properties relate to particle uptake and toxicity remains scarce. As a consequence, current risk assessment strategies are insufficient. The present study examined the mechanisms underlying particle-induced toxicity of different nano- and microparticles in different cell types. We used human dental pulp stem cells (DPSCs) and bone marrow mesenchymal stem cells as a proxy for assessing developmental effects, and Caco-2, Caco-2/HT29-MTX-E12 and Caco-2/Raji-B to assess the impact on gut health; all in line with the 3R principles and REACH regulation. Cell cycle dynamics, and related redox and mitochondrial parameters, were investigated in relation to the particle characteristics and kinetic profiles. We compared three widely implemented NP types, namely polyvinylpyrrolidone-coated silver (PVP-Ag), titanium dioxide (TiO2) and carboxylated polystyrene (COOH-PS) NPs. We further included spherical (2 µm) and fibre-/fragment-shaped (8.9 ± 10.1 µm by 1.14 ± 0.97 µm) polystyrene microplastics. The studied particles were characterized using dynamic/static light scattering and transmission electron microscopy (TEM). Cellular uptake and localization in endosomes and, in some cases, the cytosol was confirmed using TEM and confocal microscopy. PVP-Ag NPs and TiO2 NPs were cytotoxic and decreased cell proliferation, depending on the duration of exposure. COOH-PS NPs did not significantly affect cell viability at the concentrations tested, but interfered with cellular metabolism. PVP-Ag and TiO2 NPs strongly affected stem cell cycle dynamics, although particle-specific effects were observed in cell proliferation and cell cycle phase distributions. Responses depended on exposure conditions, again emphasizing the importance of studying the interaction between physicochemical characterization, uptake, and effects. Underlying these changes, particle exposure induced mitochondrial swelling, membrane depolarization and network structure alterations as determined by TEM, JC-10 assay and Mitotracker CMXRos Red imaging, respectively. Furthermore, the absence of endoplasmic reticulum dilations and the presence of lipid droplets were detected via TEM after 48 h exposure to PVP-Ag and TiO2 NPs. The detected subcellular and molecular changes, measured via Western blotting and real-time qPCR, were different for the different particles, showing the increasing need to compare different particle types in relation to their physicochemical characteristics. For the tested exposures, we hypothesize that mitochondria operate as a signaling hub, guiding the activation of downstream transcriptional processes and (stem) cell dynamics.

Gupta Govind* , Jonas Bossart, Sènan Mickael D'Almeida, Vera M Kissling, Alexander Gogos, Christoph Schwärzler, Tingting Fu, Vanesa Ayala Nunez, Marija Buljan, Emmanuel Flahaut, Miguel Garcia, Peter Wick, Tina Buerki-Thurnherr

*Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland

16:45-17:00

Boron Nitride Nanomaterials Activate Eicosanoid Lipid Signaling in Bronchial Lung Cultures that Triggers Immunomodulation

Two-dimensional carbon or boron nanosheets have been demonstrated to interact with the cell membrane and affect membrane lipid composition. However, the potential downstream effects of lipidomic changes and underlying mechanism remain poorly explored. Here, we used boron-based nanomaterials with two different aspect ratios namely hexagonal boron nitride (*h*-BN) nanosheets and boron nitride nanotubes (BNNTs) and studied their cellular interaction with healthy and asthmatic bronchial lung cultures, since lipid metabolism is involved in the progression of asthma. We used air-liquid interface-based 3D reconstituted airway epithelia (MucilairTM, Epithelix, Switzerland) of healthy and asthmatic subjects and repeatedly exposed them to the particles for 5 weeks at a total applied dose of 10 µg/cm2 (considered occupationally relevant). Our results revealed that *h*-BN and BNNTs were taken up by cells as elucidated from ICP-MS, Raman-confocal and TEM analysis, but did not trigger significant (p >0.05) effects on cell viability (LDH release) or epithelial barrier integrity (TEER) in both healthy and asthmatic lung cultures. However, we observed a higher intracellular accumulation of lipids in lipid granules after exposure to *h*-BN or BNNTs as compared to the untreated cells. Furthermore, cell surface and global lipidomic profiling by applying ToF-SIMS and LC-MS, respectively indicated significant changes in the lipid composition both at the cell surface and global lipidome of healthy and asthmatic cultures. Specifically, we observed a significant increase in the cellular content of eicosanoid lipids (i.e., leukotrienes and prostaglandins) after exposure to h-BN and BNNTs indicating that boron nanomaterials might modulate eicosanoid-mediated crosstalk between lung and immune cells.

Therefore, we next investigated whether the increase in eicosanoid lipids could modulate immune cells in human blood. We exposed human peripheral blood mononuclear cells (PBMCs) to conditioned medium (containing secreted factors including eicosanoid lipids) collected after exposure of lung cultures with h-BN or BNNTs and performed single-cell cytometry by time of flight (CyTOF). CyTOF results showed a significant induction of specific T-cell subsets and NK cells including the associated cytokines-chemokines (i.e., granzyme b, perforin). The specific effects on T-cells and NK cellscould be explained due to the known high expression of leukotriene receptors on these cells. The lipidomic changes and immune effects observed here were more pronounced for BNNT exposure to asthmatic cultures than healthy ones. Overall, our study identified adverse effects of boron nanomaterials on lipid metabolism in healthy and asthmatic human airway epithelium and indicated a potential correlation of certain lipid accumulation with enhanced immune signaling.

Langzhi He*, Zihui Li, Hongbo Wang, Hasen Bilige, Chen Li, Yun Wang

*Peking University, Peking University, China

17:00-17:15

Impacts of long-term exposure to titanium dioxide nanoparticles on chronic colitis

With the rapid development of nanotechnology, emerging studies have highlighted the presence of nanoparticles (NPs) within TiO2, suggesting potential adverse effects. Particularly, TiO2 NPs have been implicated in exacerbating acute inflammation, raising concerns about their impact on chronic ulcerative colitis (UC). This study aims to investigate the long-term effects of TiO2 NPs exposure on the onset and progression of chronic UC. Mice were randomly divided into 4 groups in 2 animal models: CT and CT-N group in healthy model, UC and UC-N group in UC model, and the CT-N, UC-N group were fed with a diet containing 1% TiO2 NPs (34.14± 6.50 nm) for 20 weeks. To induce the UC model, mice were given 2% Dextran sodium sulfate (DSS) in drinking water for 7 days at weeks 11, 14, 17, and 20 of the experimental period, and had free access to regular drinking water at other times. At the experimental endpoint, colon tissues were grossly observed and used for H&E staining, Ki-67 immunohistochemistry, and detection of 15 related cytokines. Fecal samples of all mice were collected for 16S rRNA sequencing analysis. The results demonstrated that TiO2 NPs significantly reduced body weight in both CT and UC model. Notably, TiO2 NPs also resulted in a significant increase in the disease activity index of mice in the UC model and a significant shortening of colon length, which was the key feature of chronic colitis that was not observed in the CT model. Surprisingly, exposure to TiO2 NPs did not exacerbate oxidative stress or inflammation levels in the UC model. Instead, it led to a significant decrease in IL-23, IL-17A, and TNF-α levels compared to the UC group. Besides, TiO2 NPs led to significant alterations in the microbial composition within both CT and UC model, with several differentially abundant species and predicted functions identified. These findings confirmed the role of TiO2 NPs in promoting the onset and progression of UC and explored potential mechanisms from the perspectives of inflammation-related cytokines and gut microbiota, providing robust data for assessing the safety of TiO2 NPs.

IZTOK URBANČIČ*, ALEKSANDAR SEBASTIJANOVIĆ, MARIA AZZURRA CAMASSA, VILHELM MALMBORG, SLAVKO KRALJ, JOAKIM PAGELS, ULLA VOGEL, SHAN ZIENOLDDINY-NARUI, TILEN KOKLIČ, JANEZ ŠTRANCAR

*Laboratory of Biophysics, Condensed matter physics department, Jožef Stefan Institute, Slovenia

17:15-17:30

Partriculate matter constituents trigger the formation of extracellular amyloid β and tau-containing plaques and neurite shorthening in vitro

Air pollution is an environmental factor associated with Alzheimer's disease, characterized by decreased cognitive abilities and memory.

Despite strong epidemiological correlation between exposure to airborne particulate matter and the development of the disease, the causal relationship between particulate matter in air pollution and Aβ plaque deposition with neurite degeneration in Alzheimer's disease remains controversial. Environmentally driven models of Alzheimer's disease are thus timely and necessary.
To address this, we investigated whether various types of particulate matter commonly found in polluted air, including metal oxides and carbonaceous particles, could trigger the formation of Aβ plaques and neurite shortening in an in vitro model. We exposed neuron-like cells, differentiated from the human neuroblastoma cell line SH-SY5Y, to different nanomaterials (TiO2 nanotubes, Fe2O3, diesel exhaust particles, and CeO2). We employed live-cell confocal fluorescent imaging combined with high-resolution stimulated emission depletion (STED) microscopy to follow the morphological changes of cells and the formation of extracellular amyloid β and tau plaques, visualised by fluorescently labelled antibodies.
We found that within 100h after a single high-dose exposure of in vitro neuron-like cells to particulate matter constituents, particularly for γ-Fe2O3 and diesel exhaust particles, reproduces a neurodegenerative phenotype, including extracellular amyloid-β containing plaques and decreased neurite length. In contrast, CeO2 nanoparticles did not cause any reduction in length, which is consistent with their beneficial effect observed in traumatic brain injury models. In the samples that shorten neurites the most, also more Aβ deposits were observed. Although the exact mechanism behind this effect remains to be explained, the single-exposure high-dose in vitro
model, comprising wild-type neuron-like cells, could serve as an alternative environmentally driven model of Alzheimer's disease.

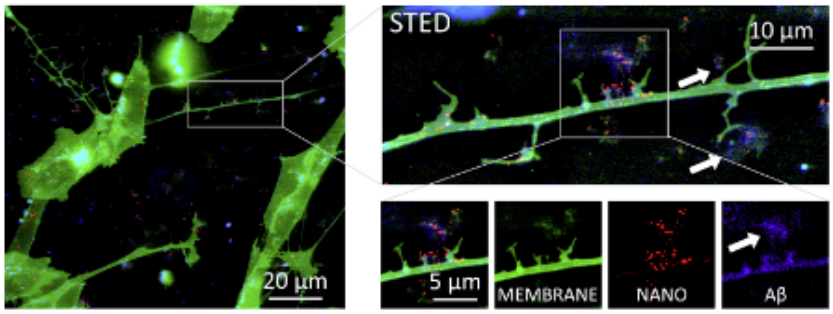

Figure: High spatial resolution fluorescence image (stimulated emission depletion STED) of synapse damage and early plaque formation (white arrows) at the sites of damaged synapses (green, MEMBRANE) by anatase TiO2 nanotubes (red, NANO) containing amyloid β (blue, Aβ).

Daniel Butterfield*, Cyrill Bussy, Thomas Kisby, Neus Lozano, Kostas Kostarelos, Sandra Vranic

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17:30-17:45
Investigating the neurotoxic and neuroinflammatory impact of intranasally administered ultra-small graphene oxide in a murine model

Current therapeutic options for diseases of the central nervous system (CNS) often suffer from poor efficacy due to low accumulation at the target disease site; this is largely due to an inability to cross the blood brain barrier (BBB). Graphene related materials (GRMs) with controllable lateral dimensions and physicochemical properties can bypass the BBB and therefore may provide novel opportunities for treating brain diseases. However, before GRMs can be employed in therapeutic applications, biocompatibility with the CNS must be thoroughly assessed. In this study, ultra-small graphene oxide (usGO) flakes with average lateral dimensions of < 50 nm were intranasally administered to mice and CNS biocompatibility assessed at 1-day, 7-days, and 28-days post administration. Our group has previously demonstrated that following intranasal administration usGO distributes throughout the brain and is retained within the CNS for at least 28 days. Here, neuron counting was performed to investigate neurotoxicity of administered usGO, with no cytotoxic effect detected in all examined regions of the brain. Surprisingly, an increase in neuron population was found in the CA3 region of the hippocampus at 28-days post usGO administration. Further work will be undertaken to determine the long-term impact of this neuronal increase and to uncover the source of this new population of cells. In addition, neuro-inflammatory response to usGO was assessed by measuring expression of inflammation associated genes and changes to resident microglia and astrocyte reactivity in specific brain regions. Acquired results indicate a non-significant inflammatory response in the CNS to usGO treatment. Taken together this evidence suggest that usGO is biocompatible with the CNS, supporting the further development of usGO-based novel nanoscale therapeutics targeted at diseases of the brain.

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*Centre of Energy and Environmental Technologies, VSB-Technical University of Ostrava, Czech Republic

17:45-18:00

Nanodiamonds are non-cytotoxic yet evoke vigorous interferon responses in primary human dendritic cells

Nanodiamonds (NDs) exhibit excellent biocompatibility and a tunable surface that can be readily functionalized, rendering them useful for biomedical purposes such as drug delivery. However, the interactions between NDs and human immune cells remain poorly understood. In this study, we explored the effects of amino-, carboxyl-, and poly(ethylene glycol) (PEG)-terminated NDs on primary human immune-competent cells. First, utilizing single-cell mass cytometry (CyToF), we assessed their impact on peripheral blood mononuclear cells. We could show an increase in the number of plasmacytoid dendritic cells (pDCs), a cell type that is critically involved in antiviral responses. Subsequent experiments provided evidence that NDs are "sensed" by pDCs, triggering a vigorous type I interferon response, and we uncovered a role for the endosomal Toll-like receptors, TLR7 and TLR9. On the other hand, titanium dioxide nanoparticles displaying similar surface functionalization did not elicit TLR7 or TLR9 activation. No cytotoxicity was observed in any of the cell types studied despite the fact that the NDs were readily internalized, as evidenced by TEM and flow cytometric analyses. These findings enhance our understanding of ND interactions with the immune system, contributing novel insights for their biomedical applications.

Acknowledgements: NDs and TiO2 NPs were provided by PlasmaChem GmbH (Berlin, Germany). The work was supported by grants awarded by the Swedish Research Council, Swedish Cancer Foundation, and the European Commission through the FP7-NANOSOLUTIONS project (grant agreement no. 309329) (for nanomaterial synthesis).

Session 2B

16:30–18:00

GROUPING AND READ-ACROSS, HYPOTHESES AND IATA EDITING/DEVELOPMENT

Similarity assessment & Hypothesis-based grouping

Chairs: Vicki Stone & Danail Hristozov

Georgia Tsiliki*, George Drakakis, Alex Zabeo, Danail Hristozov, Vicki Stone

*Purposeful IKE, Greece

16:30-16:55

Grouping of advanced multi-component nanomaterials: can machine learning help?

Multi-component nanomaterials (MCNMs) can be manufactured featuring many different physico-chemical properties. Unlike mono-component nanomaterials (NM), MCNMs consist of multiple components that may interact with one another, and for that reason their safety profile should be clearly characterised. Traditionally evaluating MCNMs is expensive and time-consuming. Similarity assessment methodologies specifically designed for MCNMs could be valuable tools to justify that existing safety-related information can

be re-used between group members, thereby reducing the need to generate new hazard data and improve their sustainability.
We present a two-step computational approach for MCNMs similarity assessment, which allows researchers to form groups using similarity between MCNMs and then use this information to read-across safety information from well-studied materials to new ones. The two procedures can be used as standalone approaches for grouping and read-across. The method primarily calculates pairwise comparisons of interest to allow researchers to focus on specific parameters of interest and evaluate their biological relevance. The agility of the method is that it can adopt to one- and two-dimensional data, and identify consistent grouping patterns across data sets. Each of the identified common groups across data sets is accompanied with an uncertainty score for regulatory decision-making purposes. Although the suggested method is less computationally intensive compared to most Machine Learning (ML) algorithms (e.g. Support Vector Machine) and it is easy to implement, it is highly influenced by the size of the available data. When rich toxicity data or high-quality biological activity data are available, more consistent and accurate results will be produced.
The approach is applied to two use cases from industry to demonstrate its effectiveness with each of the properties data separately and in combination. A comparative analysis to other grouping approaches is presented discussing applicability domain, advantages and drawbacks.

Elisa Moschini*, Agnes Oomen, Andrew McCormack, Finlay Stenton, Vicki Stone

*Heriot-Watt University, EPS, United Kingdom

16:55-17:10
Streamlining the Safe by Design of multicomponent nanomaterials via grouping – case studies

Multicomponent nanomaterials (MCNMs) and more in general advanced materials (AdMa) represent one of the current challenges for human and ecotoxicological hazard assessment. Because of their extreme heterogeneity in composition and enhanced properties it is not reasonable to do this evaluation on a case-by-case basis. The application of recently developed New Approach Methodologies (NAMs) like “grouping” may help in reducing the need of additional testing. Within the SUNSHINE project, a framework to support the development of Safe by Design Strategies for MCNMs has been proposed, and its validation on specific case studies is ongoing. The framework is based on the generation of hypothesis linking one-by-one, relevant physicochemical properties, enhanced properties, or mixture effects, to the fate of the investigated materials (“where they go”, “what they do”). The verification of the hypothesis may justify the grouping of the MCNM with the source materials or with the arbitrary chosen data-reach benchmark materials.
Here we present the application of the framework to different industrial cases to show how it could be used to guide the prioritization of testing strategies at the initial stages of product development and to help in the choice of safer alternatives.
To do that, internally generated physicochemical and hazard data obtained on Tier1/Tier2 project MCNMs (SiO2@ZnO; GO-CS; SiC@TiO2, SiC@SiO2) as well as on their individual components, has been combined with existing data, whether available, and each tailored hypothesis has been evaluated based on similarity assessment to the extent possible within the project. This exercise allowed both to highlight some additional features of the framework and to identify some limitations, especially when comparing data generated by applying slightly different procedures. In conclusion, a stepwise approach to overcome those limitations has been proposed.

Alex Zabeo*, Matteo Carisi, Fabio Rosada, Lisa Pizzol, Danail Hristozov

*Greendecision, Italy

17:10-17:25
Read-Across with Inverse Distance Weighting, OWA Similarity, and Hierarchical Clustering

This presentation introduces a novel method for read-across, a technique used to predict properties of a target molecule based on similar known molecules. It combines three powerful tools: Inverse Distance Weighting (IDW), Ordered Weighted Averaging (OWA) based similarity matrix and Hierarchical clustering. IDW prioritizes closer neighbours in the data space, leading to more accurate property predictions, OWA captures complex relationships between molecules, creating a more nuanced similarity measure while Hierarchical clustering groups MCNMs based on their similarities, facilitating efficient read-across analysis. The presented approach was tested against a real industrial case study related to core-shell nanoparticles based on Silicon carbide core and Titanium dioxide or Silicium dioxide shell. The results of applying the methodology in the case study demonstrated derived Cytostasis (CBPI) values are aligned with measured ones. The presentation explores how this approach leverages the strengths of each technique for robust property prediction and efficient data exploration.

Matteo Carisi

Greendecision, Italy

17:25-17:40
Normalised similarity assessment to inform grouping of advanced multi-component nanomaterials by means of an Asymmetric Sigmoid function

A novel procedure for similarity assessment as a basis for grouping of multi component nanomaterials (MCNMs) is presented. The approach allows for grouping of nanomaterials that is not affected by the dataset, so that group membership will not change when new candidates are included in the set of assessed materials. It can be applied to assess groups of MCNMs as well as mixed groups of multi and single component nanomaterials as well as chemicals. To facilitate the application of the proposed methodology, a software script was developed by using the Python programming language, which is currently undergoing migration to a user-friendly web-based tool. The presented approach was tested against a real industrial case study related to SiO2-ZnO hybrid nanocomposite used in building coatings, which is designed to facilitate photocatalytic removal of NOx gases from the atmosphere. The results of applying the methodology in the case study demonstrated that ZnO is dissimilar from the other candidates mainly due to its different dissolution profiles.

Daniela Hahn

Biomedical Technology Center, University of Muenster, Muenster, Germany

17:40-17:55
Meta-analysis as a quantitative tool to identify nanomaterials with high DNA damaging potential in vivo

The testing of engineered nanomaterials (NMs) for potential genotoxicity is a key element in risk assessment procedures. To obtain generally valid results and to limit the use of animals, an animal testing strategy should be based on appropriate genotoxicity test systems and relevant exposure models. To gain quantitative overall information of NM responses in different in vivo studies, we systematically extracted, reviewed and evaluated in vivo genotoxicity data from 106 different animal studies using 26 different types of NMs. These recorded data comprise nine different exposure models and five different test systems assessing a variety of different genotoxicity endpoints including DNA strand breaks, gene mutations, micronuclei formations and chromosomal aberrations. We also extracted relevant information from the selected papers, such as the core material of the applied NMs, their size, surface coating, zeta potential, the type of administration, the

administered doses (single dose, total dose), the duration of administration and study course, the treated animal species, strain and gender. This comprehensive data set covering a total of 1832 test results was harmonized to obtain consistency within the measuring units. A random effects meta-analysis was applied to estimate a weighted average from the test results. DNA damage was found in cells and tissues of 11 different organs. Study results identified several types of NMs with high DNA-damaging potential associated with different modes of exposure. A comparison with previous meta-analysis across a large-scale in vitro genotoxicity data set revealed high similarities with the results obtained for the animal studies. This quantitative and systematic approach can also be used to compare the effects of NMs on different genotoxic endpoints to receive information on their mode of action.

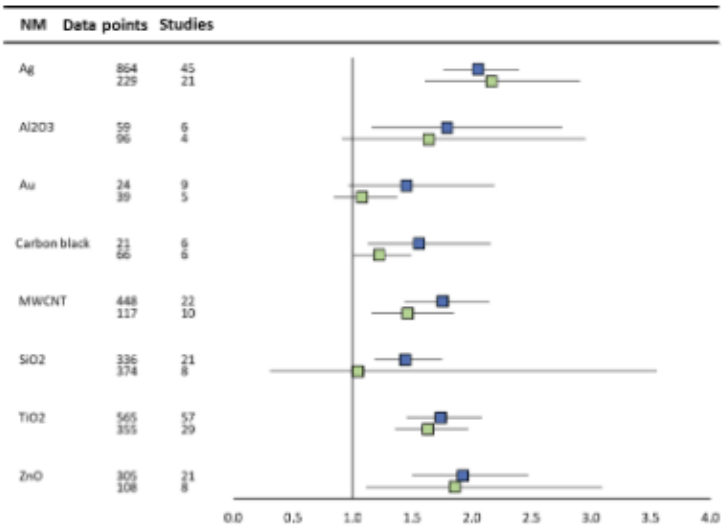


Figure: Comparison of the genotoxic effects of nanomaterials assessed with in vitro or in vivo studies. *The squares represent the mean estimate, each calculated from different in vitro (blue) or in vivo (green) test results as indicated on the left. The number of analysed studies and data points are listed for each case. The response ratio of the corresponding negative controls is normalized to 1 (vertical line indicating effect of controls). The horizontal lines represent the 95% confidence interval of each estimate.*

Session 2C

16:30-18:00

RISK ASSESSMENT AND MANAGEMENT

Exposure assessment

Chairs: Carla Martins & Wouter Fransman

Carlos Fito Lopez*, Jorge Salvador Hermosilla, Ernesto Gonzalez, Elena Barbero Colmenar, Andrea Brunelli, Manolo Lloris Cormano, Elvira Villaro, Julio Gómez, Shareen H. Doak, Magda Blosi, Ana SerranoLotina

*ITENE, Spain

16:30-16:50

New insights on the potential workplace exposure to multi-component nanomaterials

New data on the potential exposure to nanometric range materials when dealing with multi-component nanomaterials (MCNMs) is urgently needed to support risk assessment. To this end, a suite of industrial driven case studies was conducted under the H2020 SUNSHINE project (G.A. n°952924), covering exposure scenarios (ES): ES1 weighting, ES2 mixture, ES3 synthesis, ES4 integration into matrix for each of the selected MCNMs: nanoclays functionalized by essential oils, core-shell SiC-TiO2 coatings, nanostructured SiO2-ZnO photocatalytic building materials, and graphene oxide-based composites. The risk assessment of these MCNMs was conducted using a tiered approach based on two guidelines: "Harmonized tiered approach to measure and assess the potential exposure to airborne emissions of engineered nano-objects and their agglomerates and aggregates at workplaces" by the OCDE and the international standard EN 17058:2018.

Tier 1 consists on a qualitative assessment to understand the processes and identify hotspots and potential exposure scenarios, whereas Tier 2 and 3 comprises quantitative analysis for understanding the nature of the potential released particles. This includes the analysis of particle morphology as well as particle mass, concentration and chemical composition. ITENE conducted the Tier 1 assessment by analysing the processes information while Tier 2 and 3 assessments were approached by using real-time measuring instruments such as optical particle counters, condensation particle counters, DCA and/or scanning mobility particle sizers. In addition, samples of airborne-particles were taken on filters, and inspected by microscopic technics like SEM-EDX.

The results can be summarized as follow:
The real-time sensor monitoring during the synthesis of SiO2-ZnO nanoparticles on mortar matrix did not reveal any evidence of particles release. Nevertheless, SEM-EDX images taken close to fume hood displayed the presence of agglomerates consistent with process chemicals. The integration of functionalized nanoclays into polymer produced release of ultrafine particles and microparticles during the addition of micronized material (CES1.1) to the extruder.
During the SiC-TiO2 coating synthesis and formulation no release was observed while using the fume hood. The abrasion tests revealed that graphene oxide (GO) composites generated fewer particles than GO and chitosan composites. In addition, a potential reduction in particle generation was observed for each sample.

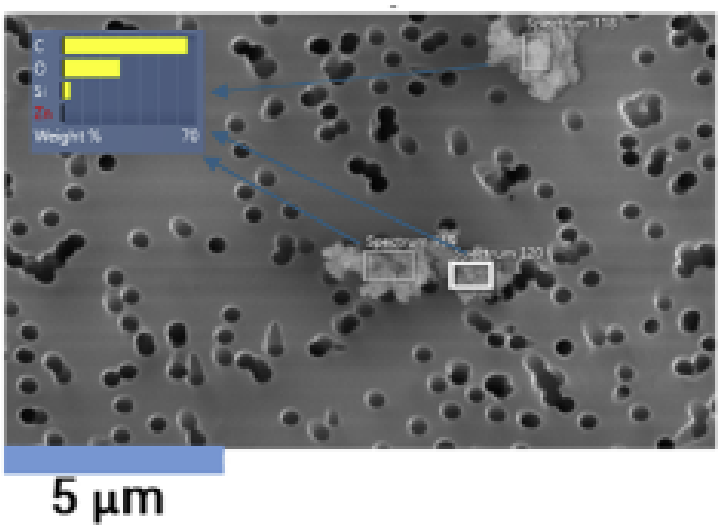


*Figure SEQ Figure * ARABIC 1: SEM-EDX of MNPs sampled on filter during SiO2-ZnOsynthesis.*

	Background	Extrusion process (ES1)	Material addition (CES 1.1)
Mean	13130	20199	31502
Max	15033	42957	42957
Min	10253	10235	10235
Std. Dev.	1027	9945	8376

The integration of functionalized nanoclays into polymer produced release of ultrafine particles and microparticles during the addition of micronized material (CES1.1) to the extruder (Table 1).

Ceyda Oksel-Karakus*, Aysel Tomak, David Winkler

*Izmir Institute of Technology, İzmir Institute of Technology / Bioengineering Department, Turkey

16:50-17:04
Protein corona formation on silver nanoparticles under different conditions

The surfaces of nanoparticles become covered by biomolecules in biological fluids. This protein 'corona' modifies materials' characteristics and biological activity. The composition of the protein corona is dynamic, abundant biomolecules that bind first are subsequently replaced by less abundant but more tightly bound ones. Here, we explore the formation of the silver nanoparticle protein corona on exposure to cell culture media containing 10 % fetal bovine serum supplemented Dulbecco's Modified Eagle's medium. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry analysis were used to monitor how different parameters such as incubation time, heating duration, cell culture medium, incubation temperature, and the number of washes affect the nanoparticle–protein corona complex. silver nanoparticles with and without bound proteins were characterized by electron microscopy, dynamic light scattering, and ultraviolet-visible-near-IR spectroscopy. The tetrazolium-based MTT assay was used to determine viability of A549 human lung adenocarcinoma cells treated with silver nanoparticles. Characterization of the nanoparticles before and after protein binding provided insights into their changing morphology on corona formation. Our results confirmed that the physiological environment directly affects protein corona formation on nanoparticle surfaces. In particular, incubation condition-dependent differences in the amount of bound proteins were observed. This work highlights the importance of environmental drivers of protein adsorption, which should be considered when predicting and/or controlling protein targets of silver nanoparticles (Tomak, Yilancioglu et al. 2022).

Alberto Martinez Serra*, Adriana S. Maddaleno, Angela Saccardo, Ana Serrano Lotina, Doak Shareen, Maria Pilar Vinardell, Montserrat Mitjans, Marco P. Monopoli

*ITENE, Spain

17:04-17:18
Exposure to multi-component nanomaterials: physicochemical characterization, biochemical profiling and cell viability

During the last years, the production of advanced and multi-component nanomaterials (MCNM) has dramatically increased, as are currently used in several industrial sectors where they enhance the materials' performances and properties. These advanced hybrid materials are formed by two or more functional components – such as NPs or organic molecules – conjugated by strong molecular bonds, or by a NM with a unique chemical origin modified by hard or soft coatings. (1, 2) However, with the increase in material production, there is also a potential increase in exposure to humans and to the environment with unknown outcomes, which requires detailed studies despite the analytical complexity of the system. After becoming in contact with complex media, nanomaterials and MCNMs are rapidly covered by biomolecules from the environment, forming the so known *corona*. (3) As these biomolecules are strongly associated with the surface, this new identity of the particles can influence the colloidal stability as well as their environmental and biological fate.(4)

We have investigated the exposure to different industrial MCNMs, targeting the effects that continuous exposure to these materials can have, characterising their intrinsic and extrinsic physicochemical properties as well as their cytotoxicity. Through a tiered innovation approach, integrating Safe and Sustainable by Design (SSbD) principles at the design phase, we evaluate the safety, efficiency, and environmental impact of MCNMs in selected industrial applications. We present case studies in construction, where photocatalytic mortars utilize safer SiO2 composites instead of TiO2, and in bakery equipment, where SiC composite coatings offer a biocompatible alternative to traditional anti-sticking surfaces. This research is fundamental to evaluate exposure scenarios, coupled with the development of materials that prioritize the enhancement of the

desired properties without compromising human health or environmental integrity.

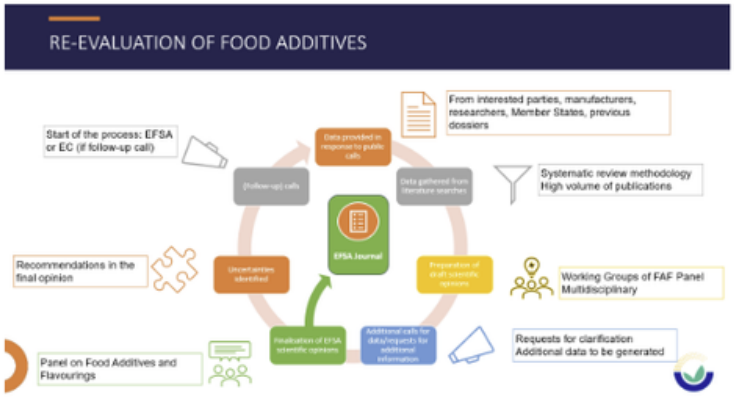
1. Banin, U., Y. Ben-Shahar, and K. Vinokurov, Hybrid semiconductor–metal nanoparticles: from architecture to function. Chemistry of Materials, 2014. 26(1): p. 97-110.
2. Saleh, N.B., et al., Research strategy to determine when novel nanohybrids pose unique environmental risks. Environmental Science: Nano, 2015. 2(1): p. 11-18.
3. Cedervall, T., et al., Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proceedings of the National Academy of Sciences, 2007. 104(7): p. 2050-2055.
4. Monopoli, M.P., et al., Biomolecular coronas provide the biological identity of nanosized materials. Nano-enabled medical applications, 2020: p. 205-229.

Ana Maria Rincon*, Camilla SMERALDI, Claudia RONCANCIO-PENA

*European Food Safety Authority (EFSA), Risk Assessment Production (ASSESS) Department, Italy

17:18-17:32
Regulatory safety assessment in the European Union of food additives containing nanoparticles

The European Union (EU) legislation contains provisions for nanotechnology and nanomaterials and a definition of nanomaterial is given in Commission Recommendation 2022/C 229/01. In the EU, the safety of substances proposed for use as new food additives as well as the re-evaluation of all food additives that were already permitted in the EU before 20 January 2009 is carried out by the European Food Safety Authority (EFSA). Since 2009, the re-evaluation of more than 230 food additives has been finalised and approximately 85 remain to be assessed in the coming years. When a food additive is already included in the Union list but there is a significant change for example in its production methods, in the starting materials used, or there is a change in the particle size of the material, the food additive must be subject to a new risk assessment. EFSA provides indications (Guidance on Particle-TR) on how to identify if nano specific aspects have to be considered when performing the risk assessment. Since the risk assessment of materials that retain properties at the nanoscale during the use of the product needs special consideration of their physicochemical properties, toxicokinetics, potential uptake into cells and tissues and physiological fate, EFSA has also issued specific Nano Guidance to perform the safety assessment of these materials. Within the programme of re-evaluation of permitted food additives, EFSA has identified some food additives for which a fraction of the material falls in the nano-range and their re-evaluations were finalised recommending further data on the physicochemical characterisation of the material used as a food additive. After a full characterisation of the material, the approach, i.e. only conventional or complemented by nano specific aspects, will be decided to complete the safety evaluation. The European Commission has an approach for the follow-up of the re-evaluation of permitted food additives for which some concerns or data gaps have been identified that implies the submission of the data needed for the completion of the risk assessment. The final decision on whether a food additive remains permitted for use in the EU will be taken by the EU risk managers based on the outcome of the EFSA's final scientific assessment.



Eun Gyung (Emily) Lee

National Institute for Occupational Safety and Health, CDC, United States

17:32-17:46

Evaluation of airborne particle emissions from a boron nitride nanotube composite lifecycle

Boron nitride nanotube (BNNT)-enabled composites show promise for applications in gas-turbine engine and aerospace industries requiring high thermal stability with electric insulation. Certain stages along the lifecycle of BNNT-enabled composites hold potential for release of respirable/ultrafine BNNT particles from BNNT composites. This study was conducted to achieve two aims, 1) characterize particles generated during sanding of BNNT-epoxy composites and 2) determine weathering effects on the airborne particle exposure. For Aim 1, three types of BNNT-epoxy composites (0% [control], 1%, and 4% BNNT loading by weight) were sanded in controlled environments using zirconium aluminum oxide sandpapers (P100 and P180). The released particles were characterized with direct-reading instruments to determine particle number concentrations and size distributions and filter-based samples to determine particle concentrations and protruded BNNTs. For Aim 2, the BNNT-epoxy composites were exposed to wet/dry condition with 18 min/102 min cycles for 2,016 hours, followed by sanding in the same manner as described in Aim 1.
Overall, 4% BNNT-epoxy released more particles than the others, while control and 1% BNNT-epoxy revealed similar concentrations regardless of sandpaper types (e.g., 41,000 particles/cm3 [4% BNNT] vs. about 30,000 particles/cm3 [control and 1% BNNT] when sanding with P100). A similar pattern was observed for the weathered blocks but the weathered 4% BNNT-epoxy generated 1.5 times higher number concentrations (62,000 particles/cm3) than the non-weathered 4% BNNT-epoxy when sanding with P100. All materials exhibited one main mode in number-based size distributions with a peak < 20 nm regardless of testing conditions. For the non-weathered composites, P100 generated higher or similar inhalable dust concentrations compared to P180. Conversely, P180 generated higher respirable dust concentrations than P100 except for the 4% BNNT-epoxy. Overall, weathered blocks generated higher or similar respirable and lower inhalable dust concentrations than non-weathered blocks for all testing materials. During the weathering test, the composites changed color from white to yellow and all composites experienced a steady reduction in mass (about 2% weekly). Electron microscopy analyses demonstrated that both control and 4% BNNT-epoxy showed irregular shapes with protrusions, but the protruding features from the control were not consistent with the reference BNNT material. The findings indicate that the addition of BNNT in epoxy impacted the emission rates of the material during sanding depending on the weight percent of BNNT embedded in epoxy. In addition, weathering of the composite may degrade BNNT surface bonding with the epoxy matrix resulting in greater number of released particles.

Sara Marchetti

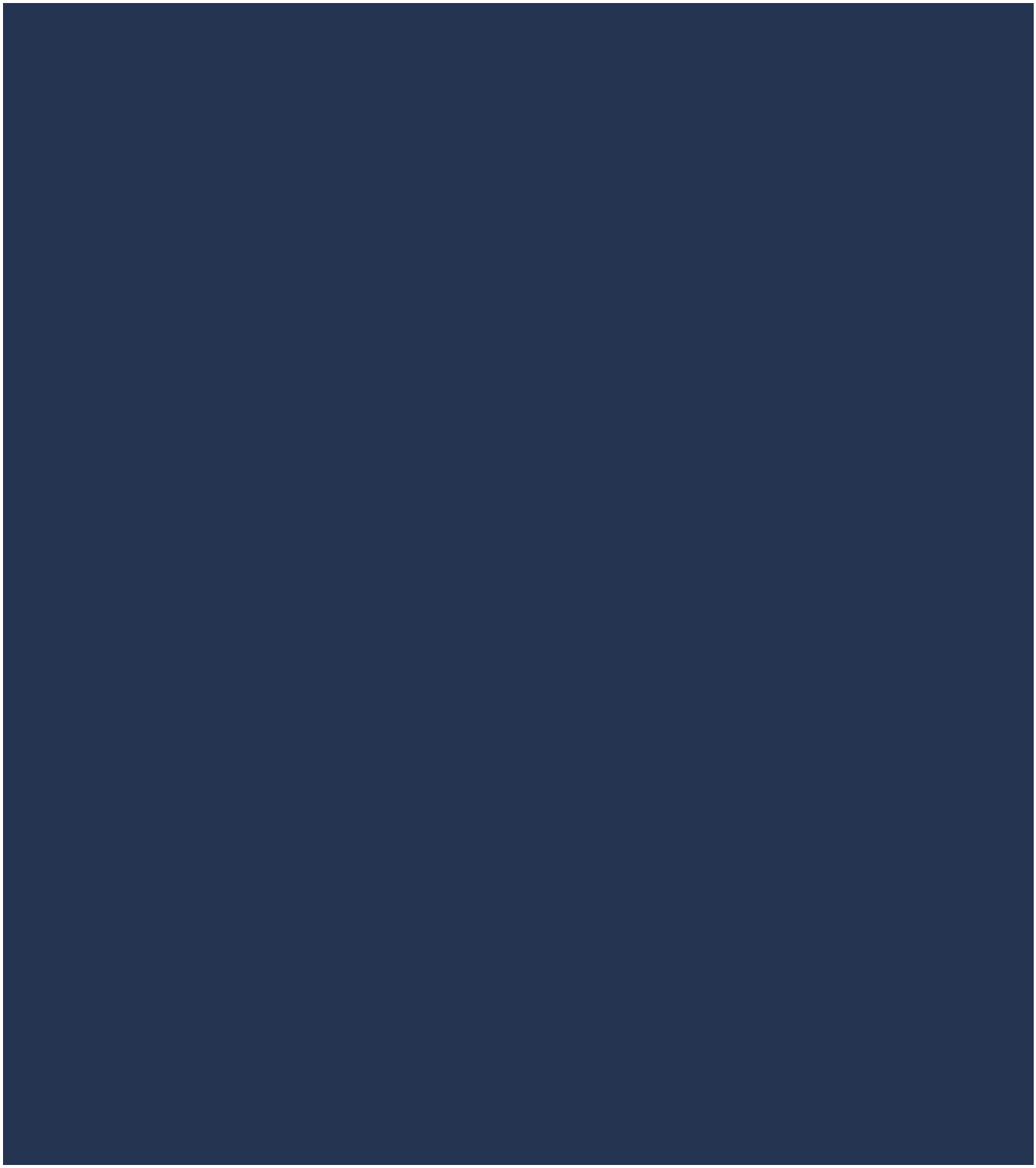
POLARIS Research Centre, Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy

17:46-18:00

How might exposure to PM2.5 increase the severity of SARS-CoV-2 viral infections?

Literature data suggest that environmental exposure to fine airborne particles (PM2.5) increases the occurrence and severity of respiratory viral infections in humans. In presence of PM2.5, viruses could potentially determine more severe infections due to altered immune homeostasis. Although it is known that physicochemical properties, such as size, determine the site of particle deposition and accumulation in the airways, recent suggestions show the possibility of interaction between PM and viruses. However, data on whether these interactions influence virus-mediated inflammatory effects are missing.
Regarding SARS-CoV-2 infection, exposure to PM2.5 has been associated with a higher incidence of the COVID-19 disease. In a previous work, we report that exposure to PM2.5 facilitates virus internalization through the angiotensin 2 converting enzyme (ACE2) - dependent pathway. In fact, pulmonary alveolar epithelial cells exposed to PM2.5 in submerged conditions over-expressed ACE2, which is exploited by SARS-CoV-2 viral particles to enter the cells and increase their inflammatory state.

Studying biological responses in more complex models, including co-cultures systems of lung cells with other cell types, could be useful to further
explore the inflammatory and immunological effects of combined exposure to air pollution and SARS-CoV-2. For this purpose, co-cultures of differentiated macrophage-like THP-1 cells and epithelial cells (1:10 ratio) exposed to PM2.5 in combination or not with viral particles (inactivated SARS-CoV-2) could be useful to mimic a more realistic in vivo situation for the evaluation of possible synergistic effects. The role of THP-1-like macrophages will be therefore useful to evaluate their influence in the response of epithelial cells to PM2.5 and SARS-CoV-2. Consequently, the inflammatory potential triggered by winter PM2.5 and the SARS-CoV-2 virus will be explored.
Our results indicate that the interaction between PM2.5 and SARS-CoV-2 influences the severity of the inflammatory responses in lung epithelial cells, exacerbating the effects of the viral infection. These findings deepen the understanding of the additional risk posed by airborne PM in facilitating and worsening virus-mediated respiratory disease in exposed populations.



Session 1

16:00–16:30

HAZARD ASSESSMENT

Novel mechanistic insights in NMs and advanced materials' toxicity

Kazuma Higashisaka

Osaka University, Institute for Advanced Co-Creation Studies, Japan

The effect of silver nanoparticles on placental syncytialization of BeWo cell

Nanoparticles have useful functions due to their large specific surface area compared to conventional sized materials and have already been put into practical use in products in various industrial fields, including food, cosmetics, and pharmaceuticals. Despite our increasing exposure to nanoparticles, however, information regarding their adverse effects on reproductive development from the implantation stage to the placentation stage remains limited. Here, we focused on the placenta, an organ that plays a central role in maintaining pregnancy, and evaluated the effect of nanoparticles on the syncytialization of placental cells, which is an essential differentiation process in the formation and maturation process of the placenta. As model nanoparticles, we selected silver nanoparticles, which are often used in various products because of their antibacterial activity. We assessed the effect of silver nanoparticles with a diameter of 10 nm (nAg10), which were treated at non-cytotoxic concentrations, on placental formation by using forskolin-treated BeWo cells, a typical in vitro model of trophoblast syncytialization. Immunofluorescence staining revealed that although the syncytialization rate was increased in forskolin-treated BeWo cells, it was significantly suppressed in co-treated with nAg10 and forskolin group. Moreover, forskolin-induced elevation of the CGB mRNA expression and concentration in the culture supernatant of human chorionic gonadotropin β, a hormone whose production increases with syncytialization, was significantly decreased in the group treated with nAg10. Besides, regarding changes in mRNA expression of ERVW-1 and ERVFRD-1, which are molecules involved in promoting syncytialization, forskolin-induced increase in ERVFRD-1 expression was suppressed in the forskolin plus nAg10 co-treated group. Furthermore, nAg10 tended to suppress the expression of sFlt-1 e15a, a placental angiogenesis marker. These results suggest that nAg10 suppressed the syncytialization process of BeWo cells. Moreover, to clarify the mechanism of toxicity on placentation induced by nAg10, we performed comprehensive genetic expression changes analysis by RNA-sequencing. Using the obtained data set, we attempted to extract a set of variable genes between the two groups and identified a mitochondria-related gene set as a gene group in which variable genes were more enriched in the forskolin plus nAg10 co-treated group than in the forskolin alone group. Mitochondria plays an important role in the progress of syncytialization, including involvement in cell survival through energy production during the syncytialization process. Thus, we now try to elucidate the mechanism by which nAg10 induce its toxicity by assessing the effects of nAg10 on mitochondrial function during the placental syncytialization.

Eva Guenther

Institute of Lung Health and Immunity, Helmholtz, Germany

Impact of repetitive particle exposure and latent viral infection on lung immunity and diseases

Our lungs are constantly challenged by several ubiquitous factors, among them latent herpesvirus infection and exposure to environmental particles. Herpesviruses persist in the host in a lifelong latent state, which can be disturbed by stress-induced reactivation, resulting in the production of lytic virus. Our previous investigations have shown that pulmonary exposure to carbon nanoparticles (CNP) can reactivate latent murine gammaherpesvirus 68 (MHV-68) in mouse lungs. This reactivation mainly localizes to CD11b+ infiltrating macrophages and is dependent on p38 MAPK signaling. Additionally, a repeated CNP administration to latently infected mice resulted in alveolar damage, leading to progressive emphysema-like changes [Han & Haefner, ACS Nano, 2023]. However, the mechanisms causing alveolar damage and the cellular communication upon exposure to CNP in latently infected lungs remain unclear and are the subject of my current project.
To enhance the clinical relevance of this study, latently infected mice were exposed to CNP up to five times, and virus reactivation and further epithelial damage are analyzed.

The repetitive triggers of viral reactivation resulted in significant changes in the composition of cells in the alveolar airspace, particularly in a gradual increase in the number of lymphocytes with each instillation. Ongoing investigations are focusing on the epithelial cell compartment and morphological changes in mouse lungs.
In order to have an in vitro model at our disposal, we additionally developed a 3D lung organoid culture using primary mouse epithelial cells in combination with persistently MHV-68 infected macrophages. This model allows for the detection of reactivation and cytokine release following CNP exposure in a controlled environment, providing insights into the communication between epithelial cells and macrophages. First results have already demonstrated inducible viral reactivation and transcription of several cytokines, including Cxcl1 and Csf2. This model will help to understand the mechanisms behind reactivation-induced epithelial responses.
By combining the in vitro and in vivo studies, we are building a solid base to uncover the molecular mechanisms underlying adverse lung health outcomes and to elucidate the cellular communication. Our goal is to identify potential targets to prevent exacerbation of lung diseases caused by two omnipresent factors: herpesvirus infection and ambient particle exposure.

Iza Rozman

National Institute of Biology (NIB), Department of Genetic Toxicology and Cancer Biology, Slovenia

(Geno)toxicity Evaluation of Vanadium Spinel Ferrite (VxFe3-xO4) Magnetic Nanoparticles in vitro

The interest in applications of metal-based nanoparticles as biocatalysts has grown tremendously, especially because bio-catalysis has outpaced conventional catalysts regarding efficiency, reusability, sustainability, and cost-benefit. Among researchers, much attention has been drawn to spinel ferrites due to their magnetic, electric, and optical properties. Thus, they are widely used in several technological applications including spintronics devices, permanent magnets, data storage, magnetic catalysts, and drug delivery. Among spinel ferrites vanadium ferrite (VFe2O4) is a very interesting compound in terms of magnetic and electrical properties, with potential applications in various fields such as catalysis and clinical uses. Furthermore, the possibility of having active V5+ centers, which contribute to its magnetic properties, makes VxFe3-xO4 spinel ferrite attractive for advanced oxidation of organic pollutants in wastewater. However, there is still a lack of data on their safety, regarding the protection of human health. In this study, we aimed to assess the potential (geno)toxic activity of three types of vanadium-containing iron oxide magnetic nanoparticles (MNPs). The VxFe3-xO4 spinel ferrite MNPs ($0.1 \leq x \leq 0.3$) were synthesized through mild oxidative hydrolysis, resulting in average diameter sizes between 38 nm and 80 nm, with octahedral, faceted morphology. The in vitro cyto- and genotoxicity were assessed in the human hepatocellular carcinoma cell line (HepG2), using the MTT assay, and the comet and micronucleus assays, respectively. Cells were exposed to graded concentrations of the tested MNPs for 4 and 24 hours. The results showed that the tested VxFe3-xO4 MNPs were not cytotoxic for HepG2 cells in the tested concentration range, up to 100 µg/cm2, except for V0.3Fe2.7O4, which decreased cell viability by about 30% at all tested concentrations. MNPs (25 - 100 µg/cm2) significantly increased DNA damage in a dose-dependent manner, observed with the comet assay, at both exposure times. On the contrary, no increase in micronucleus formation was determined. Furthermore, the induction of oxidative stress as a potential mechanism of action was examined by measuring the generation of reactive oxygen species (ROS) with ROS-GloTM assay. Results showed that the tested VxFe3-xO4 MNPs induced ROS formation after 4 hours of exposure, which may be the cause of DNA damage detected with the comet assay. The results of our study are significant since the accurate assessment of potential health risks to humans is a crucial step toward using these types of nanomaterials in biomedical or clinical applications.

Wendel Wohlleben

BASF SE, Dept. Analytical and Materials Science, Germany

ROS Activity in Carbonaceous Nanoparticles: The Influence of Surface Area and Chemistry

Carbon Black (CB), graphene, and nanodiamond are distinct carbonaceous insoluble nanoparticles. Reactive Oxygen Species (ROS) associated with these particles play a significant role in both in vivo and in vitro toxicity evaluations. Accurate assessment of ROS activity is relevant for toxicity screening and in ensuring safe working environments as well as guiding the innovation by safe-and-sustainable-by-design principles. Our study investigates the ROS activity of 13 CB, 6 graphene, and 3 nanodiamond samples, employing various techniques and assays, alongside the exploration of surface-specific physicochemical properties to understand observed differences in ROS activity among nanomaterials (figure 1). We utilized Electron Spin Resonance spectroscopy (EPR) at temperatures of -196 °C and 20 °C to evaluate bulk total spin-count. Nanoparticle-induced ROS activity at 20 °C was measured using acellular and cellular DCFH2-DA assays, as well as EPR with the CPH spin-probe (EPR-CPH). Additionally, we measured the hemolytic activity of selected test compounds, as ROS generation has also been associated with increased hemolytic potential, the capacity to disrupt red blood cells membrane. Hemolytic activity depends on a variety of phys-chem factors and may be useful as a preliminary cytotoxicity indicator. Specific surface area (SSA) determined by BET analysis and surface composition studied via x-ray photoelectron spectroscopy (XPS) were investigated to find correlations with the observed ROS activity. The elemental composition analysis focused on carbon, oxygen, sulfur, and nitrogen, and carbon sp2/sp3 hybridization. We found a high correlation in ROS activity per unit mass across different assessment methods, notably with SSA emerging as the primary predictor [r=0.86-0.92]. When normalized by SSA, the ROS surface activity covered a 4-fold range only (CB and graphene). This should be compared to a 100-fold range for the ROS activity assessed per unit mass. Interestingly, the bulk EPR measurements showed no clear trends or correlations with ROS activity or SSA, indicating a minimal contribution of bulk free electrons to particle ROS activity. The XPS analysis confirmed predominantly carbonaceous surfaces with minor traces of oxygen, sulfur, and nitrogen. Multiple linear regression analysis incorporating the surface composition suggested that ROS surface activity increased with carbon sp2-hybridization, while oxygen and sulfur surface functional groups pacified the ROS surface activity. This understanding can contribute to explaining nanomaterial toxicity mechanisms and have broader implications for carbonaceous air pollutants. However, the interpretation of our model may need refinement considering potential contributions from surface functional groups with additional, specific reaction pathways.

Vamsi Kodali

National Institute for Occupational Safety and Health, Health Effects Laboratory Division, United States

Impact of adding nanocellulose to concrete for particle aerosol characteristics and in vitro toxicity following cutting

Nanocellulose, a cellulose in the form of nanostructures, is an abundant renewable compound formed from plants, algae, tunicates, or bacteria. Nanocellulose can be split into two classes: cellulose nanocrystals or nanofibers. The morphology and physicochemical characteristics are dependent on the cellulose origin, isolation, and processing conditions. Nanocellulose can change cement properties by improving mixture stability and reducing shrinkage of cement materials. The goals of this project were to assess the impact of nanocellulose addition into concrete and the potential impact of occupational exposures following manipulation (e.g., cutting). An automated concrete-cutting chamber was utilized to simulate the concrete cutting process and collect particle characteristics. Number-based particle size distributions and particle number concentrations were measured using real-time instruments. Particles were then utilized to assess human bronchial epithelial cell toxicity and cell membrane damage. The average particle number concentration (± standard error of the mean) between control concrete and nanocellulose concrete (182,678 ± 21,642 particles/cm3 vs 146,739 ± 14,578 particles/cm3, respectively) was not different. Concrete or nanocellulose concrete particles generated during cutting did not differ in mass median aerodynamic diameter (5.24 µm vs 5.28 µm) or count median diameter (0.719 µm vs 0.719 µm), respectively. In human bronchial epithelial cells, lactate dehydrogenase (LDH) activity, a marker of plasma membrane damage, and cell proliferation via WST-1 assay were dose-dependently assessed following treatment with concrete and

nanocellulose concrete collected particulates. With increased dose, there was increased LDH activity, but there was no difference between particles at a given dose. Also, with increased dose, there was decreased cell proliferation, but this was not different between concrete and nanocellulose concrete groups. There were dose-dependent increases in toxicity with both concrete particulates, but the dose response curves showed no significant difference, IC50 concrete = 28.6 (19.0 – 38.3) and IC50 nanocellulose concrete = 30.2 (23.1 – 37.3). These results indicate that the addition of nanocellulose did not modify the aerosolized particle size distribution by number or mass following controlled cutting and did not alter in vitro toxicity compared to control concrete samples.

Francesca De Battistis

Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health, Italy

Assessing nanocellulose uptake and crossing of the human intestinal epithelium using the Caco-2/HT-29 MTX/Raji b cells triculture model

intestinal epithelium by nanocellulose (NC) was one of the main pillars of the considered IATA. Such studies are complicated by the challenge of detecting carbonaceous material as NC crystals or fibres and demonstrating their uptake and fate. In addition to the analytical limitations, studies on intestinal uptake and crossing are complicated by the lack of an entirely appropriate cell model. Incorporation of mucus secreting cells and microfold (M) cells into Caco-2 cell cultures can enhance the physiological relevance of intestinal in vitro models. A triculture model composed of three human-derived cell lines (Caco-2 cells, HT29-MTX cells, and Raji B lymphocytes) is being investigated in an OECD project focusing on internalization/translocation of inorganic nanoparticles. The analytical challenge of identifying NC crystals and fibres was addressed by fluorescence detection using two alternative staining methods. Uptake and crossing were assessed by using Confocal Laser Scanning Microscopy (CLSM). A quantitative approach was developed for screening internalisation whereby uptake of NC crystals (CNC) or fibres (NFC, BNC) was measured as percentage of NC-containing cells on the total number of counted cells at a given magnification. This methodology allowed a quantitative estimation of the proportion of cells involved in the uptake process. After a first screening of several test materials, three NCs were selected for the assessment of intestinal uptake and crossing: (i) one material composed of cellulose nanocrystals (CNC), (ii) one nanofibrillated cellulose (NFC), and one bacterial NC (BNC). Cell uptake was demonstrated for the three materials, and such uptake was greater in the triculture model as compared to Caco-2 monolayers. Evidence of greater uptake (as proportion of cells involved in the internalisation process) was obtained for CNC. Uptake was found to increase after 72 h exposure as compared to 24 exposure. CNC was further investigated under repeated exposure conditions, which led to an increase in CNC uptake as compared to single exposure; accumulation in lysosomes was also shown. CNC intestinal barrier crossing was demonstrated. A potential NC biopersistence and bioaccumulation cannot be excluded upon chronic exposure. In addition, CNC translocation across the intestinal barrier is expected to occur and other NCs are likely to translocate as well.

Amandine Le

Solvay - INSERM UMR996 Inflammation, Microbiome and Immunosurveillance, SILICA - Université Paris-Saclay, France

Role of surface chemistry surface in amorphous silica nanomaterials effects on human dendritic cells maturation

The World Health Organization estimates that by 2050, half the Western population will be affected by allergies. Nanomaterials could have an adjuvant effect on allergic response development. Synthetic amorphous silica nanomaterials (SAS-NMs) are widely used in pharmaceuticals, cosmetics, and food industries, justifying a better evaluation of their immunotoxic potential. As part of the immunization process, dendritic cells (DCs) capture and present antigens in an immunogenic or tolerogenic manner. In the presence of 'danger signals', they undergo maturation, resulting in the expression of co-stimulation and activation molecules, and migrate to draining lymph nodes where they activate naïve T lymphocytes. We have shown in an in vitro co-culture model, that SAS-NMs behave as an immunological danger signal by increasing DC maturation and T-cell response, the first steps of the adaptative immune response.

The surface of SAS-NMs contains silanol groups whose reactivity depends on their propensity to establish hydrogen bonds with the polar head of zwitterionic phospholipids in the cell membrane. Surface silanol groups, identified as critical for cytotoxicity, could also play a determining role in the activation of DCs. To explore this hypothesis, we compared the effects of two fumed and two precipitated SAS-NMs, with different physicochemical properties such as specific surface area (200 vs 36 m2/g), on the expression of co-stimulatory molecules using a surrogate model of human DCs, the monocytic THP-1 cell line. THP-1 cells were exposed for 16 hours to the SAS-NMs, and the expression of CD54 and CD86 were measured for each condition. Cytotoxicity, measured by propidium iodide labelling, was in the same range for all the nanomaterials tested, although slightly lower for the precipitated NMs with the lowest specific surface area. Our results showed that both SAS-NMs increased CD54 surface marker expression with a greater extent for pyrogenic SAS-NMs compared to precipitated SAS-NMs. A post-treatment reducing silanol density, led to a downregulation of CD54 expression, significatively for only one type of SAS-NMs. These results suggested that specific surface area or silanol density could play a role but are insufficient to explain the observed pattern of cell activation.
We hypothesize that surface chemistry is at the core of the interactions between SAS-NMs and DCs membranes. To better understand this mechanism, we are currently developing synthetic biomimetic membrane models. A more detailed understanding of these interactions could lead to a safer production of SAS-NMs through a safer-by-design approach and help to predict the immunotoxic effects of nanomaterials.

Haiyun Zhang

Helmholtz Zentrum Munich / Member of the German Center of Lung Research (DZL), Institute of Lung Health and Immunity, Comprehensive Pneumology Center, Germany

Role of extracellular vesicles in nanoparticle-induced lung inflammation

Inhalation of nanoparticles (NPs) frequently elicits a pro-inflammatory response of the lung, characterized by the influx of neutrophilic granulocytes into the airspace. Recently extracellular vesicles (EVs) released under steady state as well as under pathophysiologic conditions by different cell types, have been shown to contribute to the pulmonary inflammatory response. In this context we found that application of a membrane stabilizer (cromolyn) into mouse lungs, significantly and persistently attenuated neutrophil influx from pulmonary microvessels after Quantum dot NP (cQD-NPs) as well as carbon black NP (CNP) inhalation.
Characterizing EVs in bronchio-alveolar lavage fluid (BALF) obtained from mice after cQD-NP and CNP inhalation, showed an increase of mean EV vesicle size from 140nm to 180nm, as well as an increase in EV concentrations (1x10^9 to 1.8x10^9 /ml) as compared to EVs detected in BALF of control mice, indicating an alteration in EV release and composition.
To clarify the contribution the role of EVs in NP induced sterile lung inflammation, we currently apply nanoscale flow cytometry as well as Exoview-technology, to elucidate their cellular origin. First results indicate an increase in macrophage derived EVs. In vitro assays are applied to characterize the proinflammatory potential of the EV populations.
Taken together we have strong indications, that EVs in the alveolar region are involved in the initiation of neutrophilic immune response after NP inhalation, thus warranting further research.

Masakazu Umezawa

Tokyo University of Science, Department of Medical and Robotic Engineering Design, Japan

Functional analysis of dysregulated long non-coding RNAs (lncRNAs) related to developmental neurotoxicity of carbon black nanoparticles

Maternal exposure to low doses of carbon black nanoparticles (CB-NPs) induces persistent astrogliosis in perivascular regions in the brain (Onoda et al., P&FT 14: 4, 2017; Umezawa et al., P&FT 15: 36, 2018). This model, representing the ultrasmall fraction of atmospheric fine particles (PM2.5), is likely mimicking perinatal exposure scenarios associated with an increased risk of developmental brain disorders such as autistic spectrum disorders in children. While key genes and proteins in the perivascular pathogenesis have previously reported (Onoda et al., Sci. Total Environ. 634: 1126-1135, 2018), potential contributions of long noncoding RNAs (lncRNAs)–potent regulators of gene and protein functions–have not yet been investigated.

In this study, we analyzed the expression profiles of lncRNAs in the model exhibiting the brain perivascular lesions following maternal exposure to CB-NPs in mice. RNA expression data (GSE250286 in Gene Expression Omnibus) derived from the cerebral cortex of 12-weeks-old mice, encompassing both those with and without the perivascular lesions were subjected to the analysis. Principle component analysis and hierarchical clustering were employed to extract candidate lincRNAs whose expression pattern was similar to that of the lesion-related mRNAs (GFAP and dopamine receptors). Subsequently, sequences in common within these lncRNAs and their potentially functional targets were identified by using Clustal Omega, BLAST, and DAVID. "Cytoskeleton" and "cytocortex" were extracted as functional targets of the lncRNAs whose transcription levels were positively correlated to the level of brain perivascular lesions by CB-NP. Of particular significance is the role of the cytoskeleton in regulating the elongation of astrocyte endfeet, which is a major observation in the lesion. The lncRNAs may have a potential as novel regulators of cytoskeletal functions in these perivascular lesions. This finding will contribute to the comprehension of noncoding RNA-mediated molecular mechanisms of perivascular pathophysiology related to the developmental neurotoxicity of NPs.

Michalina Miszczak

University of Gdansk, Faculty of Chemistry, Poland

Dynamic QSAR modeling – time-dependent models of genotoxicity for advanced materials

ntroduction: New, advanced materials (AdMs) are attracting significant interest due to their wide-ranging potential applications across various scientific and engineering disciplines. Their nanoscale size and unique properties lead to different biological activity compared to bulk materials, necessitating ongoing research into their toxicity [1]. The long-term effects of AdMs on the environment and living organisms, including aggregation and aging processes, may impact particle toxicity [2]. This underscores the importance of monitoring time-dependent changes. Considering the need for integrating new approach methodologies (NAMs) in toxicity assessment, the presented study utilizes quantitative structure-activity relationship (QSAR) methods to predict AdMs toxicity, considering changes associated with different exposure times.
Results: Four predictive models based on a heterogeneous dataset of AdMs have been developed based on in vivo data for 39 materials following pulmonary exposure. To account for time-dependent toxicity changes, independent variables such as intrinsic and extrinsic properties, along with applied dose, were supplemented with exposure time. Utilizing the locally weighted least squares kernel regression method (KwLPR), dynamic models predicting in vivo genotoxicity for bronchoalveolar lavage fluid cells, lungs, liver, and neutrophil influx into bronchoalveolar lavage fluid in Mus musculus were obtained. These models exhibit good fitting quality and predictive capabilities, rigorously validated.
Conclusion: The results of the developed models indicate the significance of exposure time to AdMs for predicting their toxicity. Incorporating this parameter into the set of independent variables allows for monitoring dynamic changes in toxicity over time. An additional advantage is the ability to predict toxicity at later time points, where experimental data are often limited. The good parameters of the developed models suggest that this approach represents a significant step in assessing the toxicity risk of AdMs.

Laura Gomez Cuadrado

ICCRAM Universidad de Burgos, ICCRAM Universidad de Burgos, Spain

Biological impact evaluation of titanium carbide nanoparticles applying in silico and in vitro models

Additive manufacturing of Ti-6Al-4V titanium alloy parts with addition of varying amounts of TiC nanoparticles has raised the interest in several fields such as the aeronautic or the automotive sectors due to the special features that this mixture presents. Thus, the addition of TiC NPs provides this alloy with increased stiffness, enhanced elevated temperature strength, good creep performance, fatigue resistance, and wear resistance (Drissb, 2021) (Tao, 2011). However, the knowledge about the potential health issues related to these NPs is relatively scarce. In the present work, the biological impact of these nanoparticles was evaluated using in silico models of plasma membranes with different lipidic composition to mimic nanoparticle – cell wall interactions,

via molecular dynamics simulations and COSMOperm thermodynamic modelling, as well as docking with a database of human target proteins.
Moreover, experimental toxicological studies were carried out using different in vitro models simulating two main exposure routes: skin contact and gastrointestinal pathway. Therefore, the irritation potential of TiC NPs provided by IRIS srl was studied in skin tissues applying the OECD TG439, while their effect on the viability of a gastrointestinal model constituted by Caco-2/HT-29 cells was evaluated by the MTT assay. Finally, its effect on cells of the immune system (macrophages) was analyzed applying the MTT assay as well. In silico results probed minor biological effects of the TiC nanoparticles, which would lead to low toxicological effects, while in vitro assays showed that the viability of macrophages significantly decreased at TiC concentrations ≥ 50 µg/mL. Altogether, the reported results provide a general overview of the potential toxicity of Titanium Carbide nanoparticles.

Sabina Halappanavar

Environmental Health Science and Research Bureau, Health Canada, Canada

Does toxicity induced by micro and nanoplastics resemble the toxicity of nanomaterials?

Microplastics and nanoplastics (MP/NP) are potentially harmful to human health. However, how MP/NP impact human health, is not clear due to an insufficient number of systematic studies. Reference MP/NP reflective of real-world exposures are not available for toxicological testing, and standardized protocols to isolate a sufficient quantity of MP/NP from the complex environmental samples are yet to be developed. Thus, in this study, Standard Operating Protocols (SOPs) for laboratory-scale generation of sufficient quantity of well characterized MP/NP reflective of environmental exposure were developed and their potential to induce lung injury or toxicity was assessed using lung epithelial cells.
Commercially available polystyrene (PS) beads of various sizes, nylon powder, poly-methyl methacrylate (PMMA), polyethylene MP/NP were purchased. In-house, MP/NP were generated from plastic water bottles, storage containers and nylon tea bags (abundant source of MP/NP pollution in air). Both purchased and in-house generated MP/NPs were characterized for dry size, shape, size distribution in exposure medium and, for chemical composition. Toxicity was assessed in lung epithelial cells. The endpoints of cell viability, immune and inflammatory response, gene expression changes and genotoxicity were assessed. The gene expression profile of the most toxic MP/NP was compared to the previously generated gene expression response induced by nanomaterials in these cells.
The study successfully established laboratory-scale SOPs for isolating and characterizing MP/NP from plastics used and abandoned every day. Exposure of cells to the collection of individual MP/NP resulted in dose, time and MP/NP type-dependent reductions in cell viability. Commercially available 100 nm PS, PMMA and in-house generated polyethylene terephthalate MP from plastic water bottles induced secretions of inflammation associated proteins and the formation of micronuclei, a marker of genetic damage. The smaller size fractions of MP/NP types were more genotoxic compared to their larger counterparts. A clear difference in gene expression response was observed between cells exposed to MP/NP or nanomaterials.
In the absence of well characterized reference MP/NP reflective of real world-exposures, the methods established in this study to isolate and characterize MP/NP from everyday plastics will be useful. The preliminary results of toxicity assessment shows that
exposure to MP/NP can be toxic to cells in culture. While some apical responses are similar to that observed in cells exposed to nanomaterials, the molecular level response is different. Significant research is warranted to reveal the true human health effects of exposure to MP/NP in the environment.

Rubiyat E Islam

The National Institute of Occupational Health (STAMI), Norway

Levels and characterization of particles and bioaerosols in dental clinics and their toxicological effects on cells of the lower airways

Dental health professionals are exposed to complex aerosols in their occupational settings. The impact of such exposure on the respiratory system is not fully elucidated. It is recognized that exposure to airborne particles in a working environment can lead to asthma, allergy, COPD, fibrosis, and pneumoconiosis

Modern dental composite materials have incorporated various nano-sized filler particles to increase physio-mechanical properties and have been shown to generate <1µm-sized airborne pieces of composite particles during abrasive dental procedures. These particles might carry tooth fragments and bioaerosols namely viruses, bacteria, and fungi. The present study aims to evaluate dental clinic aerosol and bioaerosol exposure levels and comprehensively characterize the detected aerosol particles in size and composition. Furthermore, the project includes in vitro assessment of the toxicological effects of composite particles in a 3D lung cell culture including alveolar type II-like cells, macrophages, and endothelial cells exposed at the air-liquid interface (ALI). Field studies were conducted in general and specialized dental clinics to compare particle and bioaerosols' concentration levels and size during different treatment procedures in clinics with varying levels of ventilation systems. The measurement employed both stationary (scanning mobility particle sizer and aerodynamic particle sizer) and personal air sampling (respirable cyclone and conical inhalable sampler) equipment. Controlled laboratory conditions were utilized to generate composite particles by mechanical abrasion technique and respirable particle fractions were collected using respirable GK 4.162 Rascal cyclone samplers connected to sampling pumps similar to the fraction collected in the clinic. Preliminary findings indicate that particle exposure in state-of-the-art dental clinics with modern ventilation systems is generally very low. However, certain procedures such as sandblasting and air scaling may lead to elevated particle number concentrations. The mean bacterial exposure level was higher than the fungal exposure level in this dental clinic. Moreover, samples taken during surgical, and restorative procedures had higher bacterial and fungal DNA concentrations than samples taken during other procedures, suggesting that certain treatment procedures produce more bioaerosols. Future experiments involve ALI exposure of the in vitro 3D lung cell culture to in vitro-generated respiratory fraction composite particles and toxicological effects are addressed by analysis of cytotoxicity as well as biomarkers associated with inflammation and fibrosis.

Tilen Koklič

Laboratory of Biophysics, Condensed Matter Physics department, Jožef Stefan Institute, Slovenia

High throughput screening of nanoparticle-induced microtubule disruption and binucleation in cellular in vitro assay

Whole genome duplications (WGD) events - doublings of the entire complement of chromosomes are characteristic of metastatic lesions, which are a major cause of death and are associated with poor outcomes. Recently Brown et al. suggested that these events may be influenced by epigenetic or environmental factors. We have shown that binucleated cell formation can be triggered by some engineered nanoparticles, as has been shown for asbestos. To test whether WGD can be triggered by particulates found in polluted air we exposed lung epithelial cells in monoculture or coculture with murine lung macrophages to different groups of nanomaterials. From the time-lapse fluorescence and scattering microscopy images, we quantified the following responses: rate of binucleate cell formation, cell proliferation rate, microtubule, perinuclear damage, formation of extracellular tubulin-containing debris, and division cycle impediment of epithelial cells. Different types of diesel exhaust, carbon black, and one type of nano clay significantly increased WGD/binucleate cell formation rates in cocultures. We didn't see this effect in epithelial cell monocultures. Our results suggest that WGD is caused by the disruption of microtubule organization and mitosis impediment.

Vasyl Riabovol

Bogomolets National Medical University, Department of Hygiene, Occupational Safety and Health, Ukraine

Modern TiO2 and TiO2@Ag nanopowders: toxicology, physiochemistry, and biocidity. Potential Health Risks

A new TiO2 and TiO2@Ag (4wt.%) nanopowders were synthesized by the method of thermal decomposition of metatitanic acid at Frantsevich Institute for Problems of Materials Science of NASU. Experiments conducted on laboratory animals and in vitro revealed the structural-morphological, morphometric, toxicological, cytotoxic, and virucidal properties of TiO2 and TiO2@Ag nanopowders. Nano-TiO2 has dimensions of 21-28 nm.

The TiO2@Ag nanocomposite mainly consists of TiO2 nanoparticles ranging from 13 nm to 20 nm and Ag nanoparticles from 35 nm to 40 nm, with silver localized on the surface of titanium dioxide. The anatase form was confirmed for both. Acute intraperitoneal administration of nano-TiO2 to female BALB/c mice was LD50 of 4783.30 mg/kg, while it is 724.44 mg/kg otherwise. Mild accumulation is noted in rats after intragastric administration, without fatalities but with delayed body weight gain. Although nanopowders cause no skin irritation, mild conjunctival irritation and sensitization may occur. With internal organ damage observed, particularly in the liver, kidneys, and lungs. The liver tissue was most affected by nano-TiO2's toxic effect, displaying dystrophic changes at 67.7%, while nano-TiO2@Ag caused initial necrotic changes at 70%. Immunoassay analysis indicates enhanced peripheral blood mononuclear cells activity and cytokines IL-1, IL-6, TNF-α and IL-4 production induced by TiO2@Ag and TiO2, potentially leading to chronic inflammatory and allergic effects. Nanopowders also affect mitochondrial enzyme activity and damage mitochondrial membranes and boar germ cells, with TiO2@Ag showing lower toxicity to BHK-21, Hep-2, and MDCK cells compared to nano-TiO2. Significant virucidal effects were observed against human adenovirus serotype 2 and influenza A virus, with both TiO2@Ag and TiO2 nanoparticles exhibiting pronounced extracellular virucidal activity at a concentration of 100 μg/ml. Reductions in virus titers were achieved after exposure to nano-TiO2 and nano-TiO2@Ag, with reductions of 7.5 and 7.9 log10 TCID50/ml for influenza A virus, respectively. Tentatively safe exposure levels (TSEL) in the working zone air are estimated at 0.3 mg/m3 for nano-TiO2 and 0.2 mg/m3 for nano-TiO2@Ag. Experimental toxicological, virucidal, and industrial-hygienic studies facilitate the justification and implementation of various preventive measures. Parameters such as TSEL, CC50, LD50, and hazard class can potentially certify synthesized nanomaterials for biosafety, indicating promising applications of nano-TiO2 and nano-TiO2@Ag in biology and medicine.

Seokjoo Yoon

Department of Predictive Toxicology, Korea Institute of Toxicology, Republic of Korea

Evaluation of pulmonary toxicity of nano polyethylene following intratracheal instillation in rats

The objective of this study was to characterize the toxicity of nano polyethylene (Nano PE) after 13 weeks of intratracheal instillation (ITI) in Sprague-Dawley rats and to assess the reversibility of any effects during a 4-week recovery period. Plastic products like food packaging and drinking water bottles are widely used in daily life, but their limited degradability creates a huge environmental burden. Environmental factors such as mechanical wear, UV radiation, and microbial degradation can break down plastics into smaller particles including macroplastics, microplastics, and nanoplastics. Nano polystyrene has been extensively studied and found to cause toxicity in different animals. However, the health effects of more commonly used plastics like polyethylene in daily life have been much less studied.Therefore, we conducted a toxicological evaluation of Nano PE in accordance with OECD guidelines. Nano PE was administered by intratracheal instillation to Sprague-Dawley rats at dose levels of 0, 40, 80, and 120 μg/head once a week. Mortality rate, body weight, food consumption, and organ weights were measured and assessed. Additionally, ophthalmological, hematological, and histopathological parameters were evaluated. The results of our 13-week repeated-dose toxicity study revealed no treatment-related changes in clinical signs, body weight, food consumption, ophthalmologic examinations, urinalysis, hematology or clinical chemistry findings. In microscopic findings, treatment-related changes were observed in the lungs. Inflammatory cell infiltration was noted in both sexes at ≥40 μg/head Nano PE-treated groups, which was considered a toxicological change.
Additionally, thickened alveolar ducts/alveolar epithelial hyperplasia was observed in males at ≥80 μg/head and females at ≥40 μg/head Nano PE-treated groups.Therefore, under the present experimental conditions with intratracheal instillation in Sprague-Dawley rats, the no-observed-adverse-effect-level (NOAEL) of Nano PE may be considered to be under 40 μg/head in both males and females.

Session 1

16:00–16:30

RISK ASSESSMENT AND MANAGEMENT

Exposure asessment

Marco Rizzo

UNIVERSITY OF TRIESTE, Degree Course in Prevention Techniques in the Environment and Workplaces, Italy

Characterization of nano gallium arsenide exposure in a laboratory employing molecular beam epitaxy technology

Background: Gallium arsenide (GaAs) is an intermetallic crystalline solid composed of arsenic (As) and gallium (Ga), used in the electronic and optoelectronic industries. GaAs is highly toxic to the organism and is carcinogenic. The purpose of this study is to evaluate the concentration of GaAs nanoparticles in the air, on skin and on surfaces in a research facility that produces thin films of this material and to monitor As in the urine of exposed workers.
Methods: The survey was carried out utilizing a multilevel method over the course of a working week. The IOM sampler and miniature diffusion size classifier (DiSCMini) were used to implement personal monitoring. SKC Sioutas Cascade Impactor was used for environmental monitoring. The ghost wipe method was used to evaluate the surface contamination in the surrounding environment. Tape strips were used for the monitoring of skin contamination. At the start and finish of each work shift, urine was collected.
Results: Median number of nanoparticles detected during laboratory activities was low (median=913 np/cm3; 25° percentile=653 np/cm3; 75° percentile=1310653 np/cm3), however detecting the presence of exposure peaks during cutting operations of GaAs samples under a fume hood (88883 np/cm3). The analysis of the filters relating to the IOM personal sampler highlighted a median weekly concentration of 80.5 ng/m3 of gallium and 37.4 ng/m3 of arsenic. The result of the surface samples showed that laboratory floor (As max = 251 ng/cm2) and surfaces inside the hood (As max = 1418 ng/cm2) had the greatest levels of contamination. The worker's skin had a median concentration of arsenic at the end of the work shift (2.25 ng/cm2), which was more than thirteen times greater than it was at the beginning of the shift (0.17 ng/cm2). The results of weekly urine biomonitoring revealed a median arsenic content of 17.6 µg/L, which was below ACGIH guidelines (30 µg/L) but over the reference level set by SIVR (Società Italiana Valori di Riferimento) for the non-occupational population (2.0 – 15 µg/L). Conclusions: This study shows that surface contamination in the environment might be a possible source of exposure even while air exposure levels are extremely low. The findings highlight the necessity of combining aerial surveillance with skin and surface sampling as well as biomonitoring to accurately assess the degree of exposure to GaAs nanoparticles and to pinpoint potential preventive actions, like enhancing cleaning practices, utilizing PPE, and optimizing containment measures.

Swapnanil Saha

INDIAN INSTITUTE OF TECHNOLOGY DELHI, CIVIL ENGINEERING, INDIA

Risk assessment of nanoparticles in the presence of micro-plastics in aquatic system

The recent discovery of micro-plastics in our water bodies makes it imperative for us to understand their interaction with the other known components in the system like engineered nanoparticles. In an aquatic system, nanoparticles in the presence of micro-plastics and dissolved organic matter gets transformed in ways that affects it's transport in such a system. The risk assessment due to nanoparticles in such scenario thus becomes important. Nanoparticles in the presence of micro-plastics and dissolved organic matter may get adsorbed and their fate in the human digestive system will be affected as a result of these interactions. Here the wastewater contains 2 kinds of pollutants, i.e., nanoparticles and micro-plastics. The dosage of both the pollutants have to be calculated through the 2 routes of direct ingestion and indirectly through consumption of fish. So here while estimation the nanoparticle intake through consumption of fish and ingestion of water containing nanoparticles we have to take in to consideration the nanoparticle present in suspension and the nanoparticles adsorbed onto the micro-plastics as well.

We do that by taking the concentration of micro-plastics consumed (in both fish and water) and multiplying it with the mass of nanoparticles per unit mass of micro-plastics. The concentration of nanoparticles in fish tissue is the sum of the concentration due to bioaccumulation from the water and that adsorbed onto the micro-plastics from which we calculate the average daily dose of nanoparticles and in turn is used to find hazard quotient and hazard index. Experiments are performed by preparing mixture of nanoparticle and micro-plastics suspended in water. The aggregation and settlement studies of the particles are done for a 6 hour period and then filtered through filter paper of pore size 0.45 micron. The filtrate and wash water of the filter paper are analysed for metals and micro-plastics through ICP-OES and TOC respectively. Thus an understanding of the amount of nanoparticles adsorbed, settled and in suspension in the system is gained from such an experiment.

Anne Burtey

INRAE, UMR 1313 Génétique Animale et Biologie Intégrative, France

Detection of titanium nanoparticles in human, animal and infant formula milk

The sustainability of mammals on Earth depends on milk. The development and survival of offspring can be altered by maternal exposure to pollutants such as metallic nanoparticles (NPs) during lactation. Titanium dioxide (TiO2) nanoparticles have been banned from food applications in Europe due to their suspected toxicity but are still widely used in all the other industrial sectors. Despite their growing release into ecosystems, there is a lack of monitoring of this nanomaterial in milk. Here, we investigated the presence of Ti in human, animal and infant formula milk. Synchrotron X-ray fluorescence imaging and Single Particle Inductively Coupled Plasma Mass Spectrometry revealed that all analyzed milk samples, regardless of the species, location, and processing, contained micro- and nano-particles of Ti present in different minerals, phases and concentration.

Paraskevi Zagana

Metabolic Engineering and Systems Biology Laboratory, ICEHT/FORTH, Institute of Chemical Engineering Sciences/Foundation for Research and Technology Hellas (ICEHT/FORTH), Greece

Metabolomics of MCNM and HARN toxicity using earthworm as model system

Metabolomics stands out as a powerful method to assess the metabolic signatures of biological systems across different conditions, aiding in understanding metabolic changes and responses to stimuli. Metabolomics emerges as a potent tool, providing a high-throughput method to quantify the complex metabolic fingerprint of biological systems. Through this approach, researchers can thoroughly explore metabolic changes across various physiological conditions, gaining insight into how organisms respond to various stimuli and factors, shedding light on the dynamic nature of metabolic networks. In the realm of nanotoxicity and exposome studies, metabolomics has gained momentum in enhancing our comprehension of the impact of various nanomaterials on both biological systems and the broader environment. Metabolomics aids in the identification of key biomarkers crucial for the early detection of any adverse effects due to nanomaterial exposure. In the context of the H2020 project DIAGONAL #953152 ("Development and scaled Implementation of sAfe by design tools and Guidelines for multicOmponent aNd hArn nanomateriaLs"), in collaboration with the Sub-Department of Toxicology and the Laboratory of Systems and Synthetic Biology of Wageningen University & Research (WUR), we investigate the effect of MultiComponent NanoMaterials (MCNMs) and High Aspect Ratio Nanoparticles (HARNs) on human physiology and the environment, by transcriptional and metabolic profiling of particular model systems. In this study, in DIAGONAL we designed a nanotoxicity experiment in the earthworm (Eisenia fetida) model carried out at WUR. Specifically, the earthworms were exposed to soil contaminated with ZnO and Mn (in the form of MnCl2), for fourteen days (long-term exposure), when they were collected and directly frozen in liquid nitrogen. The frozen samples were lyophilized and the biological material was divided between WUR (transcriptomic analysis) and FORTH (metabolomic analysis), to where it was transferred in dry ice. We will present the metabolomic analysis results after multivariate statistical analysis and pathway enrichment interpretation of the metabolites, the relative abundance of which indicates significant changes in the samples exposed to the nanomaterials. Elucidating metabolic consequences of this model exposure to this type of nanomaterials will contribute to better understanding their environmental effect towards the development of sustainable practices for nanomaterial utilization.

16:00-16:30 Session 1
OTHER

Jenny R. Roberts

CDC/NIOSH, Health Effects Laboratory Division, Morgantown, United States

Pulmonary toxicity of reduced graphene oxide following inhalation at occupationally relevant doses

The family of graphene nanomaterials, including pristine, multiple-layer nanoplates, and oxidized forms of graphenes, are the most widely produced two-dimensional nanomaterials. This is due to a combination of unique material properties including strength, transparency, high electron mobility, and high thermal conductivity. Inhalation exposure to graphene and the potential related health effects are a concern in the industry. A previous in vivo study using a single dose aspiration in mice showed that reduced graphene oxide (rGO) caused greater pulmonary toxicity on a mass basis when compared to graphene oxide and graphene nanoplates of similar lateral dimensions. Based on these findings, rGO was selected for a subacute dose-response time course inhalation study in mice. C57BL/6J mice were exposed to rGO (2.41 um MMAD, 2.69 GSD) by whole body inhalation at 3 different doses: 5.0 or 0.5 mg/m3 for 5h/d for 19d, or 0.5 mg/m3 for 0.5 h/d for 19d to achieve depositions with a two order of magnitude range, or filtered air (control). The doses used in this study fell within the range of measurements taken in the personal breathing zone of workers in a recent exposure assessment study of several US facilities conducted by NIOSH. The majority of measures at these facilities fell in the low dose range of this study; however, several measures fell between the middle and high dose range depending on the tasks performed. Particle deposition in the lungs of mice was measured at 0d following the 19d exposure, and parameters of pulmonary toxicity were evaluated in lung tissue and lung lavage samples at 3d, 1m, and 3m post-exposure. Exposure for 19d resulted in deposition of 0.78, 6.47, and 34.4 ug of rGO for the low, middle, and high dose, respectively. Inflammation and lung injury were dose-dependent at 3d following the 19d exposure, with little to no effects at the low dose and significant increases in lung inflammatory cell influx, cytotoxicity, and lung protein levels of inflammatory cytokines and tissue remodeling proteins at the higher doses. No significant increases in indices of toxicity were observed at the low or middle dose at 1 or 3m post-exposure. Following the high dose exposure, parameters of toxicity began to resolve over time and no pathological evidence of disease was found at 3m. The findings of this sub-acute inhalation study showed that 0.5 mg/m3 of rGO caused a minimal degree of inflammation representing the low observable effect level.

Ryan Bartone

North Carolina State University, USA

House Dust Mite Proteins Adsorb on Multi-Walled Carbon Nanotubes - Forming an Allergen Corona that Intensifies Allergic Lung Disease in Mice

Background: Advancements in nanotechnology world-wide have increasingly raised concerns over the toxicity of inhaled engineered nanomaterials (ENMs) and their potential to exacerbate the pathogenesis of allergic lung disease after occupational or environmental exposure. Nanoparticles in air pollution are a major factor that exacerbate allergic asthma in humans and increasing experimental evidence shows that ENMs exacerbate allergen-induced lung disease in rodents. For example, we previously reported that co-exposures of mice to house dust mite (HDM) allergens and multi-walled carbon nanotubes (MWCNTs) intensified lung inflammation in mice in vivo. ENMs, including MWCNTs, avidly bind biomolecules to form a protein corona that can modify nanoparticle immunotoxicity. Therefore, we hypothesized that exacerbation of HDM-induced allergic lung disease in mice by MWCNTs is due to the formation of an allergen corona.
Methods: Allergen coronas were prepared in a cell-free system by co-incubating MWCNTs (NC7000, Nanocyl Inc.) with HDM extract (Greer Laboratories, Inc.), followed by sequential rinsing and centrifugation of the MWCNTs to remove free HDM proteins.

The resulting allergen corona was characterized by gel electrophoresis, western blotting and proteomics. Male and female C57BL6 mice were exposed to the following treatments (vehicle control, HDM extract, MWCNTs, mixtures of MWCNTs and HDM extract, or MWCNTs with HDM corona) delivered to the lungs via oropharyngeal aspiration six times over 3 weeks. Following the 3 weeks, necropsy and collection of bronchoalveolar lavage fluid (BALF) and tissue samples for protein and mRNA extraction, and histopathology were completed.
Results: Approximately 7% of proteins in the HDM extract adsorbed to MWCNTs, including der p 2 and der p 1, which are associated with asthma in humans. Analysis of bronchoalveolar lavage fluid (BALF) from mice showed that co-exposure to HDM extract and MWCNTs or exposure to MWCNTs with HDM allergen corona had significantly increased inflammatory cell counts, total protein, lactate dehydrogenase (LDH) and eosinophil populations compared to HDM extract or MWCNTs alone. Further experiments using qPCR of whole lung lysate showed significant increases in mRNA expression from pro-fibrotic genes (Col1A1, Arg1) and pro-inflammatory mediator genes (IL6, CCL11) when mice were exposed to the mixture of HDM extract and MWCNTs or the HDM allergen corona. Histopathological analysis of lung tissue showed exacerbation of lung inflammation, airway fibrosis, and mucous cell metaplasia following exposure to either the mixture of HDM extract and MWCNTs or MWCNTs with HDM allergen corona compared to HDM extract or MWCNTs alone.
Conclusion: Our findings indicate that exacerbation of allergic lung disease in mice induced by co-exposure to HDM extract and MWCNTs is elicited by the formation of a HDM allergen corona. This research provides new insight into the mechanisms through which inhaled nanoparticles exacerbate allergic asthma in humans.

Day 2
24 September 2024

Session 3A

9:30-10:30

HAZARD ASSESSMENT

Novel approaches and models for advanced in vitro testing

Chairs: Tobias Stöger & Shareen Doak

Carola Voss*, Lianyong Han, Eva Guenther, Hongyu Ren, Meshal Ansari, Maximilian Strunz, Verena Haefner, Carolina Ballester-Lopez, Ilias Angelidis, Christoph H. Mayr, Trine Berthing, Thomas Conlon, Qiaoxia Zhou, Qiongliang Liu, Otmar Schmid, Ali Oender Yildirim, Markus Rehberg, Ulla Vogel, Janine Gote-Schniering, Fabian J. Theis, Herbert B. Schiller, Tobias Stoeger

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09:30-09:48

Lung organoids reflect epithelial perturbations in carbon nanomaterial-induced inflammation

The development of in vitro safety tests for inhaled nanomaterials (NMs) faces challenges due to a limited understanding of cell-specific responses. Particularly inflammatory responses of the respiratory epithelium are often overlooked in vivo, usually focused on neutrophil influx, and lacks in vitro validation. To address this, we conducted a longitudinal analysis of cellular perturbations in intratracheally exposed mice to carbon black (CNP), double-walled carbon nanotubes (DWCNT), and multi-walled carbon nanotubes (MWCNT) using single-cell RNA sequencing (scRNAseq). Subsequently, murine alveolar epithelial lung organoids were investigated towards their potential to reflect signatures of epithelial injury and inflammatory activation in vitro.

In vivo, all NMs induced comparable levels of neutrophilia in the airspace at 12h, sustained until d6 for DWCNT and until d28 for MWCNT. Both CNTs caused injury of alveolar epithelial cells and alveolar macrophages (AM), evident by alveolar barrier disruption and alarmin release (Il33, Il1α) assessed in lavage fluid. The epithelial niche showed a transient accumulation of inflammatory AT2 cells marked by increased *Lcn2, Il33* and *CXCL5* for all NM exposures. Our scRNAseq data showed an inflammatory epithelial cell circuit resulting in either an acute response and resolution (CNP) versus a persistent response and chronic damage (CNT). This coincided with CNT-induced epithelial cell death peaking at d6 (TUNEL+/proSPC+ or Aqp5+). For MWCNT we identified this as ferroptosis, an iron-regulated necrotic cell death pathway, validated by immunohistochemistry. Epithelial damage induced the emergence of Krt8+ alveolar differentiation intermediates (ADI), important in balancing regeneration or fibrotic development. On d6, inflammatory AM released Spp1, Il1β and fibronectin, which triggered inflammatory and profibrotic fibroblast activation (Eln, Timp1, Mmp2, IL6). These results substantiated the detrimental Th2 cytokine driven, pro-fibrotic environment for MWCNTs.

We demonstrated the induction of a Krt8+ ADI signature (Hopx, Krt8) and inflammatory activation (Lcn2, GM-Csf; Lamp3 protein levels) in mouse lung organoids treated with IL1β as released from AM. Furthermore, increased expression of IL6 and Cxcl5 was detected after 24h of CNP exposure in vitro. The release of alarmins (Il33, Hmgb1, Tslp) fom lung organoids after carbon NM exposure in vitro shows promising preliminary results in contrast to epithelial cell lines.

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09:48-10:02

Challenges and insights in evaluating nanofiber toxicity using differentiated THP-1 cells: The importance of methodological standardization and material characterization

The Fiber Pathogenicity Paradigm (FPP) framework correlates fiber structure and durability with toxicity, as extensively documented for asbestos. Nanofibers pose a challenge due to their nanoscale diameter, which leads to reduced rigidity, increased propensity for tangling, and changes in conventional fiber characteristics. Considering the lack of rigidity assessment methods, this work evaluates the adequacy of in vitro models to determine nanofiber toxicity. Focusing on the suitability of cell models under standard submerged culture conditions, we utilized differentiated THP-1 (dTHP-1) cells to examine their capability in mimicking the toxic responses to nanofibers. The study found that changes in experimental conditions have a significant impact on the proteomic profiles of dTHP-1 cells, affecting cellular responses and emphasizing the importance of methodological standardization. Building upon these findings, further experiments were conducted to evaluate the inflammatory response, oxidative stress levels, lysosomal integrity, and overall proteomic alterations of dTHP-1 cells when exposed to selected carbon-based nanomaterials. The nanomaterial tested included two variants of multi-walled carbon nanotubes (MWCNTs) — NM-400 and Mitsui-7 — along with Printex-90, which was used a particulate benchmark, to elucidate their distinct toxicological profiles. While results showed a degree of fiber toxicity, impairment of cell viability and lysosomal disruption at high exposure concentrations, they also uncovered inherent limitations within THP-1 cells, particularly related to the material dispersion in cell culture media, often leading to significant material aggregation. This observation underlines the importance of the material selection and characterization when developing a cell model to test fiber toxicity. In conclusion, this work contributes to the understanding the impact of cell model selection, experimental methodologies, and material properties in nanofiber toxicological assessments. Addressing these challenges is key for advancing our comprehension of nanofiber-induced toxicity mechanisms and for developing more accurate and predictive in vitro models.
Acknowledgement: This work was supported by the EU H2020 project HARMLESS (grant agreement No 95318

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10:02-10:16

Development of an in vitro co-culture model system of lung cells and cardiomyocytes for Air-Liquid Interface exposures

Air pollution is a global concern impacting human health with detrimental effects not restricted only to the pulmonary system but extending to other secondary tissues. In particular, the effects on the cardiovascular system contribute to a large proportion of premature deaths linked to air pollution. However, there is a lack of cellular models allowing the study of secondary interactions between lung cells and cardiac muscle cells upon exposure to air pollutants. We utilized transwell inserts to create separate chambers for co-culturing lung epithelial A549 cells and cardiomyocytes derived from human-induced pluripotent stem cells in a 3D orientation. A549 cells were seeded on the apical side of the culture inserts while the cardiomyocytes were seeded either in a well plate or on top of a microelectrode array (see Figure) to specifically investigate electrophysiological changes during exposures. This setup allows cell-to-cell signaling mimicking the crosstalk between lung and cardiac cells. The shared culture medium was selected to have insignificant cytotoxic effects on both cell types. By implementing this approach, many biological assays were used to assess the direct effect of pollutants on lung cells as well as secondary effects on cardiomyocytes. In this pivotal study, A549 cells were exposed at the air-liquid interface to the fine fraction of Arizona Sand Dust as reference material for crystalline silica particles. The dose- and time-dependent effects on cytotoxicity and metabolic activity of both cell types were evaluated as well as DNA damage and epigenetic modifications in terms of global DNA methylation (5-mC) and hydroxymethylation (5-hmC).

Moreover, the electrophysiological parameters of cardiomyocytes were investigated up to 48 hours indirect exposure, such as the frequency of beating and the field potential duration, which reflects the action potential duration, a key parameter of the cardiac cycle. The secretion of the pro-inflammatory cytokine IL-8 was evaluated and other biological mediators are being assessed. This in vitro model was successfully used to assess both primary and secondary effects of particulate matter exposure and will also be applied by using nanomaterials with different chemical identities to depict the role of chemical identity over the size in driving secondary tissue effects.

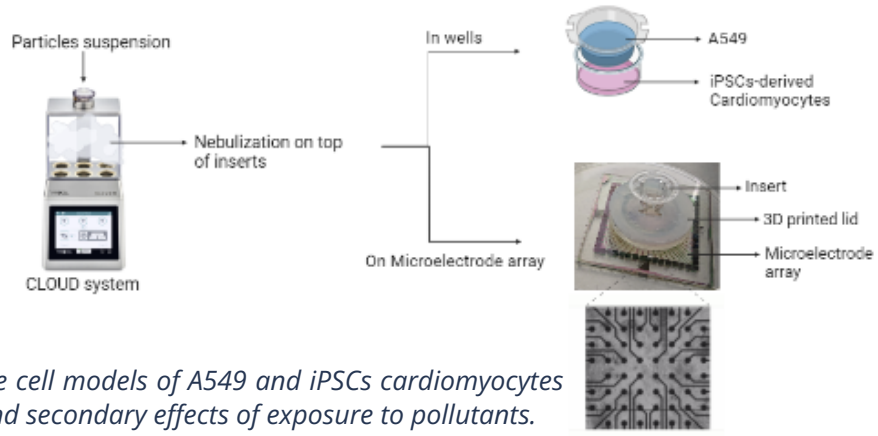

Figure: Co-culture cell models of A549 and iPSCs cardiomyocytes to study direct and secondary effects of exposure to pollutants.

Andrea Armirotti*, Valentina Casagnola, Valeria Tomati, Luca Boselli, Clarissa Braccia, Sergio Decherchi, Pier Paolo Pompa, Nicoletta Pedemonte, Fabio Benfenati

*Istituto Italiano di Tecnologia, Italy

10:16-10:30

Sources of biases in the in vitro testing of graphene oxide and gold nanoparticles: The role of biomolecular corona

The biological fate of nanomaterials (NMs) is driven by the specific interactions that biomolecules, naturally adhering onto their surface, engage with cell membrane receptors and intracellular organelles. The molecular composition of this layer, called biomolecular corona (BMC), depends on both the physical-chemical features of the NM and the biological media in which the NM is dispersed, and cells grow. In this work, we demonstrate that the widespread use of 10% fetal bovine serum (FBS) for in vitro assay is unable to recapitulate the complexity of an in vivo systemic administration, with NMs being transported by the blood. To this purpose, by using gold nanoparticles (GNP) and graphene oxide (GO) as test NM, we undertook a comparative journey involving proteomics, lipidomics, high throughput multiparametric in vitro screening, as well as single molecular feature analysis to investigate the molecular details behind this in vivo/in vitro bias. Our work indirectly highlights the need to introduce novel, more physiological-like media closer in composition to human plasma to produce realistic in vitro screening data for NMs. We also aim to set the basis to reduce this in vitro-in vivo mismatch, which currently limits the formulation of NMs for clinical settings.

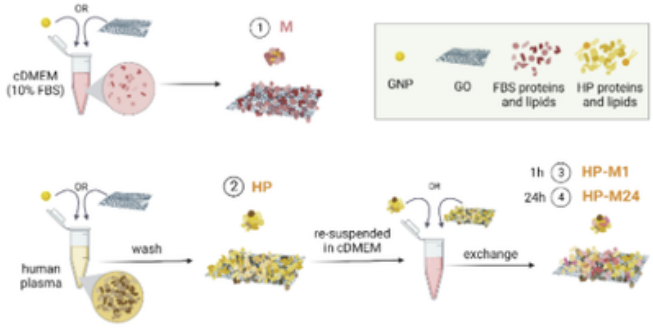

Figure: Schematics of the experimental conditions. (1) BMC formed in cDMEM (FBS proteins and lipids): M. (2) BMC formed in human plasma (proteins and lipids): HP. (3) BMC formed in HP, washed by centrifugation, then transferred to cDMEM for either 1 h: HP-M1 or (4) 24 h: HP-M24.

9:30-10:30 Session 3B

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Multicomponent nanomaterials and mixture effects

Chairs: Carlos Rumbo & Otmar Schmid

Peter Wick*, Woranan Netkuealkul, Daina Romeo, Savvina Chortarea, Bengt Fadeel, Marco Pelin, Cyrill Bussy, Kostas Kostarelos, Maurizio Prato, Alberto Bianco, Jing Wang, Tina Buerki-Thurnherr

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09:50-10:05

Release and toxicity assessment of carbon nanomaterial reinforced polymers during the production, use and end-of-life phases

Carbon-based nanomaterial (C-NM) composites represent a burgeoning class of materials, distinguished by their exceptional mechanical, thermal, and electrical properties, rendering them indispensable across various applications. Despite their promising potential, a significant challenge persists: evaluating the release of debris during manufacturing, usage, and potential mishandling of these composites, and discerning their environmental and human health impacts. In this study, we investigated the release and toxicity of abraded thermoplastic composites rein-forced with reduced graphene oxide (rGO)[1], as well as the airborne emissions resulting from the combustion of few-layered graphene-epoxy composites[2], with a specific focus on their implications for human health.

The hazard of abraded thermoplastic reinforced with 2.5 wt% rGO was assessed, focusing on the most likely exposure routes. A multi-endpoint comparison was performed between as-produced rGO, abraded PA6-rGO composite and abraded neat PA6 using a panel of robust and commonly used in vitro models as well as a mouse model of pulmonary exposure. Overall, our findings indicated a negligible impact of rGO-reinforced PA6 composites on all tested models, suggesting a low risk to human health under acute exposure conditions[1].

In a second case study, we investigated the effects of incorporating 5 wt% graphene nanoplatelets (GNP) into epoxy composites on the aerosol released during combustion, examining particle size and concentration, polycyclic aromatic hydrocarbon (PAH) concentration, and biological effects on lung cells. Our results confirmed the potential health hazards associated with aerosol emissions from epoxy composites at their end-of-life through combustion. However, we observed that incorporation of GNP did not induce any novel or additive adverse effects on alveolar epithelial cells within 96 h of culture post-exposure[2].

Conclusion: Drawing from these two individual case studies and our recent meta-analysis of further publicly available data[3], we provide a comprehensive consolidation of the current state of knowledge regarding C-NM composites, encompassing their release dynamics and potential hazards when exposed to mechanical, chemical, and thermal stresses throughout their lifecycle.

This provides a valuable contribution to a comprehensive understanding of the risks associated with C-NM composites, guiding future research and regulatory efforts in this field.

References:

[1] Chortarea S, et al (2022) J Hazard Mat 435, 129053

[2] Netkueakul W, et al (2022) NanoImpact, 100414

[3] Romeo D, et al (2023 NanoImpact, 100477

Rob Vandebriel*, Angela Saccardo, Jolanda Vermeulen, Mariajose Lopez Tendero, Jose Balbuena, Jose Cormano, Martin Clift, Shareen Doak

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10:05-10:20

Assessing the in vitro toxicity of multicomponent nanomaterials: informing the Safe and Sustainable by Design approach

Nanomaterials have unique properties and are thus applied in a wide range of industrial sectors. Innovation within the nanotechnology industry is now becoming focused on the development of multicomponent nanomaterials (MCNMs), as combinations of nanomaterials may result in novel or improved properties. However, evaluating safety of MCNM represents a greater challenge than that of single component nanomaterials. Therefore, the development and implementation of Safe and Sustainable by Design (SSbD) strategies will be essential to support future innovation of MCNM and products incorporating them. This study evaluated industrially relevant MCNMs for three hazard endpoints: cytotoxicity, genotoxicity and inflammasome activation.
The selected MCNMs were SiC@TiO2 (60 and 500 nm), SiO2-APTES, and SiO2-ZnO. All MCNMs were compared to their individual components. As a SSbD alternative to SiC@TiO2, SiC@SiO2 was developed and compared to its original version. The *in vitro* cytokinesis-blocked micronucleus (CBMN) assay on human TK6 lymphoblastoid cells measured chromosomal damage in conjunction with relative population doubling (RPD) for cell survival. Inflammasome activation was assessed through cytotoxicity (WST-1) in conjunction with IL-1ß release (ELISA), on macrophages differentiated from human THP-1 monocytes.
Both sizes of SiC@TiO2 activated the inflammasome without changes to cell survival or chromosomal breakage. Their SSbD version, SiC@SiO2, showed similar behaviour but stronger inflammasome activation. SiO2-APTES elicited inflammasome activation; however, the two methods used to assess cytotoxicity (WST-1 and RPD) displayed varying outcomes, as they evaluated different parameters underlying cell death and were conducted in different cell types. APTES coating increased inflammasome activation by SiO2. SiO2-ZnO displayed a concentration-dependent decrease in cell viability, with no inflammasome activation detected. Notably, the MCNM was more toxic than its single components; leaching of Zn ions could be responsible for the toxicity of SiO2-ZnO. The results suggest that the genotoxic and inflammatory potential of MCNMs is highly dependent upon their component composition and their interactions within the material complex.
Funding information: this research has received funding from the European Union's Horizon 2020 research and innovation programme for the SUNSHINE project under grant agreement No 952924.

9:30-10:30 **Session 3C**

RISK ASSESSMENT AND MANAGEMENT

Digital tools, including decision support systems

Chairs: Danail Hristozov & Antreas Afantitis

Shin Hyun Kil*, Yoon Seokjoo

*Korea Institute of Toxicology, Republic of Korea

09:30-09:45

NanoToxRadar: Nano-QSTR model deployment on web environment to support safe and sustainable by design on advanced nanomaterials

Great inventions have been lost throughout the history simply because the innovative technology was neither safe nor sustainable to human and environment. Advanced nanomaterials hold promising futures, but the future can benefit our society only if its safe and sustainability were sufficiently tested. As a part of the effort to predict safety and sustainability of nanomaterials, nano-QSTR (Quantitative Structure Toxicity Relationship)

models have been developed to predict toxicity of the nanomaterials based on their structure alone; however, most of models only focus on specific type of nanomaterials such as carbon-based nanomaterials, coated iron nanoparticles (NPs), or metal oxide NPs. These models can't predict toxicity of advanced nanomaterials because their structural complexity poses an obstacle to apply wide range of descriptors developed in the previous studies. As nanomaterials developed for real life application become complicated along with its advancement, nano-QSTR moodels should be improved to cover such nanomaterials in their applicability domain to give reliable prediction results on the advanced nanomaterials. In this study, we introduce NanoToxRadar (www.kitox.re.kr/nanotoxradar), a web program to predict toxicity of advanced nanomaterials. The models implemented in the website were developed based on size-dependent electron configuration fingerprint (SDEC FP). SDEC FP considers size, shape, and composition of the nanomaterials to make prediction. At the moment, NanoToxRadar predicts cytotoxicity of different cell lines to predict organ toxicity of advanced nanomaterials.
Acknowledgements: This study was funded by the Ministry of Trade, Industry, and Energy (MOTIE) and Korea Institute for Advancement of Technology (KIAT) through the International Cooperative R&D program (Project No. P0019147)

Susan Dekkers*, Veronica Di Battista, Wendel Wohlleben, Blanca Suarez Merino, Véronique Adam, Gino Kalkman, Erik Lemcke, Wouter Fransman, Eugene van Someren

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09:45-10:00

Making a simple and sensitive DSS for SSbD in the early innovation stages

Within the HARMLESS project we develop an online decision support system (DSS) to support industry in the development of innovative materials in a Safe and Sustainable by Design (SSbD) manner. Based on our experiences from applying existing methods and tools for SSbD to our case study materials, we have made the SSbD-DSS simpler and more sensitive to increase its practical applicability. The SSbD-DSS guides users through a workflow from the ideation phase up to the pilot phase of the innovation process starting with three tools, i.e. AMEA, WASP and ASDI (see Figure 1). First, we have implemented the Advanced Material Earliest Assessment (AMEA) tool for early categorization and advice as an integrated tool and starting point of the DSS. AMEA consists of only 3 questions and is used to check if the developed material or product falls within the applicability domain of the DSS and to provide initial innovation-dependent SSbD advice and early design principles for "exposure during the life cycle", "hazard" and "sustainability". If the DSS is applicable, the designer is advised to apply the second tool, named Warning flags, design Advice, Screening Priorities (WASP). WASP is based on the AMEA advice, LICARA, Stoffenmanager, NEQ and other existing tools, and developed for the ideation and business case phase of the innovation process as a simplified approach that requires less information. This approach consists of 14 questions to identify early warning flags on safety and sustainability and to provide design and assessment advice. To help industrial innovators to make an informed decision for the most optimal SSbD version in the lab phase, another approach, named Alternative SSbD Design Inspector (ASDI) was developed. Based on the early warning flags from WASP, ASDI provides guidance on which descriptors to measure and insight into the differences between the SSbD versions within the various dimensions (safety, sustainability and performance). More deeper analysis tools, including in vivo hazard prediction methods based on physicochemical and in vitro data using, for example, a Bayesian approach, are suggested and directly available in the DSS for the pilot phase.
Funding information: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 953183 (HARMLESS).

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*Greendecision, Italy

10:00-10:15
The SUNSHINE e-Infrastructure

The European Chemicals Strategy for Sustainability and the Zero Pollution Action Plan have called for a transition towards Safe and Sustainable by Design (SSbD) approach for chemicals and materials. Due to their inherently complex nature and interactions the advanced materials pose safety and sustainability concerns. Therefore, it is important to equip the European industries with the knowledge and tools needed to develop and implement SSbD strategies for products enabled by advanced materials. To support this, the EU H2020 SUNSHINE project has delivered an e-infrastructure, which has been conceived as a practical means to operationalise the Safe and Sustainable Innovation Approach (SSIA). This digital system has been aligned to all 5 steps of the EC-JRC SSbD framework, enabling tiered assessment at each step with iterative looping at each stage of the innovation process represented by the Agile Stage-Gate model. The e-infrastrucure has been designed as a digital platform to foster dialogue, collaboration and information exchange between industry actors along entire supply chains. It is also a place where (in an ideal world) innovators can communicate with regulators in a trusted environment already in the early stages of innovation. The e-infrastrucure is Inclusive as it has been developed by engaging key stakeholders (e.g. SMEs, large industry, academia, regulators) to ensure that it addresses their needs and requirements. It is State-of-the- art as it has been based on the latest knowledge and data and includes (1) guidance for cost-effective generation of new data; (2) approaches for grouping to enable read-across of existing information for SSbD purposes; (3) a tiered approach for assessing the safety-sustainability functionality balance of the materials/products at each stage of the innovation process to inform 'Go to development' and 'Go to market' business decisions. The e-infrastrucure is also Open & FAIR as it is connected to the SUNSHINE Open & FAIR database, which enables access to high-quality EHS and sustainability data. The system is also Secure as it ensures controlled exchange of information between supply chain actors by means of a highly innovative blockchain technology. The system will be able to generate a SSbD digital pass for each product assessed by it.

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*TECNALIA Research and Innovation, BRTA, Industry and Mobility, Spain

10:15-10:30
Contribution of Digital Twin technology to achieve a safer and more sustainable Graphene Oxide manufacturing process

The digitalization of production processes is a driver to overcome technical and scientific challenges and achieve cleaner and smarter, safer and more sustainable manufacturing processes for chemicals and materials.
Digital Twin (DT) is a very promising technology applicable to the real-time optimization of production processes, which allows increasing process efficiency, preventing and reducing EHS impacts and improving the protection of workers exposed to hazardous substances.
This paper presents a specific use case of applying DT technology in the dust collection and filtering cabin with cartridge filtering system and programmable automatic cleaning, installed in the solid manipulation stage (milling, sieving, and packaging) of the Graphene Oxide (GO) production process at AVANZARE (Spain).
DT's objective is to optimize the operation of the filtering cabin in real time, to prevent and reduce the risk of occupational exposure to (nano) particles, guaranteeing the manufacture of a product of the highest quality with the greatest energy efficiency.
Acknowledgment. This work has been supported by EU-project SUNSHINE. This project has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement N° 952924. This paper reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.

Session 4A

11:30-13:00

HAZARD ASSESSMENT

Novel approaches and models for advanced in vitro testing

Chairs: Alberto Bianco & Elisa Moschini

Sandra Vranic*, Rahaf Issa, Ricard Marcos, Alba Hernandez Bonilla

*University of Manchester, United Kingdom

11:30-11:50

Assessment of nanoplastic toxicity using human lung organoids enriched with macrophages

Advances in in-vitro culture systems have stimulated an interest in more reliable testing approaches that predict nanomaterial toxicity risks and align with the 3Rs framework. Lung organoids are one example, and display functions consistent with their in-vivo counterparts. We have previously established a human embryonic stem cell-derived lung organoid exposure model that utilises microinjection to deliver nanomaterials into the airspace-like lumen of organoids. These organoids exhibit the six major proximal (goblet, basal, club, ciliated) and distal (AECI/II) epithelial cell types of the adult lung and contain functional cells, evidenced by active ciliary beating and surfactant/mucin deposition.
Here, we advanced the lung organoid model by incorporating a functional immune component – human embryonic stem cell-derived macrophages and assessed its suitability as a tool for nanoplastics toxicity assessment. We evaluated the pulmonary toxicity of nanometric polystyrene nanospheres (PS, 50-500nm) and Poly-Ethylene Terephthalate fragments (PET, 50-200nm) from plastic bottles. Lung organoids, with and without a macrophage component, were exposed with PS or PET for up to 7 days. Using flow cytometry, lung epithelial cells showed an increased PET uptake compared to PS, with no significant impact on organoid viability. Histological analysis also revealed distinct areas in which the nanoplastics localise, and their interaction with airway cells and macrophages.
With further validation, our lung organoid-macrophage model may not only reduce the need for rodent inhalation studies, but also replace the current use of simple in vitro pulmonary models for toxicology studies.

Itziar Polanco*, Juliana Carrillo, Adrián García Salvador, Mari Venäläinen, Jussi Lyyränen, Satu Suhonen, Nienke Ruijter, Ana María Candalija Iserte, Apostolos Salmatonidis, Marie Carriere, Morgan Lofty, Matthew Boyles, Davide Lotti, Jesús C. Guzmán Mínguez, José F. Fernández, Flemming Cassee, Julia Catalán, Isabel Rodríguez Llopis, Socorro Vázquez Campos, Felipe Goñi de Cerio, Alberto Katsumiti

*GAIKER Technology Centre, Basque Research and Technology Alliance (BRTA), Spain

11:50-12:04

Assessment of Repeated 28-day Pulmonary Toxicity of 3D printing micro- and nanoparticles using an advanced in vitro model

Research has demonstrated that the 3D printing process can emit micro- and nanoparticles (MNPs), which upon inhalation, may lead to acute, subacute, and chronic adverse effects. Since animal models may not always provide relevant human toxicity data, the use of New Approach Methodologies (NAMs) based on advanced in vitro human models is highly recommended. Among these models, primary human bronchial epithelial models such as MucilAir seems a promise in vitro model. In this work, we evaluated the acute and subacute toxicity of top-down cryomilled polycarbonate (PC) MNPs both with and without single-wall carbon

nanotubes (SWCNTs), and polypropylene (PP) MNPs with and without silver nanoparticles (Ag) using the MucilAir model. Inserts were exposed to concentrations of 0.1, 10, and 1000 µg/mL of all MNPs for 4 hours per day, up to 28 days exposure. Cytotoxicity (LDH), barrier integrity (TEER) and inflammatory response (legendplex analysis) were assessed at time 0 and 4 hours, and on days 7, 14, 21, and 28 of exposure. Genotoxicity (Comet Assay) was assessed after 28 days of exposure. Particle size distribution analysis (based on number) using transmission electron microscopy (TEM) revealed that all samples contained a few particles > 1 µm (1–13%), with the majority ranging from 0.25 to 0.30 µm. Energy-dispersive X-ray spectroscopy (EDX) analysis confirmed the presence of Ag in the PP+Ag sample, and Raman spectroscopy identified SWCNTs in the PC+SWCNTs sample. According to the results in vitro, MNPs induced slight effects on barrier integrity and cell viability along the 28 days of exposure. Proinflammatory responses (induction of IL-6, IP-10 and MCP-1) were found in cells after long-term exposure (>14 days) to MNPs. PP+Ag and PC+SWCNTs seem to induce higher inflammation then PP and PC only. According to the comet assay results, PP, PC, PC+SWCNTs and specially PP+Ag induced genotoxity in bronchial epithelial cells after 28 days exposure. Our findings suggest that long term exposure to MNPs with and without nanomaterials induce inflammation and genotoxicity in human bronchial cells. Despite showing relatively high uncertainty, the advanced in vitro model MucilAir resulted an useful tool capable of detecting subacute effects of 3D printing MNPs and able to discern differences in particle toxicity in the presence and absence of nanomaterials, thus aiding in the inhalation risk assessment of of particulate materials. This study was supported by the EU H2020 Project SAbyNA (Grant Agreement no. 862419).

Melissa Anne Tutty

12:04-12:18

The Application of 3D Hepatic Spheroids in the Development of a NanoBioMaterial-Specific Alkaline Comet Assay for Genotoxicity Screening

The advantages and wide-reaching applications of nanomaterials are unprecedented, with these unique materials having revolutionized medicine, with the most obvious example being COVID-19 vaccine development. Despite their potential, is not without saying that nanomaterials do not induce harmful effects on humans upon exposure, and as nanomaterials and indeed NanoBioMaterials (i.e., nanomaterials with applications in medicine and medical technology) are being continually integrated into everyday life, understanding their potential adverse effects is critical. Therefore, it is imperative that hazard assessment is undertaken in the most accurate manner possible, using target organs and model systems relevant to nanomaterials. While the preclinical assessment for toxicity and sterility have been extensively developed, assessing the genotoxic, or DNA damaging, potential of nanomaterials is still challenging as common genotoxicity assays have been historically designed for small, soluble molecules and are unsuitable for nanomaterials. Therefore, there is a need for a robust genotoxicity assay for specific use with nanomaterials and NBMs. Conveniently, in the last 10 years there has been great interest in advanced tissue-mimetic 3D in vitro models, which are more closely related to the in vivo situation, for the pre-clinical risk assessment of nanomaterials. These have ranged from 3D models of the liver for toxicity screening, to 3D lung models to study inflammation, and immune response to viral and bacterial infections. Recently, using 3D models has also been applied to nanomaterial genotoxicity screening, i.e., the ability of nanomaterials to cause DNA or chromosomal alterations or damage, with the development and validation of EpiSkin™, used with a 3D modification of the 2D in vitro micronucleus assay for applications in skin corrosion and irritation assessment. One specific 3D model, 3D liver spheroids, has been shown to be more faithful model for the pre-clinical toxicity screening of nanomaterials compared to its 2D counterparts. Regarding genotoxicity assessment, most in vitro genotoxicity assays, including the micronucleus assay and the focus of this work, the alkaline comet assay, exhibit poor specificity and do not mimic how nanomaterials induce DNA damage in the human body. Whilst a 3D Skin Comet and Reconstructed Skin Micronucleus (RSMN) assay has been developed, standardized and validated, with the intention to close the gap between 2D and in vivo genotoxicity assessment, and as a European Commission EURL ECVAM ToxTracker assay, no such protocols or guidelines exist for a 3D alkaline comet assay. Therefore, in this work, gold standard 2D cell cultures and alternative 3D hepatic spheroids, are exposed to two nanomaterials, inorganic TiO 2 nanoparticles and organic 20 nm AUNPs, with acute exposure times used to assess genotoxic potential of these materials, as determined via DNA unwinding and the alkaline comet assay.

Jürgen Schnekenburger*, Anne Marzi, Kai Moritz Eder, Alvaro Barroso Pena, Bjoern Kemper

*Muenster University, Biomedical Technology Center, Germany

12:18-12:32
Quantitative Phase Imaging as Sensitive Screening Method for Nanoparticle Induced Cytotoxicity Assessment

Nanoparticle in vitro cytotoxicity quantification is challenging due to the lack of customized and standardized guidelines for nanoparticle testing. Nanoparticles with their unique properties can interfere with biochemical test methods, so multiple tests are required to fully assess their cellular effects. For a more reliable and comprehensive assessment, it is therefore mandatory to include methods in nanoparticle testing routines, which are not affected by particles and allow the efficient integration of additional molecular techniques into the workflow. Digital holographic microscopy (DHM), an interferometric variant of quantitative phase imaging (QPI), was demonstrated as a promising method for the label-free assessment of the cytotoxic potential of nano-particles. Due to minimum interactions with the sample DHM allows further downstream analyses of cells and supernatants. In this study, we investigated the potential of DHM in a multimodal approach to assess cytotoxicity by directly comparing DHM-detected effects on the same cell population with two downstream biochemical assays. The dry mass increase of RAW 264.7 macrophages and NIH-3T3 fibroblast populations measured by quantitative DHM phase contrast after incubation with poly (alkyl cyanoacrylate) nanoparticles for 24 h was compared to the cytotoxic control digitonin and the control cell culture medium. Viability was then determined using a metabolic activity assay (WST-8). Moreover, to determine cell death, supernatants were analyzed for re-lease of the enzyme lactate dehydrogenase (LDH assay). In a comparative analysis, in which the average half maximal effective concentration (EC50) of the nanocarriers on cells was determined, DHM was more sensitive to nanoparticle effects compared to the biochemical assays. DHM can be easily integrated into the experimental routine and existing workflows or even replace established assays for cytotoxicity assessments in a time efficient manner. Our results show that QPI with DHM is highly suitable to identify harmful or low-toxic nanomaterials. The presented DHM assay with a newly developed commercial DHM microscope for the imaging of native cells and the compatibility with common 96-well plates allows high-throughput systems and future embedding into existing experimental routines for in vitro cytotoxicity assessment.

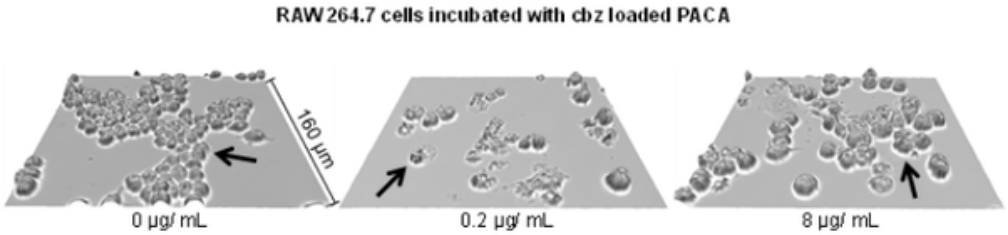

Figure 1. Pseudo 3D surface plots of representative DHM QPI image overlaid with the texture of correlatively recorded bright field images of RAW 264.7 macrophages 24 h after incubation with 0.2 and 8 µg/mL of cbz loaded PACA compared to medium control cells (0 µg/mL). Black arrows indicate cell debris due to apoptotic or necrotic cell death.

Francesco Cubadda*, Stephanie Blanquet-Diot, Susanne Bremer Hoffmann, Lucie Etienne Mesmin, Valerie Fessard, Francesco Sirio Fumagalli, Kevin Hogeveen, Jan Mast, Eveline Verleysen, Olimpia Vincentini

*Istituto Superiore di Sanità - Italian National Institute of Health, Italy

12:32-12:46
NAM-based hazard assessment of nanocellulose oral exposure: the NANOCELLUP project

Nanocellulose (NC) is an emerging material in the food sector with several application areas, including prospective use as a novel food or food additive. Three main types of NC exist, i.e. bacterial NC (BNC), nanofibrillated cellulose (NFC), and cellulose nanocrystals (CNC). The biological sources and processing conditions affect several physicochemical parameters of NC. All NC materials have a high aspect ratio and can have very small diameters (5-10 nm).
In the EFSA-funded project NANOCELLUP, a NAM-based IATA for addressing data gaps in the assessment of potential hazards associated to NC oral exposure was considered. This IATA focused on three main pillars, i.e. (i) assessment of the uptake and potential crossing of the intestinal barrier, (ii) assessment of local effects, including inflammation and genotoxicity, on the gastrointestinal epithelia, and (iii) assessment of any digestion or degradation of NC by the human microbiome. Eight samples belonging to the three NC types, plus a comparator in the micro-range, were selected as study materials.
All experimental studies were performed to ensure relevant and reliable results in the perspective of their use for regulatory risk assessment. A detailed physicochemical characterisation was performed and material-specific dispersion protocols ensuring maximal deagglomeration were developed, with a maximum concentration applicable in in vitro studies of 30 µg/mL. For in vitro testing, a number of requirements were complied with, including (i) detailed cell characterisation and description of cell culture methods, (ii) exposure and post-exposure times defined and justified with respect to the individual tested parameters, (iii) check for the absence of interference, (iv) quality controls including negative and positive controls and assay reagent

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Mihaela Roxana Cimpan*, Ivan Rios-Mondragon, Laura Maria Azzurra Camassa, Victor Franco Puntes, Agnieszka Gajewicz Skrętna, Marti Busquets, Eleonora Marta Longhin, Espen Mariussen, Ole-Bendik Hofshagen, Neus Gómez Bastus, Elisabeth Elje, Sergey Shaposhnikov, Maria Dusinska, Terje Espevik, Asbjørn Magne Nilsen, Elise Rundén Pran, Shan Narui

*UiB - University of Bergen, Department of Clinical Dentistry, Norway

12:46-13:00
Towards a reliable assessment of nanomaterial health effects using advanced biological models and assays (NanoBioReal)

The aim of the "NanoBioReal" project funded by the Research Council of Norway (2019-2023) was to create and implement realistic biological models and new advanced methods (NAMs) for the hazard assessment of nanomaterials (NMs) *in vitro, in vivo, and in silico*. The project addressed two of the main sources of uncertainty that hamper the assessment of real-life impact of NMs on human health: i) most of the existing *in vitro* models do not reflect real life exposure to NMs and ii) potential interferences of NMs with assays or detection systems. A set of NMs representing widely used NMs in Europe and worldwide, consisting of TiO2, nano-silver, and CeO2 with various physicochemical characteristics, were produced and characterized by Applied Nanoparticles SL (Barcelona). The NM-300K was used as a reference NM.

A 3D respiratory model of human epithelial A549 cells, endothelial EA.hy926 cells and differentiated monocytes (dTHP1) cultivated at the air-liquid interface (ALI); an astrocytes-neurons co-culture model; a microvasculature model; a human in situ inflammation whole blood complement system; and a mouse model of inflammation were established and used for hazard assessment. Key biological processes and mechanisms were investigated, i.e., cellular uptake, cytotoxicity, genotoxicity, DNA damage, DNA repair, inflammation, oxidative stress, and key biological markers were identified. To avoid NM-induced interferences, label-free electric cell-substrate impedance sensing and cyclic voltammetry were implemented to evaluate the viability and proliferation of cells and oxidative stress, respectively. To mimic real-life exposure, we have designed and produced multicompartment microfluidic chambers for the culture of 3D lung and microvasculature models, which were monitored in real-time by bright-field and fluorescence microscopy. In addition, we have built a module to monitor transepithelial electrical resistance (TEER).
In silico modelling was performed on the data to search for descriptors of toxicity.
The outcome of the NanoBioReal project contributes to a reliable and more realistic assessment of NMs' health effects through the development and implementation of new approach methodologies for next generation risk assessment (NGRA). Further, the in silico modelling showed promising results on identification of descriptors for cytotoxicity and grouping of NMs, to support integrated approaches for testing and assessment (IATA) of NMs for NGRA.
The project was funded by the Research Council of Norway, grant no. 288768.

11:30-13:00
ADVANCED MATERIALS: TOWARDS SAFE INNOVATION
Multicomponent nanomaterials and mixture effects

Chairs: Antonio Marcomini & Santiago Aparicio

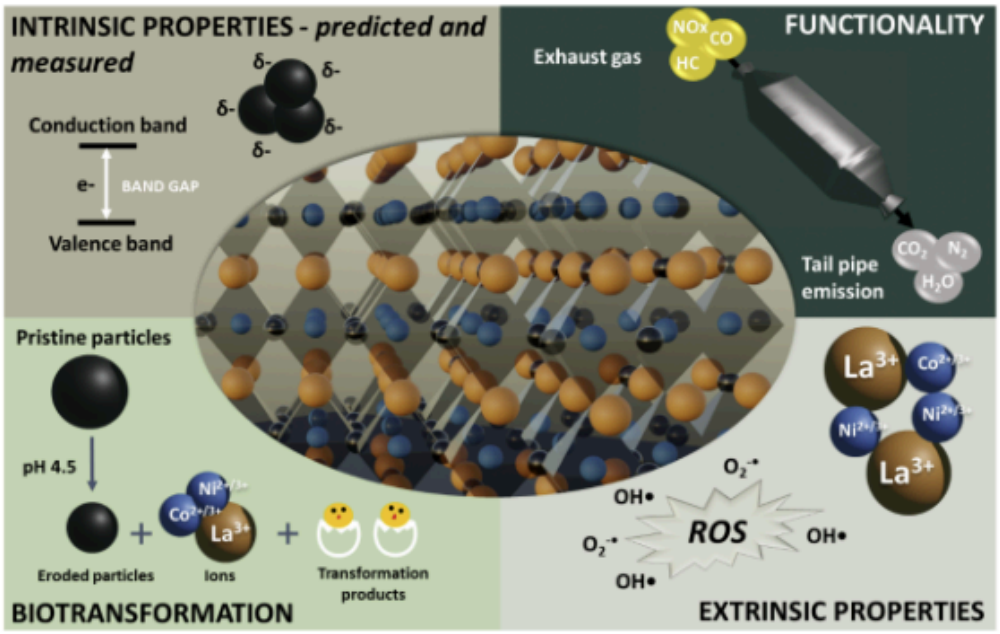
Wendel Wohlleben

BASF SE, Dept. Analytical and Materials Science, Germany

11:30-11:55
Inhalation hazard of multicomponent perovskites by New Approach Methodologies (NAMs) for SSbD comparative screening

Oxide-based perovskites designed for automotive catalysts contain multiple metal elements whose presence is crucial to achieve the targeted performance. Due to their multicomponent character and their enhanced functionality, they are regarded as Advanced Materials. They are highly stable in the exhaust operating conditions; however, little is known about their stability in physiological conditions.
Within HARMLESS (GA no. 953183), for the screening of safety and sustainability at earlier design stages, we rely on New Approach Methodologies (NAMs) due to ethical reasons, speed, and comparative testing. Here we amended the testing advices that the AMEA and WASP tools of the HARMLESS DSS provided for multicomponent advanced materials.
We found that the performance under realistic and varying exhaust catalyst conditions depends specifically on doping, whereas hazard-related properties depend primarily on Nickel content, in accord with the predictions by quantum chemistry modeling of the electronics properties. We also found that the composition and surface properties of six different Lanthanum-based perovskites compromise their stability in the physiological conditions of the lung, influencing the oxidative damage of the particles and the bioaccessibility of leaching metals. We observe biotransformation in lysosomal conditions, where the leached Lanthanum ions, but not other metals, re-speciated into lanthanum phosphate nanoparticles, increasing the observed oxidative damage in a non-synergistic way.
The in chemico results were confirmed by the measured multi-component clearance from lungs into urine after in vivo inhalation by male Wistar rats and were benchmarked against well characterized spinels

materials for ranking purposes.
In summary, we followed the HARMLESS DSS, implemented the SSbD re-design advices and re-tested hazard and performance during the lab phase, evaluate sustainability trade-offs, and propose decisions for the next gate.



Chang Guo*, Sanghamitra Mukhopadhyay, Laura Zanetti Domingues, Robert Lees, Esther Garcia Gonzalez, Benji Bateman - Andy Ward, Sameirah Macchiarulo, Ludmila Mee, Rachel Smith, Ian Mudway

*UKHSA, Harwell Science Park, United Kingdom

11:55-12:10
Exploring the impact of inhaled (nano)particles on the blood-brain barrier (BBB) through interdisciplinary investigation

The increasing prevalence of human-produced (nano)particles in our environment has become a pressing concern in recent years, with mounting epidemiological evidence linking air pollution to a range of human neurological diseases including Alzheimer's disease, Parkinson's disease etc. It is postulated that inhaled (nano)particles could have negative effects on the brain either directly, by entering the brain via the olfactory nerve or through the blood circulation, or indirectly, i.e. that chemical messengers released in the lung when exposed to air pollution particles then reach the brain and induce a range of deleterious effects including promoting oxidative stress, neuro-inflammation etc. This study is employing an interdisciplinary approach, integrating cellular models, advanced analytical techniques, and state-of-the-art facilities to gain a deeper insight into how inhaled (nano)particles could affect the blood-brain barrier (BBB).
By utilizing ambient particulate matter (PM) and samples from specific sources such as diesel exhaust particles (DEPs), the study exposed a BBB cellular model composed of brain endothelial cells to PM. The interaction between particles and the BBB were investigated using the Zeiss Crossbeam 550 focused ion beam scanning electron microscope (FIB-SEM) in conjunction with Confocal microscopy (Central Laser Facility). Unlike a typical scanning electron microscope (SEM), which uses a single electron beam, the FIB-SEM incorporates a second beam, the ion-beam, for material cutting while the SEM performs high-resolution imaging. This combination allows for high-resolution (10-20 nm), material-sensitive ultrastructural 3D imaging of cellular volumes. Initial observations indicate that FIB-SEM effectively visualises the electron-dense carbon-based particles in biological samples, enabling direct observation of nano-sized particles derived from PM. Images collected show that particles (individuals/clusters) adhere to the plasma membrane or are taken up by cells inside lysosome-liked vesicles, particularly the nano-sized particles.

To further probe the interactions between (nano)particles and plasma membrane lipids, quasi-elastic neutron scattering (QENS) experiments were conducted at the OSIRIS spectrometer (ISIS Neutron and Muon Source). QENS was employed to measure the scattering signal arising from the dynamics of intra-cellular water, which serves as an indicator of lipid membrane behaviour. Prior to exposure to the neutron beam, cell samples were rinsed with deuterated PBS to eliminate any signal from extracellular water. Initial findings suggest that QENS experiments offer a promising approach for examining how the dynamics of lipid membranes are impacted by particle entry and traversal of barriers in heterogeneous biological models, such as the BBB. Further analysis is ongoing, and the results will be presented in the conference.

Sonia Cambiaso*, Roshan Shrestha, Hafez Razmazma, Davide Bochicchio, Giulia Rossi, Luca Monticelli

*University of Genoa, Physics Department, Italy

12:10-12:25
In silico study of the interactions of chitosan with biological membranes

This work has been developed in the framework of the H2020 SUNSHINE project (No 952924). Chitin, made of N-acetylglucosamine units, is one of the most abundant natural polysaccharides, synthesized by living organisms, such as arthropods, cell walls of fungi, and yeast. Its properties, including low nanotoxicity and biodegradability, make it ideal for biomedical and pharmaceutical applications, as well as for treating industrial pollutants. Chitosan is a cationic polymer derived from chitin partial deacetylation. It consists of a random distribution of β-(1→4) D-glucosamine (GlcN) and its acetylated derivative (GlcNAc). Polyelectrolyte complexes made of chitosan with proteins, polyanions, or DNA, have promising applications as advanced materials in transmucosal drug delivery [1] and other industrial applications.
Molecular simulations are valuable tools for understanding how chain composition and solution physicochemical conditions influence polymer solubility, size, flexibility, and aggregation. To this purpose, we developed a coarse-grained model of chitin and chitosan compatible with the last release of the popular Martini forcefield [2], which allows the simulation of complex, multi-component systems with submolecular resolution while retaining a high degree of chemical specificity. Our chitosan model can accurately reproduce the structural features of polymer chains with different degrees of acetylation and charge states, which depend on the pH of the solution. The transferability of the model between different solvent environments allows the simulation of several applications involving chitin and chitosan.
In the framework of the H2020 SUNSHINE project, we simulated a new multi-component nanomaterial (MCNM) consisting of graphene oxide flakes functionalized by chitosan and dispersed in a polyamide matrix. In the automotive sector, this innovative MCNM provides superior mechanical and flame-retardant properties, offering a non-toxic alternative to halogenated compounds. Our molecular simulations investigate its mechanical properties and the interactions with graphene oxide and with biological membranes, to gain insight into potential mechanisms of biological activity.

[1] Rinaudo M., Prog. Polym. Sci. 2006, 31, 603-632.
[2]Souza, P. C. T. et al., Nat. Methods 2021, 18 (4), 382–388.

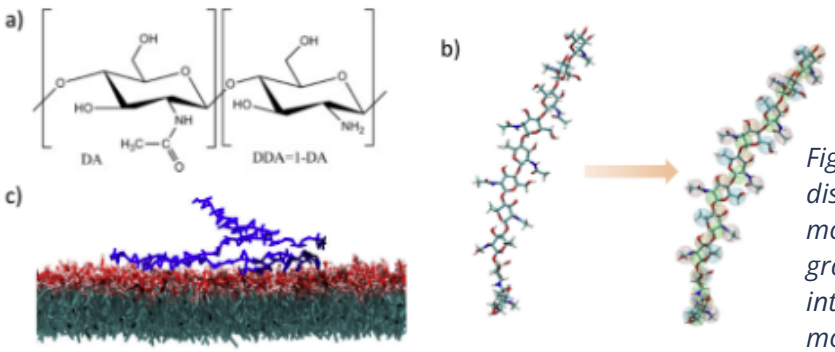


Figure 1: a) Chitosan, made of a random distribution of GlcN and GlcNAc monomers. b) atomistic (left) and coarse-grained model (right) of chitosan. c) interaction of chitosan (blue) with a lipid monolayer.

Angela Saccardo*, Rob J. Vandebriel, Jolanda P. Vermeulen, Mariajosé López Tendero, José Balbuena, José M. Lloris Cormano, Castor Salgado, José F. Fernandez, Martin J. D. Clift, Shareen H. Doak

*Swansea University, In Vitro Toxicology Group, Institute of Life Sciences, Faculty of Medicine, Health and Life Sciences, United Kingdom

12:25-12:40

Evaluating the in vitro genotoxicity of multicomponent nanomaterials to inform the Safe and Sustainable by Design approach

In recent years, the nanotechnology industry has focused its attention on multicomponent nanomaterials (MCNMs), due to their novel and/or improved functional properties. However, evaluating the MCNMs' safety is a significant challenge; hence, developing and implementing Safe and Sustainable by Design (SSbD) strategies is vital to support future innovation of MCNMs and products incorporating them. This study assessed the dose-response interactions and potential in vitro genotoxicological hazards of industrially relevant MCNMs: four silicon-based materials and one essential oil (EO) encapsulated in nanoclay. Their single components were tested alongside to evaluate whether the response was different when combined.
The in vitro cytokinesis-blocked micronucleus (CBMN) assay performed on human lymphoblastoid (TK6) cells was used to assess chromosomal damage, paired with relative population doubling (RPD) for cytotoxicity evaluation. The initial selection of MCNMs comprised SiO2-APTES, SiO2-ZnO, SiC@TiO2 (60 and 500 nm), and one with eugenol encapsulated in bentonite clay. An additional SSbD alternative was subsequently included for SiC@TiO2, replacing TiO2 with SiO2 (SiC@SiO2).
SiO2-APTES and both sizes of SiC@TiO2 showed no biologically significant changes in cytotoxicity and chromosomal breakage. Its SSbD version (SiC@SiO2) displayed a similar behaviour. SiO2-ZnO revealed a concentration-dependent decrease in cell viability. Furthermore, the MCNM was more toxic than its single components, possibly due to Zn(II) leaching. In contrast, the eugenol component of the EO-nanoclay MCNM showed significant cytotoxicity, whilst the EO-nanoclay was non-cytotoxic, demonstrating the efficient encapsulation of this essential oil in the MCNM. Collectively, these results suggest that the genotoxic potential of MCNMs is highly dependent upon their component composition and their interactions within the material complex.
Funding information: this research has received funding from the European Union's Horizon 2020 research and innovation programme for the SUNSHINE project under grant agreement No 952924.

Alicja Mikołajczyk

QSAR Lab Ltd., University of Gdansk, Poland

12:40-12:55

New Nanoinfomatics Approach for Joint Effect Prediction of Advanced Multicomponent Nanomaterials: Computational Tool to Support SSbD Strategy

The rapid development of Advanced Nanomaterials (AdvNMs) and the emergence of new Multicomponent Nanomaterials (MCNMs) inevitably expose organisms to multiple nanoparticles (NPs) at varying levels. Understanding the combined impacts of MCNMs and their individual components, as well as predicting the toxicity of AdvNMs mixtures, is crucial.
The innovative design of AdvNMs, which taking into account the environmental health and safety concerns alongside functionality, has become an integral part of the Safe and Sustainable by Design (SSbD) approach that has gained prominence in recent EU Commission policies. Accelerating the implementation of SSbD can be achieved through the application of artificial intelligence (AI) and digital tools, including Machine Learning (ML) algorithms and Quantitative Nano-Structure-Activity Relationship (Nano-QSAR) models, enabling early consideration of safety aspects in product design in a cost-effective and policy-compliant manner. However, due to the complex structure of AdvNMs, computational methods such as predictive ML-based models or Nano-QSAR methods to forecast specific joint responses, such as antagonistic, synergistic, or additive effects, remain limited.

In our work, we propose an approach to support the prediction of AdvNMs' joint effects at the early design phase (prior to synthesis). This approach is based on determining the mixture concentration effect of AdvNMs (EC50) that causes a 50% effect (e.g., immobilization) in organisms, based on theoretically derived mixture concentrations of its individual components. The approach allows to calculate different mixture toxicity indexes such as: Sum of Toxic Unit (STU), Additivity Index (AI), Mixture Toxicity Index (MTI) and Model Deviation Ratio (MDR). We believe that providing knowledge based on theoretical evidence is critical for developing efficient SSbD strategies to assess hazards induced by combined exposure to multiple components of AdvNMs and to facilitate their modification with non-animal alternative methods at the earliest possible stage.

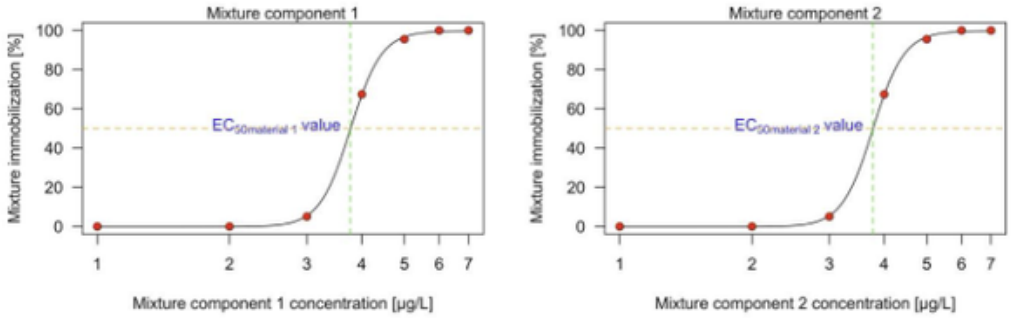


Figure 1. Graphical explanation of two DRC curves method

11:30-13:00 Session 4C

RISK ASSESSMENT AND MANAGEMENT

Dose-response, biodistribution

Chairs: Lang Tran & Otmar Schmid

Otmar Schmid*, Lin Yang, Pramod Kumar, Arunima Sengupta, Ali Farnoud, Ruolin Shen, Darya Trofimova, Sebastian Ziegler, David Kutschke, Wolfgang G. Kreyling, Marie Piraud, Fabian Isensee, Tobias Stoeger

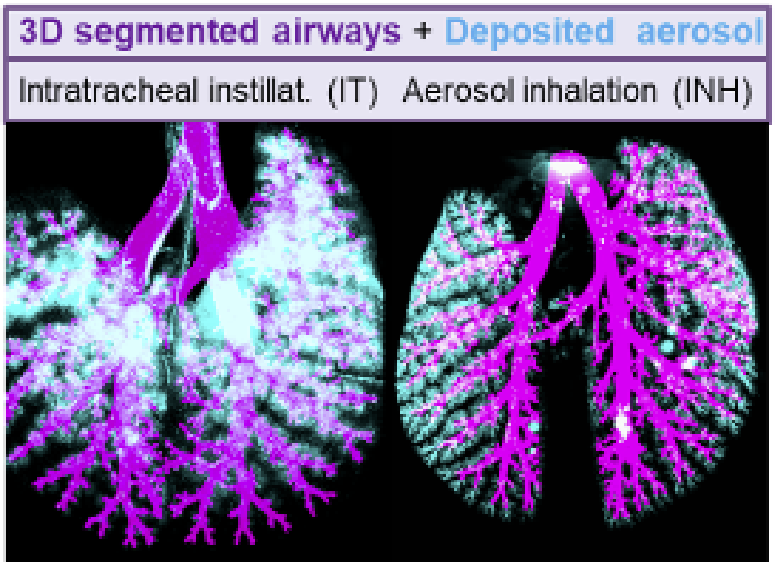
*Helmholtz Munich, Institute of Lung Health and Immunity, Germany

11:30-11:45

Monitoring deposition, spatially resolved dosimetry and biokinetics of inhaled nanoparticles with cellular resolution throughout the entire murine lung

Background: Understanding the dynamic process of spatially resolved particle deposition and subsequent particokinetics in the lung is of utmost importance for toxicological understanding as well as therapeutic (nano-) particle applications. In this study we provide new insights into these aspects leveraging holistic fluorescence lung imaging for spatially resolved nanoparticle dosimetry in non-dissected murine lungs.
Methods: We have recently applied optical clearing of murine lungs and combined it with light sheet fluorescence microscopy (LSFM) for holistic 3D co-mapping of lung morphology and aerosol deposition with cellular resolution in non-dissected murine lungs. Artificial intelligence (AI)-based algorithms were developed for automated 3D segmentation of whole-lung morphology and spatially resolved quantitative analysis of the dosimetry of fluorescent melamine resin (nano-)particles (VMD=480nm). This allowed investigation of differences in pulmonary distribution and biokinetics of nanoparticles depending on the method of application (e.g. intratracheal instillation or aerosol inhalation).

Results: Both nose-only and ventilator-assisted inhalation of micron-sized aerosol containing nanoparticles yields homogenous nanoparticle deposition throughout the entire lung with the highest dose per area in the distal bronchial and proximal alveolar region. In contrast, the widely used preclinical routes of bulk liquid (non-aerosol) application such as intratracheal instillation (IT) or intra-nasal aspiration result in patchy substance deposition preferentially in the central airways. The highly central deposition of bulk liquid applications is evidenced by high central-to-peripheral (C/P) deposition ratios of up to 3.8±0.7 for intra-nasal application (for IT: 2.3±0.3) as compared to 1.3±0.1 for aerosol inhalation. Analogously, the bronchial deposition fraction decreases from 0.32±0.06 for intra-nasal delivery, over 0.18±0.03 for IT to 0.08±0.01 for aerosol inhalation. Interestingly, albeit for IT application the initial bronchial deposition fraction (0h) is initially higher 0.18±0.03 than for aerosol deposition (0.08±0.01), it reaches the value for aerosol application within 2h after application. Yet, even for bulk liquid application a surprisingly high dose fraction reaches the acinar region (ca. 4-fold higher than bronchial dose) which is likely due to secondary spray formation. We also show that phagocytic uptake by macrophages facilitates both clearance and redistribution of nanoparticles within the acinar region.
Conclusions: Whole lung imaging combined with AI-supported image analysis provides quantitative evidence that - in spite of some shortcomings - IT application is a reasonably good approximation of aerosolized nanoparticle delivery to the lung.



Whole murine lung images after deposition of IT and aerosol inhalation of nanoparticles (blue)

Acknowledgements: European Union's Horizon 2020 program grant agreement No. 953183 (HARMLESS).

Luisa Diomede*, Ada De Luigi, Ahmed Subrati, Carmina Natale, Aline Chary, Tommaso Serchi, Luana Epicoco, Jolanda Spadavecchia, Alex Zabeo, Irina Estrela Lopis, Beatriz Alonso, Danail Hristozov, Amaya Ortega, Sergio Moya, Paolo Bigini

*Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy

11:45-11:57
Correlation between the physico-chemical properties of graphene oxide and its biological effect

Europe´s need for technological autonomy in digital industries has strengthened research to provide a reliable assessment of advanced nanomaterials from their system to large-scale production. To this aim, a multidisciplinary and integrated platform optimization to enable nanomaterial safety assessment for rapid commercialization has been built up through the POTENTIAL European project, which involves thirteen beneficiaries. The project's philosophy stems from validating harmonized test methods for physico-chemical characterization and safety in vitro and in vivo testing.

One of the study materials considered in POTENTIAL is graphene oxide (GO), selected for its broad and prospective development in future years. Four GO types with three different oxidation levels, commonly used for industrial aims, were produced: GO1 with a high oxygen content, GO2 in a reduced version, and GO3 with a medium percentage of oxygen. GO4 was prepared with the same oxidation level as GO1 but with a significantly lower lateral dimension. These materials were extensively characterized, showing that GO samples possess various features of periodic crystallinity, lamellar structure, and flake size. Additionally, the nature and density of defect-probed functionalization of GO flakes were estimated from the integrated intensity ratios of GO-characteristic Raman bands. Mass loss variances between GO samples confirmed various degrees of oxidation that were corroborated by the X-ray photoelectron spectroscopy-found carbon-to-oxygen atomic ratios.
Since inhalation and ingestion are the main routes of unintentional exposure, in vitro methods based on human pulmonary and intestinal cells were adopted to investigate the acute toxicity of the different GO. No toxic effects were observed in pulmonary cells with GO materials at any concentration. In contrast, only the highest GO3 dose of 0.1 mg/ml significantly reduced the cell viability of intestinal cells, suggesting a cell-specific acute toxic effect exerted explicitly by a material with a medium oxidation level. In vivo studies were performed on the nematode Caenorhabditis elegans, which is widely used as a good indicator of animal and eco-toxicological toxicity. GO1, GO3, and GO4, but not GO2, caused a dose-dependent reduction of worm viability, exerting comparable IC50 values.
These findings pointed out that GO, depending on its oxidation state more than its flake size, can exert an in vivo acute toxicity in C. elegans, raising concern about the possible effect on living organisms of accumulating this material in the environment. Data generated from this study will be exploited for grouping and read-across approaches for safety and sustainability.

Eyüp Bilgi*, Çiğdem Bilgi, Fırat Barış Barlas, Ceyda Oksel Karakus

*İzmir Institute of Technology, İzmir Institute of Technology / Bioengineering Department, Turkey

11:57-12:09
Statistical Optimization of Polypropylene Nano-micro Plastic Synthesis and in vitro/in vivo Toxicity Evaluation

Plastics are the general name given to polymers that consist of synthetic or semi-synthetic organic compounds and can be molded into different shapes. If current production and transformation policies continue as they are today, it is predicted that 12 billion metric tons of plastic waste will accumulate in the world by 2050. The most common plastic-related effects in the aquatic environment are the entanglement with plastics (aquatic mammals and birds) and the ability of plastic residues to attract microalgae, other microorganisms, and various invertebrate aquatic creatures, like natural residues. Unlike natural polymers and substrates, the fact that plastics do not biodegrade over time and can remain in that environment for years and transform into smaller sizes (micro/nano) because of various effects can cause the relevant habitats to radically change. Ultimately, the fact that creatures in these habitats can be included in the human diet can cause even ocean pollution to expose people to plastic. This can potentially cause adverse effects on various systems and organs, especially the endocrine system. In addition to diet, exposure to nano- and micro-plastics can also occur through skin contact, intraocular and respiratory.
In nature, polymers can be degraded into smaller parts by physicochemical (photodegradation, thermal degradation, or thermal oxidation with different wavelengths of light), enzymatic, or chemical means. Since polypropylene is resistant to physicochemical degradation due to its structure and no enzyme can perform hydrolysis, in this study, we focused on the chemical degradation of PP with organic solvents, namely xylene, toluene, and chloroform. By employing a design of experiments (DoE) approach was used instead of the one variable at a time (OVAT) approach, the capacities of xylene, toluene, and chloroform to break down polypropylene (PP) into nano and micro dimensions at different temperatures and reaction times were examined. Analyzes showed that chloroform could not decompose PP, and xylene could be used as the most suitable solvent to obtain PP with hydrodynamic radii below 400 nm. Optimization of PP synthesis at nano and micro dimensions continues using xylene digestion, reaction conditions, and response surface methodology. Characterization of the obtained nanomaterials will be carried out via SEM and DLS, and their toxicity potential will be evaluated via 2D cell culture and in vivo zebrafish model.

Acknowledgement:
This research will be supported by The Scientific and Technological Research Council of Turkey (TUBITAK)-BIDEB- 2218 National Postdoctoral Research Fellowship Program.

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12:09-12:21
The surface charge of polystrene nanoparticles impacts their penetration and safety

Many industrial plastics commonly used for food packaging are degraded into micro- and nanoplastics (MNPs). MNPs can interact with various matrices representing an "eco-toxicology" issue impacting animal and human health. In particular, through the food chain, MNPs may accumulate in animals and human bodies, deposit in various tissues, and enter the cells, activating an endosomal process known as the "trojan horse" mechanism.
Among the most commonly used non-biodegradable polymers is polystyrene (PS), which covers many applications due to its high molecular weight and low hydrophilicity. Like other nanomaterials, PS-MNP toxicity can be strongly influenced by different physico-chemical parameters (e.g., size, geometry, external charge). Therefore, robust indications about the PS-MNPs' biological effect after interacting with environmental-biological fluids and possible contaminants are crucial to forecasting their possible toxicity.
This study evaluated how modifying the modification of the surface charge of PS-nanoparticles (PS-NPs) may affect the interaction with the host and the toxicity in vitro and in vivo. First, amine-modified and carboxylate-modified PS-NPs (positively and negatively charged, respectively), both rhodamine-labelled, were characterized through dynamic light scattering and atomic force microscopy to assess the dimension, surface charge, and geometry. Then, we treated HEK293 cells (epithelial cells from human kidney embryos widely used in toxicology research) with PS-NPs and developed a platform combining fluorometric measures, imaging, and toxicity analyses. The internalization pathway was investigated using pharmacological inhibitors of endocytosis (chlorpromazine and amiloride) and labelling intracellular vesicles. Finally, the potential in vivo toxicity of PS-NPs was evaluated by administering them to C. elegans and determining their effect on the main physiopathological features.
Both positive and negative NPs had a spherical shape, their diameter ranged around 100 ± 30 nm, were monodispersed, and only differed for the z-potential (+51 mV and -9 mV). NPs entered the cells mainly by clathrin-mediated endocytosis and were rapidly entrapped by lysosomes. The internalization amount in HEK293 cells was markedly higher for positive PS-NPs, probably due to their electrostatic attraction to the cell membrane, which caused dose-dependent toxicity. Similarly to what was observed in vitro, only positive NPs were toxic in worms, causing dose-related motility and pharyngeal dysfunction and, at higher concentrations, also defects in reproduction and development.
These findings underline the crucial role the surface charge of PS-NPs plays in their interaction with the biological matrices.

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12:21-12:33
AI-powered 3D imaging unveils fresh perspectives on pulmonary nanoparticle delivery in healthy and fibrotic murine lungs

Pulmonary nanoparticle (NP) delivery plays a crucial role in localized therapy and assessing the health implications of inhaled substances. However, a holistic understanding of the interconnected lung networks, spatially-resolved delivery patterns, and NP biokinetics along with NP-induced innate immunity remains elusive.
Intratracheal instillation and ventilator-assisted aerosol delivery (3 µm droplets of a fluorescent NP suspension) were conducted under homeostatic and diseased conditions. Whole lung samples were collected from healthy and bleomycin-induced fibrotic mice at various time points (0h, 2h, 1d, and 14d) after NP administration. Employing tissue clearing and light sheet fluorescence imaging, we comprehensively assessed

multiple lung anatomical networks and the biodistribution of NPs at cellular level. In particular, we established active learning AI pipelines (convolutional neural networks) to achieve precise segmentation and reconstruction of the entire bronchial airway tree and complete vasculature systems.
Leveraging AI-driven quantitative morphological analysis, we observe significant alterations in multiple lung networks during lung fibrogenesis, which subsequently exhibit recovery during the resolution phase. Spatially resolved NP delivery characteristics, including inter- and intra-acinar distribution patterns and regional NP dosages, show distinct features in the lung fibrosis model. Notably, NPs exhibit limited deposition to highly diseased areas, such as regions with fibrotic foci. Furthermore, phagocytosis and the migration of tissue-resident macrophages (TRMs), responsible for intra-acinar NP transport, are compromised under diseased conditions.
In conclusion, this study offers impartial evidence of alterations in multiple structural networks of the lung, in aerosol-based NP deposition profiles and in NP translocation in a pulmonary fibrosis mouse model. Our openly-shared, diverse 3D murine lung network models are expected to enhance the power of future studies in lung diseases and targeted pulmonary drug delivery.

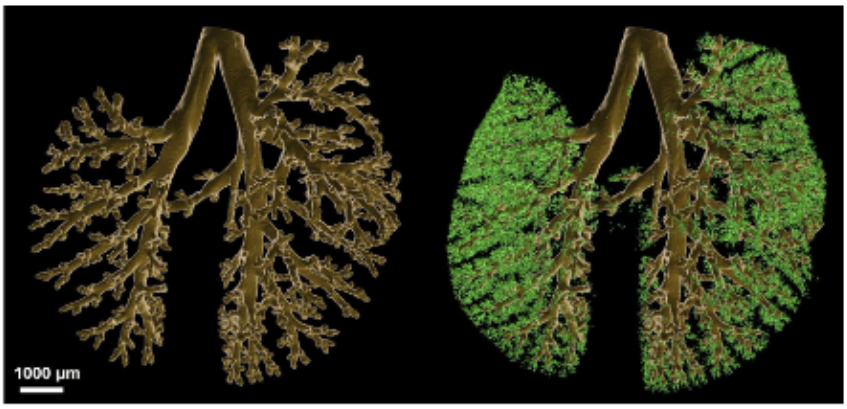

Figure: Holistic 3D projections of AI-driven segmentation of a full respiratory airway tree (brown) and uniformly-distributed NP aerosols (green) directly after ventilator-assisted aerosol delivery in a healthy mouse lung. It is evident that the vast majority of NPs (> 93%) deposits not in the bronchial, but acinar region of the lung.

Soyeon Jeon*, Dong-Keun Lee, Gyuri Kim, Sinuk Lee, Songyeon Kim, Wan-seob Cho

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12:33-12:45

Multimodal Pulmonary Clearance Kinetics of Carbon Black Nanoparticles Deposited in the Lungs of Rats: A Role of Alveolar Macrophages

Alveolar macrophages (AMs) are predicted to have some effects on the pulmonary clearance of nanomaterials, but their qualitative and quantitative role is poorly understood. In this study, carbon black nanoparticles (CBNPs) were instilled into the lungs of Wistar rats at 30, 100, and 300 μg/rat, and evaluated concentrations of particles in organs, including lung, lung-associated lymph node (LALN), liver, spleen, and kidney, at days 0 (immediately after instillation), 1, 7, 28, 60, and 90 post-instillation. The results showed that CBNPs showed a multimodal pulmonary clearance pattern: slow clearance till day 28, fast clearance at days 28 to 60, and slow again at days 60 to 90. Then, CBNPs at 100 μg/rat were instilled into AM-depleted rats using clodronate liposomes (CLO) to prove the mechanism of this unique clearance pattern. At 28 days after instillation, the CBNP levels in the lungs treated with CLO showed about 31% reduction than those in normal rats. In addition, the concentration of CBNPs in LALN treated with CLO significantly increased on day 28, while those of normal rats showed no detectable levels. This result highlights that prolonged retention of poorly soluble NPs in the lung till 28 days is mediated by phagocytosis of AMs, and fast clearance at days 28 to 60 is due to the turnover time of AMs, estimated around 1 to 2 months after birth. Likewise, new generations of AMs mediate the slow phase at days 60 to 90. However, further studies, such as testing in AM-depleted rats with genetic engineering, are needed to support the conclusion of this study.
Funding: This study was supported by the National Institute of Food and Drug Safety (22212MFDS233).

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12:45-12:57

Impact of particle size and limit of detection on the design of toxicokinetic studies for nanomaterials: a review focused on TiO2, CeO2 and SiO2

Currently there are no internationally agreed protocols or guidance for assessing the biodistribution and toxicokinetics of nanomaterials for regulatory purposes. This gap is currently being addressed by an OECD project to develop a Guidance Document on toxicokinetic study design for nanomaterials following oral and inhalation exposure. The work reported here is being undertaken in support of this OECD project.
Following intakes of nanomaterials, biodistribution of both nanoparticles and their chemical constituents (i.e. following dissolution) can occur. Particle size has been demonstrated to influence biodistribution and biodistribution kinetics of inhaled nanoparticles and their chemical constituents (e.g. via effects on solubility) and as such may be an important factor to consider when designing a toxicokinetic study for nanomaterials. Quantification of nanoparticle translocation rates and subsequent particle accumulation in secondary tissues requires detection methods with high specificity and sensitivity and very low limits of detection. Thus, available analysis techniques and their sensitivity, considered primarily in relation to limits of detection (LOD) and limits of quantification (LOQ), are also important factors to consider in the design of a toxicokinetic study.
A literature review of nanomaterial toxicokinetic studies is being undertaken to investigate the impact of size and LOD on the design of toxicokinetic studies for nanomaterials. This is considering both inhalation and oral exposures for three exemplar nanomaterials (TiO2, CeO2 and SiO2) chosen as representative nanomaterials with different physicochemical properties and detection limits. TiO2 was included as an insoluble high-volume nanomaterial and an example of a nanomaterial with a relatively high detection limit in tissue due to polyatomic interference. CeO2 nanoparticles were included as relatively insoluble nanoparticles with low detection limits. SiO2 nanoparticles are high volume nanomaterials with intermediate solubility at neutral pH and have a very high limit of detection in biological tissues due to high background levels of SiO2.
To assess the effect of particle size, it was decided to focus on translocation to the liver, as this is a key organ given its role in the reticuloendothelial system. Translocation to the kidney and spleen were also considered as tissue burdens for this organ were also provided in a number of the studies. The results obtained thus far for the inhalation studies indicate no clear particle-size related trends, although overall the data is limited, and comparisons are hampered by the wide range of study designs. Information on analytical approaches is often limited with LODs/LOQs provided for only a small number of studies.

Session 5A

14:30-16:00

HAZARD ASSESSMENT

Ecotoxicity

Chairs: Teresa Fernandes & Willie Peijnenburg

José Maria Navas*, Gregorio Molés, Ana Valdehita, Mona Connolly

*INIA CSIC, Department of Environment and Agronomy, Spain

14:30-14:50

Induction of cytochrome P4501A dependent detoxification activities in fish caused by graphene oxide

Graphene oxide (GO) is the most widely investigated graphene based material. Yet potential issues related with environmental safety, in particular with the possible fate and metabolization of GO in organisms still need to be addressed. Considering the polyaromatic nature of graphene, we hypothesized that GO could induce detoxification processes in fish similar to those triggered by hazardous polyaromatic hydrocarbons

(PAHs) and related substances. When planar PAHs enter cells they activate the aryl hydrocarbon receptor (Ahr) and the activated receptor induces the production of cytochromes P4501A (CYP1A), whose enzymatic activities (e.g. ethoxyresorufin-O-deethylase (EROD)) play a key role in the catabolism of the inducing substances. Using the knowledge of this pathway for PAHs, we investigated the possible induction of such enzyme activities and their possible contribution to the metabolism of GO in fish. Using both an in vitro testing approach with the RTL-W1 rainbow trout liver cell line, and in vivo toxicity assays with juvenile rainbow trout, Oncorhynchus mykiss (OECD TG 203), any effects at the cellular level as well as on a whole organism were assessed. In RTL-W1 cells a time- and dose-dependent loss in plasma membrane integrity and lysosomal function associated with increased reactive oxygen species (ROS) levels and an enhancement of metabolic activity was evidenced only after 96h of exposure. In in vivo exposures, GO did not provoke mortality in rainbow trout juveniles following 96h exposure (≤88.5 mg/L) but led to a significant induction of EROD activity in liver. Strikingly, such induction was concomitant with a down regulation of ahr and cyp1a gene expression. Simultaneously, histological alterations in liver and gills (in this last case related with inflammatory processes) were evidenced, and an upregulation of pro-inflammatory cytokines il1b and il8 was observed. Taken together, the results show that while the GO tested did not cause short term acute toxicity in fish it was able to provoke the induction of CYP1A related detoxification activities that probably contribute to its own metabolism and the observed inflammatory phenomena are likely associated with the down regulation of ahr and cyp1a genes. Further investigations of these observed disturbances caused by GO exposure at the molecular, cellular and tissue level is warranted to fully understand the material fate in organisms such as fish.

Acknowledgements: The Graphene Flagship Core 3 project has received funding from the European Union's Horizon research and innovation programme under grant agreement No. 881603.

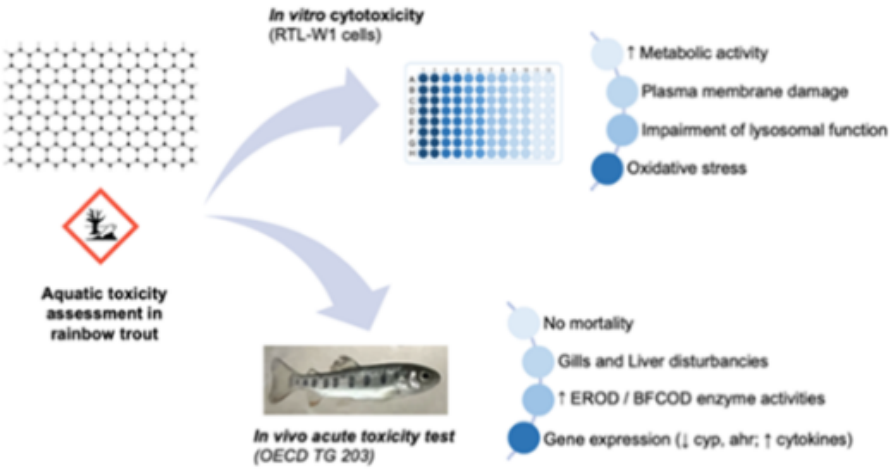

Figure: GO effects and disturbances in fish

Ruben Martinez*, Patricia Solorzano, Melissa Faria, Ana Candalija, Thierry Douki, Niels Leijten, Yvonne Staal, Socorro Vázquez-Campos

*Leitat Technological Center, Spain

14:50-15:04
Safety assessment of SURPASS materials using Daphnia magna as model species and SAbyNA guidance platform

The worldwide problem of plastic waste has risen and increased concerns during the last decades. In addition to their high persistency, their leached substances, possible breakdown up to micro- and nano-plastics (NPs) and therefore, their impact in human and environmental health need to be considered (e.g., possible endocrine disrupting properties). In this regard, the SURPASS project aims to design and formulate safer, more sustainable and recyclable polymeric materials, applying the SSbD (Safe and Sustainable by Design) concept, following the assessment steps defined in the SSbD framework recently released (December 2022) by the European Commission, to guide the innovation process for chemicals and materials. In this context, human and environmental safety assessment of the SURPASS project's materials will be performed and results will be compared with traditional final materials and reference compounds used in their synthetic

routes. Both final products and intermediates from 3 different case studies from the transport, building and packaging sectors, will be studied. In this study, acute aquatic environmental toxicity of both final and intermediate compounds will be experimentally determined using Daphnia magna as a model specie (following the OECD 202 guideline) while the hazard assessment of potential releases of nanoplastics during their whole life cycle (of those insoluble solid polymeric materials) will be performed following the SbD SAbyNA Guidance Platform recently developed by the European project SAbyNA (https://platform.sabyna.eu/). These results will contribute to the integrated assessment of the innovative products in SURPASS, which will take into account not only their safety but also their sustainability (including recyclability) and performance, selecting new polymeric materials that better balance all these factors.

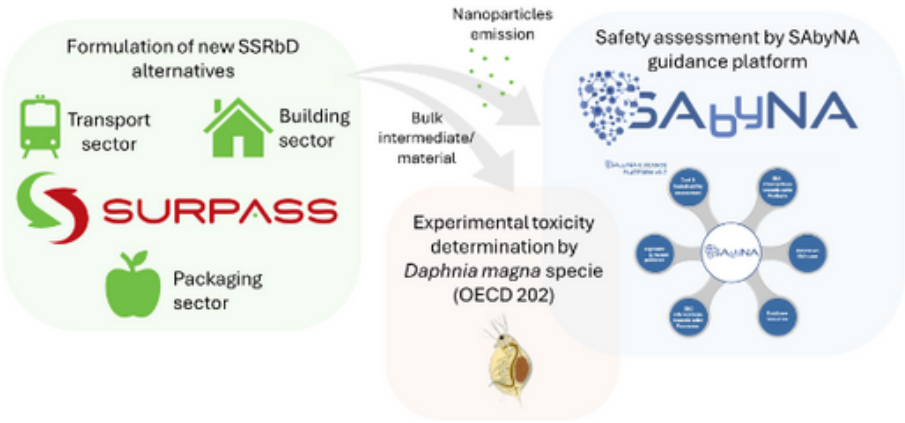

Figure 1: schematic summary of the safety assessment proposed in the herein study, as part of the SURPASS project. SSRbD: Safe, Sustainable, and Recyclable by Design.

Yi-Chin Hsieh*, Jia-Sin Cheng, Jing-Rong Chen, Wen-Che Hou

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15:04-15:18

Development of particle number-based analytical method to characterize nanoplastic bioaccumulation in fish under environmental relevant conditions

Nanoplastics (NPs) are plastic fragments with particle sizes smaller than 1 μm. Their environmental ubiquity has prompted great concerns regarding their potential hazards and accumulation in biota. Detecting and quantifying NPs in complex biological matrices, however, is a challenging task. The ecological and human risk of emerging NPs is currently wide-open, requiring methods to quantify their accumulation in biota. NPs are characterized by their small sizes and mass concentrations alone may not be sufficient or even misrepresents their environmental importance. The research is the first study to develop a particle number concentration-based method to detect NPs in fish. Fish represents a major source of human diets and is also a recognized model to evaluate the adverse ecological impacts resulting from emerging contaminants as NPs. In this study, a method to label NPs with gold nanoparticles (Au NPs) is first presented that accomplishes NP counting by using single-particle inductively coupled plasma mass spectrometry (spICP-MS). Various fish tissue digestion methods to extract NPs from fish tissue were explored to evaluate of their compatibility with subsequent AuNP labeling of extracted NPs and spICP-MS analysis. Preliminary results in aqueous solution indicate a recovery efficiency of 74.4 to 113.8% over 1.0 × 108 to 4.6 × 1012 #/L of 70 nm polystyrene nanoplastics (PS NPs) added, covering the likely environmental concentrations of 1.2 × 108 to 1.7 × 1011 #/L in drinking waters and surface waters. Various NPs (105 nm polymethyl methacrylate nanoplastic (PMMA NP), 70 nm and 500 nm PS NPs spiked into fish tissue over 2.6 × 1010 to 7.3 × 1014 #/kg-fish show good recovery efficiencies of 92.5 to 99.1%, manifesting the promise for the analysis of NPs with different particle size and materials in fish. In this study, the bioaccumulation of NPs in fish organs and tissue is investigated under environmental relevant conditions such as low exposure concentrations of NPs. The fish biochemical responses are also examined concurrently.

Radhika Sharma*, Arun Kumar

*Indian Institute of Technology Delhi, Department of Civil Engineering, India

15:18-15:32

Accumulation of zinc in spinach roots due to irrigation with zinc oxide nanoparticles

With the rise in nanotechnology, variety of daily usage products now contain metal oxide nanoparticles. They are increasingly being utilised in cosmetics, sunscreen, paints, electronic industry, packaging materials, water treatment, etc. Organisation of Economic Cooperation and Development (OECD) has listed representative nanomaterials (such as zinc oxide, CNTs, fullerene etc.) to generate data for toxicology. There is rising evidence presented by researchers detecting the presence of nanomaterials in environmental matrices, such as river water, wastewater effluent, raw sewage, leachate etc. This presents opportunity for the exposure of nanoparticles (NPs) to living components like plants. Earlier research has focused upon the ill effects of NPs irrigation on plants at unrealistically high concentrations. As plant roots present the first line of defence against entry of nanoparticles, it is essential to observe how they affect plant roots at environmentally relevant concentrations. To counter this, in the current work, zinc oxide nanoparticles at 10 ppm concentration were used to irrigate spinach (Spinacia oleracea) plants throughout their growth period. For comparison, an ionic control group (having same molar concentration as zinc content in 10 ppm ZnO NPs) of plants were irrigated with zinc ion as well for the same duration. The plants were harvested upon maturity, and the root biomass was observed. The plant roots were dried and investigated for metal content using ICP-MS. The results revealed no significant difference in the root biomass for the two groups studied. However, a significant difference ($p<0.05$) was found amongst them for zinc accumulated in root. It was observed that the NPs exposed spinach plants accumulated zinc 11.1% higher than their ionic equivalent. The results indicate that although at lower concentrations of nanoparticles, the root biomass may not be affected, however the difference in the metal accumulation can persist. Further investigation is required to ascertain how the uptake of metal oxide nanoparticles by root cells differ in comparison to ions, and if the size of the nanoparticles may have facilitated its entry in the roots.

Mona Connolly*, Elena García Sánchez, Gerardo Pulido Reyes, José María Navas

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15:32-15:46

Use of a fish model and the oxidative stress paradigm in an integrated testing strategy for NM hazard assessment; a proof of principle study

Fish represent important model organisms for environmental hazard assessment and fish cell lines can be used as valuable tools for assessing early effects at the cellular level. These cell lines afford the development of new approach methodologies (NAMs) avoiding the use of animals. Such cellular NAMs can be integrated into tiered in vitro/in vivo testing strategies for hazard assessment and thus the fish model serves as a complete model to investigate in vitro-in vivo translation and the predictive capabilities of cell lines. Such a tiered integrated approach was developed in this study using the epithelial cell lines RTgill-W1 and RTL-W1, from the gill and liver of rainbow trout (Oncorhynchus mykiss), respectively, in tier 1 testing. The use of both cell lines was considered relevant as the gill epithelia is a primary uptake site, while following entry into systemic circulation the liver is a target organ for nanomaterials (NMs). Specific endpoints for analysis were selected according to the key events (KE) of the most relevant adverse outcome pathways (AOPs) for NMs (AOP-Wiki), allowing the development of new NAMs. Oxidative stress was identified as a KE or related KE in many, and it is an accepted paradigm for NM mediated toxicity. Thus, the tier 1 testing strategy focused on hazard screening based on the ability to predict the oxidative stress potential of NMs. Cellular level upstream events such as increases in cellular ROS levels (KE 1940), decrease in ATP (KE 1771), mitochondrial dysfunction (KE 177), oxidation of glutathione (KE 926) and increased lipid peroxidation (KE 1445) are assayed for using a multi-parametric approach. In vivo anchorage and translation of effects seen at the cellular level

to downstream effects on a tissue and whole organism level are investigated in tier 2 following in vivo exposures in juvenile rainbow trout (96h and 14 days). Any increases in lipid peroxidation (KE 1445) and/or oxidation of glutathione (KE 926) in fish tissues are used to affirm a direct in vitro/in vivo correlation for oxidative stress-mediated effects.
A multi-component perovskite case study material composed of La, Co and Ni was used to apply the developed testing strategy. Results highlight both the feasibility and limitations of using in vitro NAMs and AOP directed data for predicting apical toxicity endpoints in vivo.

Acknowledgement: HARMLESS project has received funding from the EU's Horizon 2020 research and innovation programme under grant agreement No. 953183.

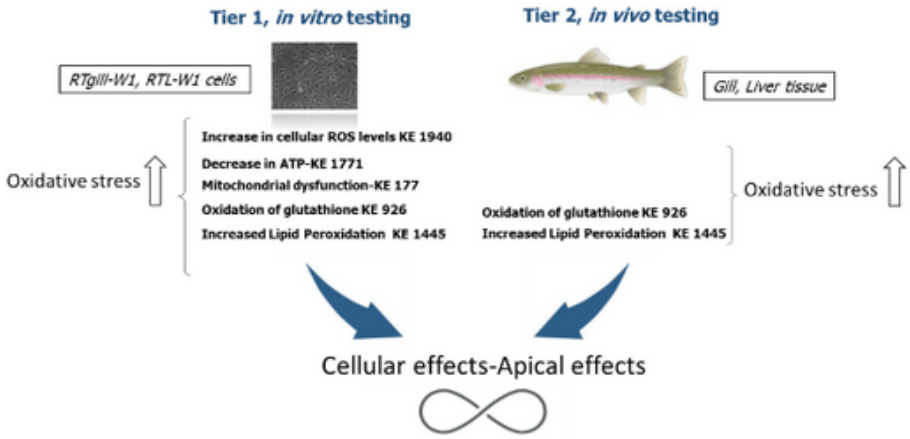

Figure: Fish Oxidative stress-NAM for hazard assessment.

Jasreen Kaur*, Alexander Khort, Inger Odnevall, Suelen Barg, Bengt Fadeel

*Karolinska institutet, IMM, Sweden

15:46-16:00
Ti3C2 MXenes alleviate the adverse effects of the environmental pollutant benzo[a]pyrene

Advanced materials are materials that have been engineered to exhibit novel or enhanced properties that confer superior performance when compared to conventional materials. Here we evaluated the impact of Ti3C2Tx MXenes, a two-dimensional (2D) material, on the adverse effects caused by polycyclic aromatic hydrocarbons (PAHs). To this end, benzo[a]pyrene, denoted here as B[a]P, was selected as a model compound. MXenes, in the presence or absence of natural organic matter (NOM), were subjected to characterization using XPS, XRD, and FTIR. Subsequent studies indicated that MXenes were well tolerated, with no impairment of the survival of zebrafish embryos. Internalization (ingestion) of MXenes was determined by quantifying the Ti content using inductively coupled plasma mass spectrometry (ICP-MS) while Raman confocal mapping was applied for the label-free identification of MXenes in exposed zebrafish. Next, the interaction of MXenes and B[a]P was monitored by using UV-Vis spectroscopy, and the potential impact of MXenes on aryl hydrocarbon receptor (AhR) induction by B[a]P was assessed by evaluating the induction of downstream genes including cyp1a. These studies were complemented by using the transgenic zebrafish reporter strain tg (cyp1a-eGFP) to monitor cyp1a induction. The potential impact of MXenes on genotoxicity was also evaluated. MXenes were shown to ameliorate B[a]P-triggered AhR induction and DNA damage in zebrafish. These results were corroborated by using the human colon-derived cell line HT-29 as a model of the gastro-intestinal tract. Overall, B[a]P was found to adsorb to MXenes thereby mitigating the impact of B[a]P on AhR induction and DNA damage in vitro and in vivo. MXenes are thus non-hazardous and also seem to alleviate the adverse effects caused by the environmental pollutant B[a]P.

Session 5B

14:30-16:00

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Safe-and - Sustainable-by-Design strategies and case studies

Chairs: Wendel Wohlleben & Ana Serrano Lotina

Stefano Manfredini*, Elena Cesa, Paola Ziosi, Erika Baldini, Irene Gugel, Stefania Costa, Anna Luisa Costa, Dario Fornara, Mustafa Culha, Giuseppe Valacchi, Filippo Marchetti, Begona Espina, Arantxa Ballestreros Riaza, Sebastien Artous, Silvia Vertuani

*University of Ferrara, Department of Life Science and Biotechnology, Italy

14:30-14:50

A Safe, Sustainable and Effective by Design (SSEbD) surface modified nano-sunscreen UV filters upcycled from agro-food industry side products

In recent years, there has been a growing awareness of safe and sustainability attributed to cosmetic products, including sunscreens. In particular, the toxicity of titanium dioxide nanoparticles (TiO2 NPs), preferred in terms of spread ability over the microsized form, has assumed increasing relevance and concerns about both environment compatibility and human exposure in relation to the risk of ingestion, skin penetration, or inhalation.

The global nanotechnology sector faces a significant challenge in creating safe and functional engineered nanomaterials (ENMs) and nano-enabled products (NEPs). Our approach followed two recent concepts Safe by Design (SbD) and Safe Sustainable by Design (SSbD) developed in the frame of our Horizon 2020 (SbD4Nano) and Horizon Europe (SUNRISE) projects, with the aim to establish a comprehensive e-infrastructure to facilitate collaboration among supply chains and develop optimized SbD setups through case studies.

In particular, often by surface modification of nanoparticles to improve safety, efficacy is lost, and this from an industrial point of view is a major limitation. In addition, environmental and human sustainability is something that is increasingly perceived as a value. We focused therefore on an upcycling approach to safe, sustainable and effective by design (SSEbD) TiO2 NPs selecting an appropriate coating from side products of food industry.

The project's surface engineering approach aims to reduce (eco)toxicity, exposure, and release of active materials, without losing sight of its properties and effectiveness in formulation.

Indeed, the photo-toxicity of pristine TiO2 NPs was significantly reduced with surface active molecules, avoiding photo-reaction during UV exposure and, totally unattended, also improving UV filtering activity.

By meeting standards, regulations, and exploring alternatives, the project successfully designs and offers a next generation of sunscreen products that fulfill consumer demands while safeguarding human health and the environment.

Supported: Horizon 2020 and Horizon grants No. 953206 and 101137324 respectively (Ambrosialab, SBD4Nano and SUNRISE).

Vesa Hongisto*, Roland Grafström

*Misvik Biology, Toxicology, Finland

14:50-15:04

High-throughput screening generates useful toxicity reference data for Safe- and Sustainable-by-Design production of materials

Aiming to establish confirmatively applicability of HTS technology for SSbD and NAM-testing coupled materials production, we assessed within the EU-funded SABYDOMA project an extensive set of material and particle production parameters, including for silver, copper, and titanium nanoparticles. Different sizes, compositions, synthesis temperatures/times and reagent ratios were among many aspects evaluated under blinded standard operating procedures. The human bronchial epithelial line BEAS-2B (with or without 10% foetal bovine serum) was applied as test model under "Misvik's Tox5 scoring concept", i.e., five endpoints covering changes in cell numbers, apoptosis, mitochondrial functional integrity (ATP content), DNA damage and oxidative stress was analysed at 6, 24 and 72 h exposure times over three orders of magnitude concentration ranges. Overall, 73 material variants were assessed within four case studies spanning technology readiness levels of 4-6 (TRL4-TRL6), where totally 116 000 data points formed the basis for deriving "hazard estimates" for each material and variants. As expected, the selected materials had variable toxic potency. More interestingly, variably few or none of a range of intermediate assessed production variants differed significantly in toxicity from the starting material. Absence of marked serum influences, such as quenching of the hazard estimates, indicated further that the applied synthesis protocols agreed with stable and safe production routines. Our study confirms that HTS 384-well protocols with the human epithelial BEAS-2B cell line serve effectively for dose-response toxicity screenings producing overall levels of 105 data points; such data can be analyzed and FAIRified for broader utility within 2-3 months. Biological and technical variables can be captured and integrated into standardized safety testing and production protocols, including typically cell density, exposure time, culture conditions (e.g., with or w/o serum), different dispersion/solvent protocols, storage stability, batch variations, etc. Dose-dependency of functionality and toxicity over broad concentration ranges is captured. Ranking and even grouping based on toxicity profiles opens for deepened mechanistic follow up and NAM-driven regulatory application. Primarily, we demonstrate in this study that NAM-based HTS assessment can be a useful component of SSbD where the evaluation of safety (e.g., expected stable toxic/hazard potency with low variability under production protocols), is a key element (SABYDOMA received funding from the European Union´s HORIZON 2020 Research & Innovation Programme under grant agreement no. 862296).

Andrea Brunelli*, Ana Maria Serrano Lotina, Miguel Banares, Victor Alcolea-Rodriguez, Magda Blosi, Anna Costa, Serena Ortelli, Willie Peijnenburg, Carlos Fito, Ernesto Gonzalez Fernandez, Jorge Salvador Hermosilla, Lya Soeteman-Hernandez, Irantzu Garmendia Aguirre, Hubert Rauscher, Fiona Murphy, Vicki Stone, José Balbuena, José Cormano, Lisa Pizzol, Danail Hristozov, Antonio Marcomini, Elena Badetti

*Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Italy

15:04-15:18

Safety assessment of the SiO2@ZnO multi-component nanomaterial embedded in a cement mortar for photocatalytic NOx degradation

The safety assessment of the SiO2@ZnO multi-component nanomaterial (MCNM) embedded into a cement mortar for NOx degradation was performed in line with the safety dimension steps of the Safe-and-Sustainable-by-Design (SSbD) framework proposed by the European Commission. First, in line with step 1 of the SSbD framework, the hazard assessment of the MCNM was carried out by i) investigating the physicochemical identity of both the individual NMs and the MCNM, ii) assessing the hazard of the precursors for the MCNM synthesis and iii) evaluating the cytotoxicity of both the individual components and the MCNM through in vitro testing. Physico-chemical characterization showed that the mesoporous SiO2 core present an incomplete coating of a thin layer of ZnO, did not impacting on the overall SiO2 size, but reduced the surface area covering the surface pores. As far as the hazard assessment of MCNM precursors is concerned, while SiO2 NM powder was considered a non-hazardous substance, ZnAc2·2H2O showed both health and environmental hazards, (i.e., acute toxicity, serious eye damage and long-term (chronic) aquatic hazard). Moreover, the in vitro hazard screening using THP-1 cells to inform the early innovation stages suggested the hazard of the MCNM was comparable to ZnO NM. Afterwards, according to step 2 of the SSbD framework, human health and safety aspects of the MCNM-based material were also investigated. Starting from the very early stage of the design phase, a questionnaire-based standard industrial hygiene survey showed that a

release of nanoscale particles into the workplace air could not be reasonably excluded during production, handling, processing or maintenance and cleaning phases. Therefore, an occupational exposure assessment was carried out, evaluating three different exposure scenarios, i.e., material synthesis, calcination and MCNM-based mortar formulation, by a monitoring campaign. The results highlighted that none of the exposure scenarios considered generated particles concentration higher than recommended limit values (Recommended Benchmark Level – RBL, or nano reference values – NRV) proposed by international bodies. Lastly, following the final application/use phase of step 3 of the framework, the potential leaching of inorganic elements constituting the MCNM-based mortars was investigated. The results showed no or negligible release of Zn and Si, suggesting that their potential exposure is unlikely once the MCNM is embedded in the mortars. In conclusion, the methodological approach presented allowed to follow the steps of the safety dimension proposed by the EU SSbD framework for MCNMs, providing useful information on their risk profile for future assessment of their sustainability.

Lisa Pizzol*, Arianna Livieri, Beatrice Salieri, Lucian Farcal, Lya G. Soeteman Hernández, Hubert Rauscher, Alex Zabeo, Magda Blosi, Anna Costa, Willie Peijnenburg, Stella Stoycheva, Neil Hunt, Maria José López-Tendero, Castor Salgado, Julian J. Reinosa, Jose F. Fernández, Danail Hristozov

*GreenDecision Srl, Italy

15:18-15:32

Screening level approach to support companies in making safe and sustainable by design decisions at the early stages of innovation

The European Chemicals Strategy for Sustainability and the Zero Pollution Action Plan have called for adoption of the Safe and Sustainable by Design (SSbD) paradigm for chemicals and materials. To facilitate this, the H2020 SUNSHINE project has developed a tiered SSbD assessment approach, which has been tested in real industrial case studies involving advanced multi-component nanomaterials (MCNMs). This approach allows the assessment of safety and sustainability aspects from a lifecycle perspective and at each stage of product development. This is done by application of qualitative (Tier 1), and quantitative (Tier 2) methods. The focus of this study is on Tier 1, a self-assessment methodology which enables the evaluation of safety, functionality, and sustainability in the early stages of innovation. This approach is designed to be easily implementable by industries, especially SMEs, addressing the common problem of insufficient resources and expertise for in-depth safety and sustainability studies. The approach was thoroughly tested in two industrial case studies, the first consists of a nanocomposite composed of silica carbide and titanium dioxide (SiC@TiO2) coatig which provides non-stick properties on its applications in bread baking trays. This innovative material is a substitute candidate for Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)-based non-stick coatings. The second is composed of nano drops of essential oil anchored to the surface of nano-clays and encapsulated in a polymeric film to be used as a substitute for Low Density Polyethylene (LDPE) and it's produced to develop food packaging to keep the packaged food free of insect pests. The results demonstrate that the SUNSHINE tiered approach was successful in informing the development of innovative materials, which demonstrate improved safety, functionality, and sustainability compared to their conventional counterparts. Indeed, the approach was instrumental in supporting key 'go to development', product optimisation and 'go to market' business decisions by the industrial companies, which has proven its practical value.

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15:32-15:46

Safe and Sustainable by Design Strategies for the H2020 SUNSHINE case studies

The European Green Deal policy ambitions, the Chemicals Strategy for Sustainability, and the Zero Pollution Action Plan identify the transition to a Safe and Sustainable by Design (SSbD) approach to chemicals and materials. The H2020 SUNSHINE project has developed an approach to operationalize SSbD, specifically addressing advanced multi-component nanomaterials (MCNMs). The SUNSHINE SSbD approach enables the assessment of safety and sustainability aspects at each stage of product development from a lifecycle perspective. This is achieved via a tiered approach that uses qualitative (Tier 1), and quantitative (Tier 2) assessment methods. Tier 1 consists of a (self-assessment) questionnaire to evaluate the safety, functionality, and sustainability in the early R&D stages of the lifecycle of chemicals and materials. This approach has been developed to be implementable by industries as often there is a lack of time and/or expertise to engage in resource-intensive safety and sustainability evaluations. Tier 1 uses a scoring system to calculate indices for the impacts and plot those on a chart that visualizes safety and sustainability-related 'hotspots of concern' along the lifecycles of the materials/products which can be further assessed in Tier 2. Tier 2 consists of LCA, LCC, and S-LCA methodologies applied to the MCNMs and the product(s) incorporating them to compare the results to a benchmark. The application of the tiered approach supports the development of SSbD strategies for MCNMs, which consist of action plans to identify, mitigate, and ultimately resolve the hotspots identified during the SSbD assessment. SSbD strategies have been developed for 4 SUNSHINE industrial case studies: 1) a novel PFAS-free anti-sticking coating used in the bakery industry (i.e., coating of baking trays and pans), produced by the company Laurentia, 2) a nano-drops of essential oil anchored at the surface of nano-clays and encapsulated in a polymeric film produced by the company Encapsulae, 3) a nanocomposite of graphene oxide functionalized with chitosan which provides flame retardant properties produced by the company Avanzare, 4) an additive for construction materials with photocatalytic decontamination properties (NOx gas removal) produced by CIAC.
The results prove the added value of the SUNSHINE SSbD approach in guiding early stages of innovation, along with the opportunity it provides in enabling companies to assess their sustainability performance in an easy and affordable manner, which can make them more competitive on the market while leading the design of more environmentally friendly nanotechnologies of high social and economic benefit.

Arianna Livieri*, Lisa Pizzol, Agnes Oomen, Kathrin Schwirn, Elmert Schwirn, Hubert Rauscher, Mar Gonzalez, Kimiko Yamamoto, Danail Hristozov

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15:46-16:00

Advancing the development of Safe-and-Sustainably-by-Design toolboxes for advanced materials: Similarities between the SUNSHINE SSbD approach and Early4AdMa

The Chemicals Strategy for Sustainability (CSS) and Zero Pollution Action Plan have called for a transition towards a Safe and Sustainable by Design (SSbD) approach for chemicals and emerging materials. As a result, the European Commission has proposed a European assessment framework for SSbD chemicals and materials. To support the implementation of this framework, the H2020 SUNSHINE project developed a tiered SSbD assessment approach. Concurrently, the OECD adopted Early4AdMa, an early awareness and action system for advanced materials, which is an adaptation of a system developed by the RIVM (NL), BfR (DE), BAuA (DE) and UBA (DE).. The SUNSHINE SSbD approach aims to assist industries in developing SSbD strategies for specific advanced materials and products. The Early4AdMa system offers a structured methodology for identifying potential safety, sustainability, and regulatory issues associated with emerging advanced materials. The two approaches are complementary, and to assist users in selecting the system that is most suited to their needs, a comparative analysis of the two methodologies was carried out in the frame of the OECD Working Party on Manufactured Nanomaterials Steering Group on Advanced Materials. The comparative analysis assessed common aspect categories such as safety, environmental impact, economic and social sustainability, and functionality. Early4AdMa included an additional category focusing on applicability to regulatory frameworks. Further comparison of the output of the two approaches was illustrated through the assessment of a case study, a nanocomposite of graphene oxide (GO) functionalized with chitosan to be used as a substitute for classic flame retardants such as melamine cyanuric acid. The results demonstrated that the two approaches complement each other well. Early4AdMa is effective in identifying safety and sustainability issues not covered by current regulations, making it suitable for regulators. In contrast, the SUNSHINE approach is designed to pinpoint safety and sustainability concerns throughout the lifecycle of specific materials and products to aid in SSbD decision-making by industries, including SMEs. Ultimately, both approaches can work synergistically to facilitate the implementation of the EC SSbD framework for different purposes and by different stakeholders. The SUNSHINE approach is tailored for industries (including SMEs) and consultants, while Early4AdMa is well fitted to the needs of policy-makers, the standardization community, and regulators.

14:30-16:00 **Session 5C**

DATA ANALYSIS WITH BIG DATA MANAGEMENT AND MODELLING SOLUTIONS

Data-driven & multi-scale modelling

Chairs: Phil Demokritou & Luca Monticelli

Tomasz Puzyn

University of Gdansk, QSAR Lab, Poland

14:30-14:45
Regulatory readiness of in silico NAMs for nanoforms

According to the reports for EUON/ECHA from 2023 [1,2] and the NAMs Network database [3], more than 200 various in silico NAMs have been proposed. However, while the focus of scientific community is rather on profoundly exploring phenomena, which certainly takes time, regulators urgently need to have tools that are practical in use and regulatory relevant. Do we already have such in silico tools?
In addition, both groups (scientists and regulators) declare high concern about quality, but the term 'quality' may be understood differently. In the context of in silico NAMs, one can ask about defining 'minimum quality criteria' to evaluate the applicability of the computational tools and models in the regulatory context. For instance, to what extent is the OECD QSAR Assessment Framework published last year sufficient to evaluate nano-QSARs? Is there anything to be added, considering the specificity of nanostructure [5,6]?
Finally, New Generation Risk Assessment methods require various approaches (e.g., in vitro, ex vivo, in silico) to work together since no one believes that a simple replacement of animal testing by just a single in vitro or in silico model is possible. Thus, what are the needs, and what is the most appropriate role for in silico NAMs?

The lecture aims to provoke discussion on the readiness of the available and recently developed computational tools to be used in the regulatory context for risk assessment of substance nanoforms. It will be illustrated with three case studies: (i) a new classification model for predicting water solubility of nanoforms according to the criteria included in recently published IATA to support grouping and read-across of nanomaterials in aquatic systems [7]; (ii) a set of models for predicting genotoxicity (comet and micronucleus tests) in the context of EFSA guidance [8] implemented in newly launched nQTb software [9] and (iii) new models for predicting points of departure in AOP173 [10] and allowing to group multiwalled carbon nanotubes according to structure-related potential of inducing the toxicity.

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*ProtoQSAR, Spain

14:45-14:57

ProtoNANO: assessing the toxicity of inorganic nanomaterials using nano-QSAR models

Development of new materials focuses in enhancing a few properties of interest, but it is essential to assess the risks for the safety of humans and the environment. Computational methods are very convenient to reduce the economical, ecological and ethical impact of this assessment. Among them, the most outstanding are the QSAR models (from Quantitative Structure-Activity Relationships), which are widely used and are accepted for regulatory purposes for discrete organic molecules. In recent years, QSAR models on nanomaterials (NMs), from herein labelled as nano-QSARs, are being developed and improved [1].

Describing substances by their chemical structure, often represented by the SMILES code, is insufficient for NMs. In this case, structural aspects such as the size and the complex composition affect the physicochemical and biological behaviour. We recently reviewed the range of numerical descriptors used in the literature for nanomaterials [2], and proposed a classification for descriptors including direct descriptors of the structure (composition of the core/surface and geometry of the particles) and indirect experimental parameters (related to the NM properties, its synthesis or the endpoint measurement). However, the use of experimental data creates another challenge for the nano-QSAR models, the lack of consistence among the methods and parameters used to characterize and evaluate NM in the literature that hinder the creation of modelling databases. ProtoNANO (a module of the in silico prediction server ProtoPRED®) [3], facilitates a series of nano-QSAR models for different inorganic NMs (such as noble metals, metallic oxides and quantum dots). The models concern toxicity to humans, ecotoxicity and physicochemical properties with a role in risk assessment and NM characterization. In this presentation, we will introduce the challenges found developing nano-QSAR projects and discuss some of the models in ProtoNANO as example. The examples will serve to discuss the effect of different features, including calculated descriptors and experimental measurements.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 896848 (NanoQSAR).

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14:57-15:09
A data-driven machine learning approach for predicting the hazard of advanced materials based on the integration of in silico, in chemico, and in vitro data

Prediction of nanosafety and nanomaterial properties is essential in modern science. Different research and academic institutions and regulatory authorities are striving to develop and implement new approach methodologies (NAMs) for better risk assessment and property estimation of industrially-relevant advanced materials (AdMa). Experimental testing is a troublesome and time-consuming process; thus, scientists and regulatory agencies rely predominantly on in silico techniques (e.g., quantitative read-across, quantitative structure-activity relationship, structural alerts, etc.) to fill toxicity/property data and knowledge gaps. However, these computer-aided methods encounter several challenges, the most significant being limited dataset sizes. More efficient and inclusive algorithms that can process sparse datasets are needed.
In the present study, emphasis has been placed on the methodology of a new approach and the discussion of three recently developed prediction-oriented quantitative models for predicting the hazard associated with advanced materials (AdMa). These models integrate in silico predictions with in chemico and in vitro data. Due to limited dataset sizes, modeling was conducted using the locally weighted kernel linear regression (KwLPR) approach. The theoretical framework supporting the proposed approach for filling data gaps will be elucidated. Subsequently, we will provide examples of three models for predicting the hazard of (i) oxide-perovskites, (ii) core-shell quantum dots, and (iii) various advanced materials. Finally, we will discuss further perspectives for developing computational models that integrate data from in silico, in chemico, and in vitro tests.
Acknowledgements:
Project HARMLESS received funding from the European Union's HORIZON 2020 Research & Innovation Programme under Grant Agreement no. 953183.

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15:09-15:21
Identifying factors controlling cellular uptake of gold nanoparticles by machine learning

There is strong interest to improve the therapeutic potential of gold nanoparticles (GNPs) while ensuring their safe development. The utility of GNPs in medicine requires a molecular-level understanding of how GNPs interact with biological systems. Despite considerable research efforts devoted to monitoring the internalisation of GNPs, there is still insufficient understanding of the factors responsible for the variability in GNP uptake in different cell types. Data-driven models are useful for identifying the sources of this variability. Here, we trained multiple machine learning models on 2077 data points for 193 individual nanoparticles from 59 independent studies to predict cellular uptake level of GNPs and compared different algorithms for their efficacies of prediction. The five ensemble learners (Xgboost, random forest, bootstrap aggregation, gradient boosting, light gradient boosting machine) made the best predictions of GNP uptake, accounting for 80–90% of the variance in the test data. The models identified particle size, zeta potential, GNP concentration and exposure duration as the most important drivers of cellular uptake. We expect this proof-of-concept study

will foster the more effective use of accumulated cellular uptake data for GNPs and minimise any methodological bias in individual studies that may lead to under- or over-estimation of cellular internalisation rates (Bilgi, Winkler et al. 2024).

Reference:
Bilgi, E., et al. (2024). "Identifying factors controlling cellular uptake of gold nanoparticles by machine learning." Journal of Drug Targeting 32(1): 66-73.

Michal Kalapus*, Tomasz Puzyn

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15:21-15:33
Predicting Dissolution Rates of Engineered Nanomaterials: Insights from Machine Learning and Environmental Factors

While solubility is an important factor in ENM toxicity, dissolution rate provides a more nuanced understanding of how quickly ENMs break down and release potentially harmful components into the environment. Specifically, understanding whether ENMs will undergo rapid dissolution with a swift release of ions or exhibit minimal dissolution and will undergo other potential transformations.
We address the question: how can we predict the dissolution rate of ENMs in a system where both the ENMs and the surrounding medium are subject to change?
We compiled experimental dissolution rate data for 114 metal and metal oxide nanoparticles from the literature. Ten different classifiers were employed to categorize ENMs into three distinct groups: quick, partial, and very slow dissolution. The models were performed based on OECD guidelines. Most classifiers achieved outstanding performance, with a mean accuracy, precision, recall, f1 score, kappa, and Matthew Correlation Coefficient of 0.92, 0.96, 0.92, 0.92, 0.85, and 0.87, respectively. Evaluation metrics for very slow dissolution were 1.0, 0.67, and 0.80 for precision, recall, and f1 score, respectively. For partial dissolution, they were 0.94, 0.94, and 0.93, and for quick dissolution, they were 0.96, 1.00, and 0.98.
This research demonstrates the feasibility of accurately predicting dissolution rate classes for ENMs using machine learning techniques. These models not only enable us to classify the dissolution rate of ENMs into one of three groups but also provide valuable insights into how changes in the environmental conditions or the ENM itself might affect its dissolution behavior. Our findings further emphasize the influence of both intrinsic (solvation enthalpy, primary size, bond dissociation enthalpy) and extrinsic factors (pH, total concentration, ionic strength) on ENM dissolution rates. These models have the potential to contribute significantly to risk assessment and regulatory decision-making related to ENMs. Future research should explore the impact of additional environmental factors such as dissolved oxygen and specific ion concentrations.
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15:33-15:45
Advancing Safe and Sustainable materials design through Multiscale Modelling and Data Integration

The pursuit of Safe and Sustainable by Design (SSbD) stands as a cornerstone in contemporary materials science and engineering. In this context, the synergy between multiscale modelling and data integration techniques becomes crucial for realizing the principles of SSbD. By leveraging advanced computational methodologies, researchers are empowered to explore the intricate interplay between materials structure, properties, and performance across multiple length and time scales. At the same time, the integration of

comprehensive datasets spanning experimental, theoretical, and computational domains offers unprecedented insights into the lifecycle assessment and environmental impact of materials and products.
In this context, our approach is based on multiscale modelling, applied for the advancement of materials design and characterization, in order to elucidate the safety and sustainability profiles of materials. In particular, atomistic, mesoscale, and continuum modelling are used to gain insights into the fundamental physics governing material properties, thereby enabling the tuning of materials and processes to achieve enhanced performance and functionality. In order to exploit the potential of the multiscale modelling, HPC infrastructures play a critical role, allowing to efficiently perform computationally intensive simulations and explore complex and large-scale materials systems with unprecedented detail and accuracy. The automation of computational tools and the development of automatic workflows complements the modelling process, combining experimental data, computational results, and predictive models to create a unified framework for materials analysis. Within this framework, integrating artificial intelligence (AI) methods for analysis and support of automatic workflows enhances the efficiency and effectiveness of multiscale modelling efforts, enabling intelligent decision-making and optimization of design strategies. By employing state-of-the-art computational techniques within the SSbD principles, we aim to accelerate the discovery and development of next-generation materials that not only exhibit superior performance but also meet the stringent criteria for safety, sustainability, and environmental impact. The BIO-SUSHY project is funded by the European Union under the Grant Agreement Number 101091464. University Leeds is funded by the UKRI Horizon Europe Guarantee Fund: Grant Number 10056199. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

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15:45-15:57

In silico Characterization of Neat and Doped Zinc Oxide Nanoparticles: Implications for Stability, Electronic Properties, and Biological Interactions

This research delves into the intricate behavior of Zinc Oxide (ZnO) nanoparticles, both in their pure form and when doped with Manganese, employing multiscale modeling simulations. First principles simulations were utilized to unravel the properties of these nanoparticles, focusing on size, shape, and the underlying mechanism of nanoparticle growth through aggregation from small nanoparticle seeds. The adsorption and Manganese doping processes were found to efficiently modify the electronic properties of the nanoparticles, unveiling novel avenues for tailoring their functionalities. The investigation extended to the behavior of nanoparticles in aqueous solutions, revealing efficient solvation and minimal solubilization. This observation indicates the formation of stable nanoparticles in aqueous solutions, setting the stage for potential applications in various domains. In-silico studies explored the interaction between these nanoparticles and model cell membranes, as well as human proteins. The results indicated minor disruptions to these biological structures, signifying the stability of nanoparticles in aqueous environments in contact with cells. Importantly, the low toxicological effects observed at reasonable concentrations following human and environmental exposure suggest the potential for safe utilization of products containing these nanomaterials. This comprehensive study offers insights into the intricate interplay between Zinc Oxide nanoparticles, their electronic properties, and their interactions with biological structures. The outcomes of this research not only contribute to the fundamental understanding of nanoparticle behavior but also pave the way for the development of safer and more efficient nanomaterials for various applications.

16:30-18:00 Session 6A

HAZARD ASSESSMENT

Novel mechanistic insights in NMs and advanced materials' toxicity & AOPs

Chairs: Phil Demokritou & Angela Saccardo

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16:30-16:45

Initiation of the pulmonary innate immune response upon NP-inhalation -New insights by intravital microscopy

To visualize and elucidate in real-time the initiation of the cellular pulmonary innate immune response elicited by inhaled NPs, events that could not be observed so far, we apply state of the art intravital microscopy on the alveolar region of the murine lung, combined with ventilator-assisted inhalation of nebulized NP aerosols.
Inhaled fluorescent carboxyl-quantum-dot-NPs (cQD-NPs, deposited dose 16 cm2/g) accumulated within seconds as distinct fluorescent spots at the alveolar walls. At the administered dose, one third of alveoli in the recorded field of views exhibited deposited cQDs. Already at 30min, cQD-NPs, as well as a bioequivalent dose of carbon black NPs (CNPs) elicited an increase in microvascular neutrophil numbers, which subsequently transmigrated into the alveolar space and ingested cQDs. Neutrophils were preferentially recruited in pulmonary microvessels near the sites of cQD-NP deposition.
AMs, visualized by PKH-dye labeling, actively accumulated cQD-NPs over time, starting few minutes after inhalation. Significantly more neutrophils were attracted in proximity to cQD-NP-laden AMs, as compared to NP-free AMs or control areas with epithelial associated cQD-NPs. In addition, AM patrolling velocity was increased after cQD-NP and CNP inhalation and AMs accumulated over time in alveoli with deposited cQD-NPs.
Inhibition of Fcγ-receptor mediated AM NP phagocytosis by prior airway application of anti-CD64 mAbs, as well as utilizing a cellular degranulation inhibitor, or neutralizing of TNFα by anti-TNFα mAbs in the airspace, likewise abolished the early immune response induced by cQD-NPs and CNPs.
Taken together, our data demonstrates the crucial role of AM patrolling and NP uptake in the alvoli and links NP induced AM degranulation and cytokine release to the rapid and site-specific recruitment of neutrophils, suggesting this processes to be key events in mounting the immune response upon NP inhalation in the lung.

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16:45-16:57

Photochemically aging of residential wood combustion emissions induces inflammatory responses in mice and air-liquid interface exposed RAW264.7 murine macrophages

Residential wood combustion (RWC) substantially contributes to the burden of disease attributable to air pollutants, which has been associated with adverse human health effects. However, the impact of atmospheric aging on toxicological outcomes remains poorly understood. This is one of the first studies aiming to compare the toxicological profiles of nanoparticles containing fresh and aged RWC aerosol emissions. Emission source comprised combustion of spruce logs in a modern chimney stove, with the Photochemical Emission Aging flow tube Reactor (PEAR) used for photochemical aging of the RWC emissions. C57BL/6J mice were exposed to fresh and photochemically aged (equivalent to 2±0.5 days in the atmosphere) RWC emission aerosols in an inhalation chamber for 4 hours at three consecutive days. Simultaneous 4h exposures were performed for RAW264.7 murine macrophages at the air-liquid interface (ALI) in an automated exposure system. Physical aerosol properties revealed particle sizes of 72 nm for fresh and 76 nm for aged RWC emissions. Aging slightly increased particle mass, while causing distinct changes in the particle chemical composition. Detailed offline chemical analyses identified specific wood combustion species such as polycyclic aromatic hydrocarbons (PAHs) and their oxygenated analogues (o-PAHs and OH-PAHs), resin acids and nitrophenols. The ageing of the RWC emissions led to a drastic decrease in the total concentration of PAHs and resins, but to a net increase in OH-PAH and nitrophenols. Considerable toxicity-related outcomes were detected both in vivo and in vitro model systems after the exposure to either fresh or aged RWC emissions (e.g., DNA breaks and cytotoxicity). Greater adverse effects were measured for aged RWC emissions compared to fresh RWC emissions. No signs of inflammation were observed in mice after the exposure to fresh RWC emissions. Remarkably, the aging of RWC emissions led to a different response. Influx of neutrophils and release of pro-inflammatory cytokines increased in both the BAL and serum of mice exposed to aged RWC emissions. Moreover, activation of inflammatory related canonical pathways was revealed by transcriptional changes in BAL and RAW264.7 murine macrophages. In addition, the first evidence of macrophage polarization was observed in in vitro and in vivo, especially after the exposure to aged RWC emissions. This study highlights the importance of atmospheric aging and chemical speciation on driving specific adverse health-related effects and the use of realistic in vitro exposure model systems to better understand toxicological outcomes.

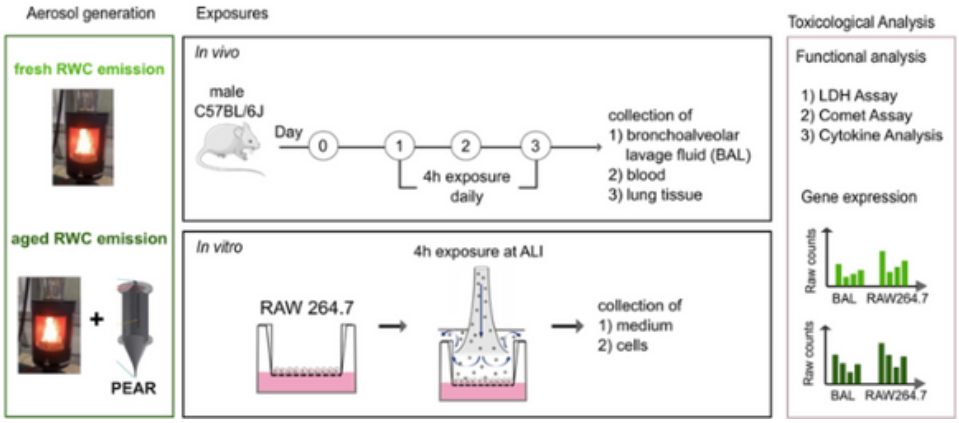

Figure: A schematic overview of the experimental set-up

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16:57-17:09

Resolving conflicting small validation databases and numerous mechanisms-of-action by refocusing in vitro monitoring onto functional level

Residential wood combustion (RWC) substantially contributes to the burden of disease attributable to air pollutants, which has been associated with adverse human health effects. However, the impact of atmospheric aging on toxicological outcomes remains poorly understood. This is one of the first studies aiming to compare the toxicological profiles of nanoparticles containing fresh and aged RWC aerosol emissions. Emission source comprised combustion of spruce logs in a modern chimney stove, with the Photochemical Emission Aging flow tube Reactor (PEAR) used for photochemical aging of the RWC emissions.

C57BL/6J mice were exposed to fresh and photochemically aged (equivalent to 2±0.5 days in the atmosphere) RWC emission aerosols in an inhalation chamber for 4 hours at three consecutive days. Simultaneous 4h exposures were performed for RAW264.7 murine macrophages at the air-liquid interface (ALI) in an automated exposure system. Physical aerosol properties revealed particle sizes of 72 nm for fresh and 76 nm for aged RWC emissions. Aging slightly increased particle mass, while causing distinct changes in the particle chemical composition. Detailed offline chemical analyses identified specific wood combustion species such as polycyclic aromatic hydrocarbons (PAHs) and their oxygenated analogues (o-PAHs and OH-PAHs), resin acids and nitrophenols. The ageing of the RWC emissions led to a drastic decrease in the total concentration of PAHs and resins, but to a net increase in OH-PAH and nitrophenols. Considerable toxicity-related outcomes were detected both in vivo and in vitro model systems after the exposure to either fresh or aged RWC emissions (e.g., DNA breaks and cytotoxicity). Greater adverse effects were measured for aged RWC emissions compared to fresh RWC emissions. No signs of inflammation were observed in mice after the exposure to fresh RWC emissions. Remarkably, the aging of RWC emissions led to a different response. Influx of neutrophils and release of pro-inflammatory cytokines increased in both the BAL and serum of mice exposed to aged RWC emissions. Moreover, activation of inflammatory related canonical pathways was revealed by transcriptional changes in BAL and RAW264.7 murine macrophages. In addition, the first evidence of macrophage polarization was observed in in vitro and in vivo, especially after the exposure to aged RWC emissions. This study highlights the importance of atmospheric aging and chemical speciation on driving specific adverse health-related effects and the use of realistic in vitro exposure model systems to better understand toxicological outcomes.

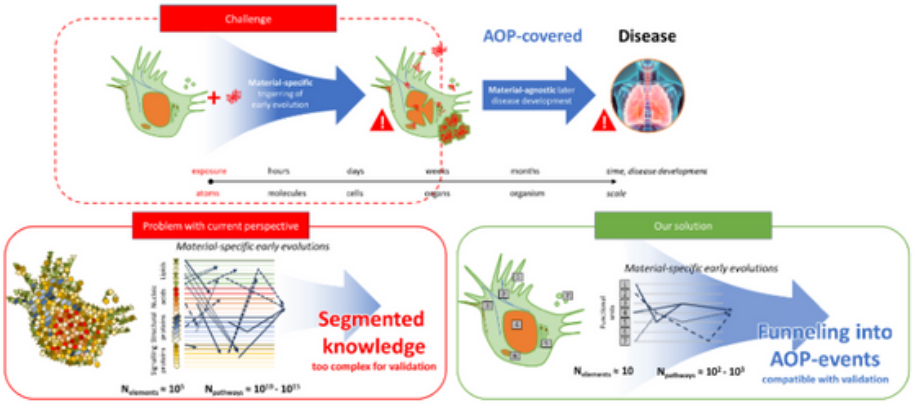

Figure: Resolving conflicting small validation databases and numerous mechanisms-of-action by refocusing in vitro monitoring onto functional level

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17:09-17:21

Basic research on the health risks of oral intake of titanium dioxide nanoparticles

Titanium dioxide containing nano-fractions is widely used in the field of food and medicine due to their excellent whitening and brightening capability. Recently, European Commission has banned titanium dioxide as a food additive, raising public concern about its health risk, especially the nanoparticles contained therein. It is crucial to evaluate the health risks of oral intake of titanium dioxide nanoparticles (TiO2 NPs). Our research group estimated the dietary exposure level of TiO2 NPs among Chinese population by measuring the content of TiO2 NPs in food sold in China, and found that children are the highest exposure population due to their love of sweets. Through animal experiments of oral exposure, it was found that young rats are more susceptible than adult rats, mainly manifested as liver and heart damage, which worsens with the increase of exposure dose and exposure time. The combination of TiO2 NPs and glucose showed an interaction, but TiO2 NPs had no significant effect on glucose absorption. TiO2 NPs can alter the intestinal epithelial structure and thereby affect intestinal function, manifested in changes in amino acid absorption and metabolism levels, and increase intestinal barrier function when LPS coexists. TiO2 NPs play an important role in the course of acute colitis by affecting the gut microbiota and activating the

ROS-TXNIP-NLRP3 inflammatory pathway. It can also be observed that TiO2 NPs is absorbed into the blood through the intestine, and nanoparticles are found in the liver, but the absorption and transportation amount is relatively low. TiO2 NPs can also induce DNA double strand breaks in rat bone marrow cells and HPRT gene mutations in V79 cells. To sum up, oral intake of TiO2 NPs carries certain health risks. Foods supplemented with TiO2 NPs should be carefully consumed by people with high protein requirements, such as children, the elderly, and patients with high metabolic disease or intestinal inflammation.

Keywords: titanium dioxide, nanoparticles, oral toxicity, intestinal injury, nanosafety

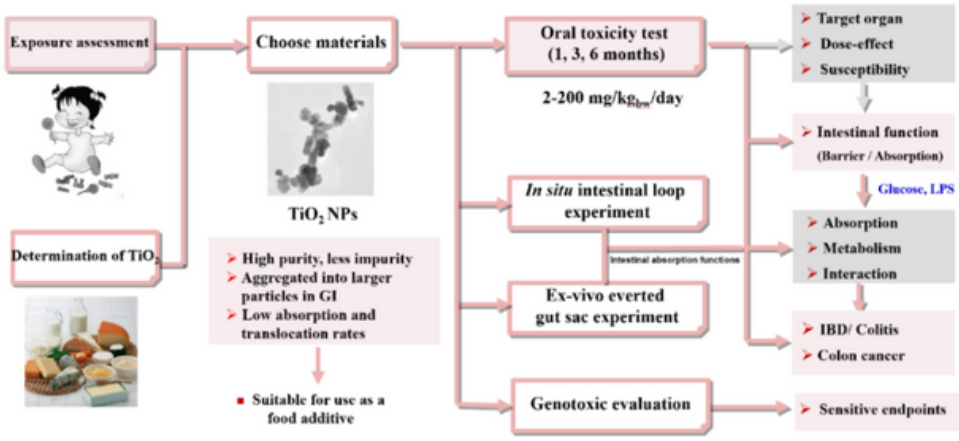

Figure 1 Basic research on the health risks of oral intake of titanium dioxide nanoparticles

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Jared Brown*, Arthur Stem, Keegan Rogers, Carlos Roncal-Jimenez, Richard Johnson

*University of Colorado Anschutz Medical Campus, Department of Pharmaceutical Sciences, Aurora, United States

17:21-17:33

Agricultural Worker Exposure to Environmental Silica Nanoparticles is Associated with Mesoamerican Nephropathy

Chronic kidney disease of unknown etiology (CKDu), also known as Mesoamerican nephropathy (MeN) in Central America, is a global epidemic of kidney disease that is primarily impacting young otherwise healthy agricultural workers. Those most impacted in Central America are sugarcane workers of which many are dying from this disease and for which the cause remains unknown. We have hypothesized that the routine use of sugarcane burning to facilitate harvest in developing countries contributes to this devastating disease. Elemental analysis shows that sugarcane stalks constitute >80% amorphous silica (SiO2). Therefore, we hypothesized that burning of sugarcane generates silica nanoparticles (SiNPs) that present an inhalation hazard to these agricultural workers. To determine if SiNPs are present in kidney biopsies of patients with MeN, we utilized single particle inductively coupled plasma mass spectrometry. We found SiNPs, which ranged in size from ~220-240 nm, were present in kidney biopsies from a small cohort of MeN patients from El Salvador and Mexico, while biopsies from patients with other kidney diseases or healthy individuals had significantly less SiNPs present. Further, we found that Si and SiNP concentrations increased in the urine of sugarcane workers across a harvest season (from November to April). In addition to patient studies, we have exposed rats to sugarcane ash or SiNPs and found increased kidney injury similar to that observed in patients with MeN including tubular interstitial nephritis. Lastly, we have reported changes in kidney proximal convolute tubule cells related to oxidative stress and cellular metabolic changes following exposure to sugarcane ash derived SiNPs. Overall, these findings suggest SiNPs present in sugarcane ash contribute to development of Mesoamerican nephropathy.

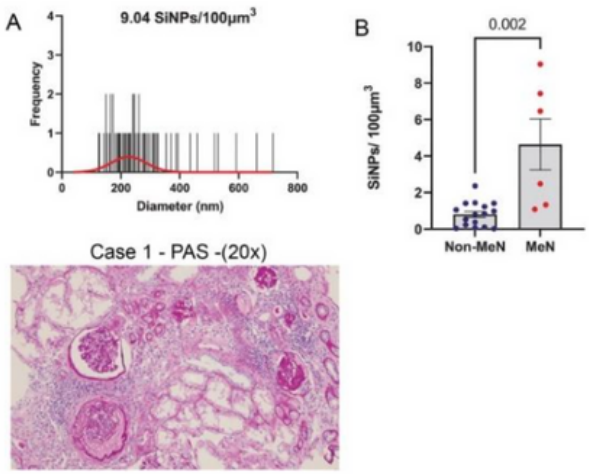

Figure 1: Detection of silica nanoparticles in biopsies of Mesoamerican nephropathy (MeN) patients. (A) Patient in case 1 had high silica nanoparticle (SiNP) content and a characteristic size distribution pattern (220-240 nm) in the biopsy determined by single-particle inductively coupled plasma mass spectrometry. Histologic changes were consistent with MeN, with chronic tubulointerstitial fibrosis, tubular atrophy, interstitial inflammation, and global glomerulosclerosis. (B) Silica nanoparticles depicted as number of nanoparticles per 100 µm3 tissue. Biopsies of patients with MeN (n = 6) had significantly more silica nanoparticles compared to those of subjects from El Salvador who were biopsied for other conditions (n = 16). Mean ± SEM, P = 0.002, Mann-Whitney rank test. Non-MeN refers to biopsies performed for kidney disease of various known etiologies.

Vanessa Auer*, Benjamin Punz, Magdalena Weiß, Lisa Kleon, Patricia Farias, Gabriele Gadermaier, Victor Puntes, Andreas Stingl, Martin Himly

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17:33-17:45

Functional testing of anti-viral green synthesized nanoparticular blockers against a SARS-CoV-2 pseudovirus infection model using submersed and air-liquid interface in vitro models of human lung epithelia

Exposure to aerosols is formed during sneezing, speaking or coughing, which contributes to the spreading of microorganisms and viruses, in some cases leading to severe diseases. To provide a reliable and reproducible system for monitoring the health impacts upon unintentional exposure or (intentional) administration of (bio)pharmaceutics or viral blockers, the NAVETTA workplace was established at the Paris Lodron University of Salzburg (Frijns et al, EST, 2017). Its main advantage is that it allows generation and deposition of various types of aerosols onto lung epithelial cell in vitro models to determine different types (acute toxicity, inflammatory, barrier integrity, etc.) of cellular responses. Deeply inhalable aerosols are generated by the Bio-AerosolizerTM (Vitrocell, Waldkirch, Germany), run over a diffusion dryer, and ionized by a corona charger before entering the in vitro cell exposure chamber, which enables the deposition of aerosols onto air-liquid interface-cultured cells. Basal nutrient support and apical exposure to the low laminar horizontal airflow containing the produced aerosols resembles the anatomic situation of the alveolar cells as realistically as possible. In addition, an electric field enhances the deposition of the charged aerosols making deposition more efficient. These main features make the NAVETTA workplace a powerful tool for in vitro cell exposure experiments in nano-carrier and vaccine development. As shown before, nanoparticles can impair microbial growth, which is already used widely, when thinking of silver nanoparticles in clothes, washing machines or cosmetics, whilst being harmful to the environment. Alternative functional candidates, less harmful to the environment and with a better safety/sustainability profile, for these nanoparticles are explored in this study. Moreover, the COVID-19 pandemic showed the restricted availability of preventative tools for viral infection, surface disinfectants and drugs to inhibit viral infection, suggesting nanoparticles as a promising tool in various application areas. In this study, nanoparticles aiming to block the ACE-2 receptor of the host cell are aerosolized with the NAVETTA, and SARS-CoV-2 pseudovirus infection of cells is analyzed. The pseudovirus with a lentiviral backbone carries plasmids with ZsGreen and Luciferase tags to enable quantification of viral uptake and copy number. Identified alternatives pose a diminished cyto- and immunotoxic threat to the exposed cells, while being able to inhibit pseudoviral uptake. The novel utilized NAVETTA system provides an enhancement to current systems by mimicking the inhalation into the deep lung and reproducibly & realistically facilitates in vitro monitoring of cellular responses.

Tina Buerki-Thurnherr*, S. Chortarea, G. Gupta, L.A. Saarimäki, W. Netkueakul, P. Manser, L. Aengenheister, A. Wichser, V. Fortino, D. Greco, J. Boos, A. Hierlemann, P. Wick

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17:45-17:57

Mechanisms of micro-/nanoplastics toxicity in pregnancy

Objectives: Micro- and nanoplastics (MNPs) are global pollutants and harmful to the environment, yet there is a lack of knowledge concerning human health effects, in particular for sensitive populations such as the pregnant women and the unborn child. In particular, the impact of MNPs on the placenta, as central mediator of maternal-fetal crosstalk, is largely unexplored. Here, we investigated the translocation and impact of polystyrene nanoplastics (PS NPs) on global gene expression in ex vivo perfused human placental tissue in comparison to CuO NPs, a common known hazardous material. Furthermore, we established a placenta-embryo chip as a promising model to assess direct and indirect placenta-mediated embryotoxicity mechanisms of MNPs.
Results: Transcriptome profiling revealed alterations in global gene expression after 6 h of placental tissue perfusion with sub-cytotoxic concentrations of CuO (10 µg/mL) and PS NPs (25 µg/mL). Pathway and gene ontology enrichment analysis of the differentially expressed genes showed highly material-specific responses in placental tissue. PS NPs affected the expression of genes related to inflammation and iron homeostasis whereas CuO NPs induced pathways related to angiogenesis, protein misfolding and heat shock responses. The observed effects on protein misfolding, cytokine signaling, and hormones were verified by western blot (accumulation of polyubiquitinated proteins) and qPCR analysis. Further data from the newly established placenta-embryo-chip indicated adverse effects on embryoid body viability only from an indirect exposure via the placenta barrier but not upon direct particle contact.
Conclusions: Collectively, our data suggest a central role of the placenta in mediating indirect embryotoxicity of MNPs even in the absence of fetal particle transfer. While our transcriptomics profiling study provides a valuable source of lead candidates for further studies into indirect placenta-mediated toxicity mechanisms, the placenta-embryo chip model offers a novel screening platform to gain broader insights into size-, additive-, or plastic chemistry-dependent responses of MNPs in pregnancy.

Session 6B

16:30-18:00

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Safe-and-Sustainable-by-Design strategies and case studies

Chairs: Elena Badetti & Magda Blosi

Wendel Wohlleben*, Veronica Di Battista, Karla R Sanchez-Lievanos, Nina Jeliazkova, Fiona Murphy, Georgia Tsiliki, Alex Zabeo, Agnieszka Gajewicz-Skretna, Alicja Mikołajczyk, Danail Hristozov, Vicki Stone, Otmar Schmid, Neil Hunt, Agnes G. Oomen

*BASF SE, Dept. Analytical and Materials Science, Ludwigshafen, Germany

16:30-16:45

Concepts of similarity in a SSbD context: finding a balance of safety and functionality for five multicomponent Quantum Dots

Concepts of similarity, such as grouping, categorization, and read across, enable a fast comparative screening of hazard, reducing animal testing. These concepts are established primarily for chemicals and have been explored for nanoforms in the GRACIOUS project. The development of concepts of similarity is now further motivated by the increased industrial need for comparison of innovative against conventional materials in the Safe and Sustainable by Design (SSbD) context, aiming for an informed balance of functionality and safety.
We investigated similarity assessment methods that can be applied to multicomponent nanomaterials (MCNMs) on the example of core–shell quantum dots (QDs). The term 'multicomponent' refers to their structural composition, which consists of up to four different heavy metals (cadmium, zinc, copper, indium) in different mass percentages, with different morphologies and surface chemistries. The choice of properties that are subjected to similarity assessment, with respect to safety, is guided by the Integrated Approaches to Testing and Assessment (IATA) for the inhalation hazard of simple nanomaterials, which recommends characterizing QDs by (i) dynamic dissolution in lung simulant fluids and (ii) the surface reactivity in the abiotic ferric reducing ability of serum (FRAS) assay. With respect to functionality, the quantum yield derived from QDs fluorescence spectra was used as a measure of their performance.
We propose two different approaches to compare and rank the case study materials amongst themselves and against well-known benchmark materials, here ZnO NM110, BaSO4 NM220, TiO2 NM105, and CuO. In the first approach, specific descriptors for each assay (i.e., leachable metal mass (%) and mass based biological oxidative damage (mBOD)) were selected based on expert knowledge and used as input data for generation of similarity matrices. The second approach introduces the possibility of evaluating multidimensional raw data by a meaningful similarity analysis, without the need for predefined descriptors. Relative differences in the sample set are calibrated against the biologically relevant range.
Strengths and weaknesses of the two approaches in informing SSbD decisions will be discussed. We anticipate that the similarity assessment approach is transferable to the assessment of further advanced materials (AdMa) that are composed of multiple components.

Irini Furxhi*, Anna Costa, Massimo Perucca

*CNR, ISSMC, Bologna, Italy

16:45-17:00
Safe and sustainable by design roadmaps. A glimpse of the ASINA case studies.

The Safe and Sustainable by design (SSbD) framework from the Joint Research Centre (JRC) seeks the definition of SSbD criteria and evaluation procedures for chemicals, (nano)materials and processes. The framework foresees the assessment of the entire life cycle of a compound, capturing the human and environmental safety aspects, and the environmental and economic sustainability dimensions shown in Figure 1 to stimulate sustainable research and innovation, beyond the current regulatory requirements.

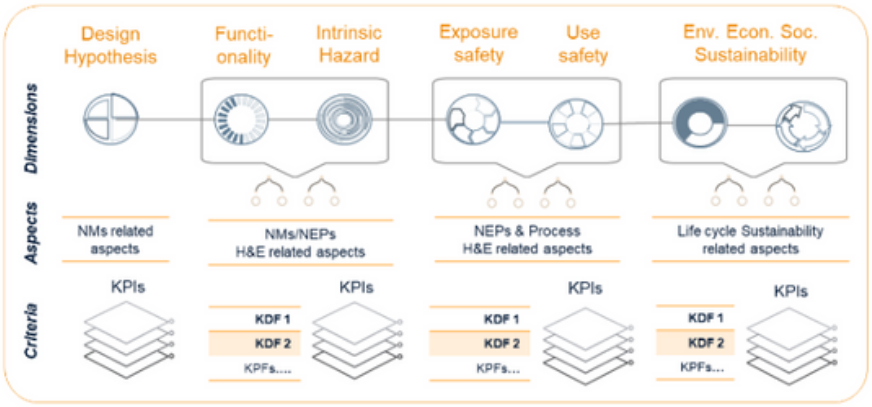


Figure 1. Dimensions, aspects and criteria of the SSbD framework. The SSbD approach within ASINA captures Key Performance Indicators (KPIs) in each dimension, Key Decision Factors (KDFs) and Key Performance Factors (KPFs).

ASINA utilizes the structure of the framework in case studies, within the context of nanotechnology. The application of SSbD concept involves identification of material design alternatives at the early stage of the innovation to reduce the potential for release of hazardous materials and/or decrease their hazard, while retaining functionality for their uses. In this presentation we will show the
1.measurable quantitative or qualitative criteria as Key Performance Indicators (KPIs)
KPIs are based on defined aspects (e.g. health or environmental) measured with an assessment method (experimental, modelling) and compared with thresholds when available.
2. KPIs are strongly depended on Key Decision Factors (KDFs) that allow the differentiation among the final KPIs and thus, the SSbD alternative solutions. Those KDFs can be altered by the designer and allow a degree of freedom in the "re-design" aspect.
3. KPIs are depended of Key Performance Factors (KPFs) that differentiate the results but are not manageable by the designer.
In this manner, we provide KPIs in each dimension of the frameworks followed by their KDFs and KPFs providing future SSbD implementations a scientifically sound basis as a starting point of departure.
Being transparent can help ongoing/future projects trying to achieve similar objectives, to get inspired and reach sound scientific approaches. In addition, demonstrating the ASINA cases, might align the efforts towards a common roadmap for executing a SSbD approach, ultimately, promoting the EU ambitious Green Deal goals. Finally, the roadmap acts as an illustrative tool to stakeholders to facilitate engagement and dissemination of results.

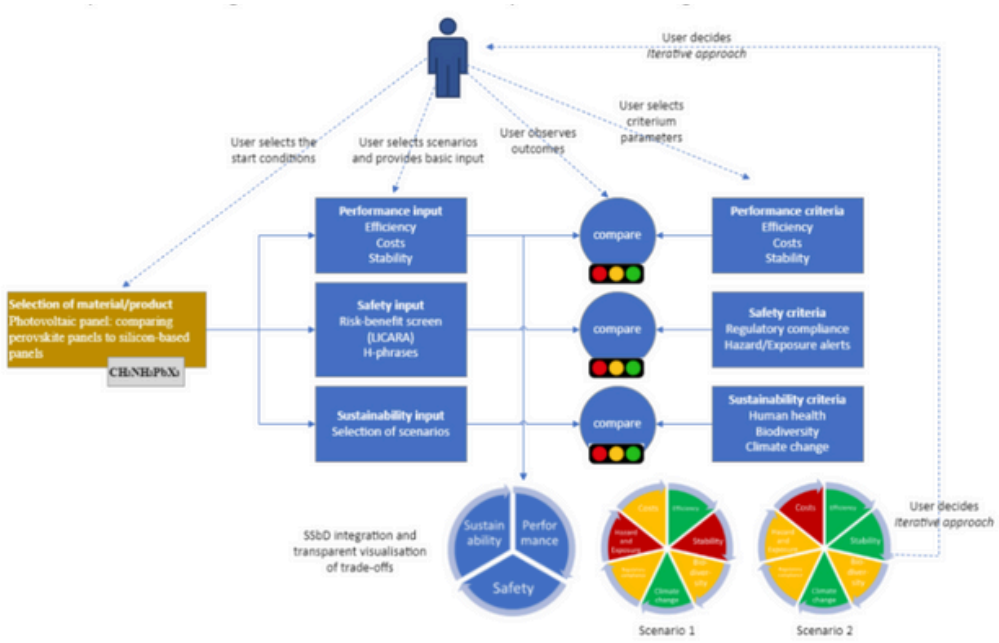
Wouter Fransman*, Hedwig Braakhuis, Neeraj Shandilya, Susan Dekkers, Thomas Hennequin, Felipe Blanco Rocha, Wouter Tabingh Suermondt, Frank Lenzmann, Mark Huijbregts

*TNO, Risk Assessment of Products in Development, Utrecht, Netherlands

17:00-17:15

Enabling Safe and Sustainable Innovation: transparent decision support

To move towards a safer, healthier, and more sustainable future, the European Commission introduced the Chemicals Strategy for Sustainability. This strategy aims to transition to a circular economy that is safe, climate-neutral, and sustainable. A pivotal element of this strategy is the adoption of chemicals and materials that adhere to the principles of Safe and Sustainable by Design (SSbD).
SSbD represents a paradigm shift where safety and sustainability considerations are integrated at the earliest stages of product development, running parallel to functionality and cost considerations. This proactive approach allows for the identification of potential opportunities to enhance safety and sustainability early in the innovation process. By doing so, companies have the flexibility to adapt their products, minimizing adverse effects and mitigating the risk of market failure.
SSbD presents an opportunity to proactively design innovative chemicals and materials that not only provide solutions to societal challenges, such as the energy transition, but also ensure no harm is caused to humans or the environment – embodying the principles of being safe and sustainable by design.
Achieving this delicate balance between substance functionality, safety, sustainability, and costs necessitates an integrated approach during the early phases of product innovation. We present a Multi-Criteria Decision-Support-System (MCD) that integrates relevant aspects (based on the EC-JRC SSbD framework) at a low Technology Readiness Level (TRL) based on basic information input. The MCD is being further advanced by incorporating multi-object optimization to help the user make optimal design choices. To demonstrate the MCD, multiple technological design choices for perovskite photovoltaic panels are included as a case study and compared to more conventional thin-film silicon-based panels. The main challenge is to balance efficiency, stability, and costs with a minimum or no use of potentially toxic substances in the perovskites. The output shows that the SSbD MCD empowers industries to test scenarios and effectively balance risks and benefits in the initial stages of their design process, contributing to the creation of a healthier and more sustainable society.
This work is funded by the Dutch government via the Early Research Program of TNO.



Christoph Olscher*, Florian Part, Stefanie Prenner, Anna Pavlicek, Sabine Jung-Waclik, Patricia Farias, Andreas Stingl

*Institute of Waste Management and Circularity, BOKU University, Vienna, Austria

17:15-17:30

Application of the safe and sustainable by design concept to nanoscale zinc oxide: An Austrian case study

The European Green Deal, the European Chemicals Strategy for Sustainability and the Austrian Circular Economy Strategy have set ambitious environmental goals to produce more sustainable and recyclable chemicals, materials, and products in the future. To meet these challenges and achieve the overarching goals, the European Commission's Joint Research Centre (JRC) has promoted the so-called "Safe and Sustainable by Design" (SSbD) concept. SSbD assessment methods, such as safety assessments in accordance with the REACH Regulation or LCA, should enable to newly design or re-evaluate ("redesign") chemicals or materials already on the market. In this process, harmful substances should be avoided or subsequently replaced with more safe and sustainable substitutes.

The objective of our research project Safety and Sustainability of Nanomaterials and Advanced Materials (SiNa) was to analyse the initial situation for the operational implementation of the SSbD framework in Austria, test its practical applicability using a specific case study and derive recommendations for the future establishment of the SSbD concept. To this end, literature research was used to analyse existing frameworks, initiatives, and platforms whose objectives overlap with those of the SSbD concept. Different stakeholders from the SiNa value network were involved through an online survey and qualitative expert interviews to analyse their knowledge and experience of the SSbD concept (see Figure below). The applicability of the SSbD assessment method to the production of zinc oxide nanoparticles (for cosmetics, textiles, sensors, etc.) was tested with a small Austrian company specialising in the production of nanomaterials.

The project results show that the SSbD concept is already known in many companies and research institutions. There are also similar initiatives and platforms in Austria that already deal with SSbD-relevant topics (e.g. the Green Chemistry platform). However, the case study and online survey show that the operational implementation of the SSbD framework in its current form is a major challenge for many companies, especially SMEs. The reason for this is the increased workload, the partial lack of expertise in their own company to carry out the SSbD assessments, and the current lack of financial incentives. To the end an Austrian SSbD roadmap, including proposed measures and recommendations, was derived from the project results to enable and promote the implementation of the SSbD concept in Austria companies and further institutions in the future.

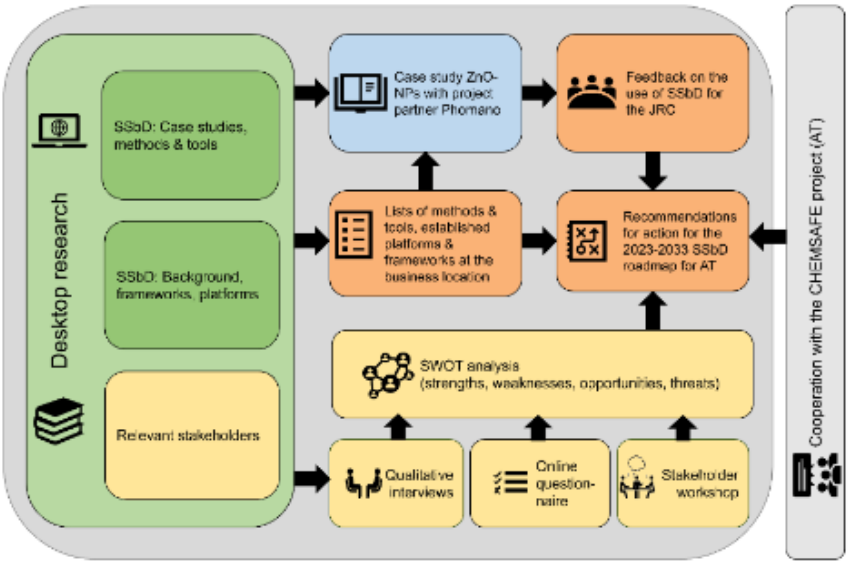


Figure: Workflow during the "SiNa" project.

Steffi Friedrichs

AcumenIST SRL, Etterbeek, Belgium

17:30-17:45

Interim Results of the MACRAMÉ R&I Approach – Ensuring regulatory Alignment in the Research & Innovation of five market-relevant Advanced Materials

The R&I Approach of the MACRAMÉ Project ('Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials') (Figure 1) aims to widen the development of harmonised test guidelines (TGs), guidance documents (GDs) (OECD) and standards (CEN, ISO) to market-relevant AdMas in their complex product matrices. This will be achieved by defining the R&I Strategy through life-cycle assessment for five market-relevant industrial MACRAMÉ Use-Cases. These define the selection of the MACRAMÉ R&I Activities and development of MACRAMÉ Methods, and the benchmarks chosen for monitoring the progress R&I. MACRAMÉ R&I Activities include a range of novel sample preparation techniques and ambitious quantitative detection and imaging methodologies that support reliable and reproducible determination of AdMas in different complex matrices (AdMa@CMs) and using inhalation as their main exposure route. By applying, combining and evaluating both established and novel inhalation toxicity tests a tiered approach to toxicity testing will be developed that will provide data on state-of-the-art characterised control materials for the MACRAMÉ Control Material Library. The library will serve future AdMa toxicological research. The ultimate MACRAMÉ Outcomes are proposals for harmonisation and (pre-)standardisation projects to be provided to and further elaborated with the relevant bodies, (i.e. OECD, VAMAS/CEN/ISO). The proposals will be founded on robust summary datasets, scientific documents and recommendations for hazard- and risk-assessment methodologies for AdMa@CMs. All data and information, obtained from external sources and generated during the Project, will be handled and stored in the MACRAMÉ Information Hub - the Project's central information processor, whose interoperability is based on a Data Stewardship concept, designed according to IndustryCommons principles.
The resulting efficiency and effectiveness of MACRAMÉ Methods will be demonstrated through their application in Use-Case evaluations, using LCA-, LCC- and 'Safe & Sustainable by Design' (SSbD)-based (EWARN (2022)), highlighting benefits like reduced costs of regulatory compliance, by following a MACRAMÉ Safety & Sustainability Matrix. This matrix will be a modular building block for MACRAMÉ's information-transferring interfaces for different scientific and regulatory communities, and thus provide a stepping-stone for Europe's route towards a 'one substance – one assessment' approach (European Green Deal (2019)) and promote an open strategic autonomy (ETUI (2021)) through key enabling and emerging technologies, including digital ones.

Figure 1: Illustration of the MACRAMÉ R&I Approach (AdMa@CMs: Advanced Materials in complex matrices; CF: Characterisation Factor; GRM: graphene-related material; IATA: integrated approaches to testing and assessment; LCA: Life-Cycle Assessment; LCC: Life-Cycle-Costing; MFA: Material-Flow Analysis; RA: Risk-Assessment; SSbD: Safe-&-Sustainable-by-Design).

Benjamin Punz*, Lisa Kleon, Vanessa Auer, Magdalena Weiß, Patricia Farias, Andreas Stingl, Martin Himly

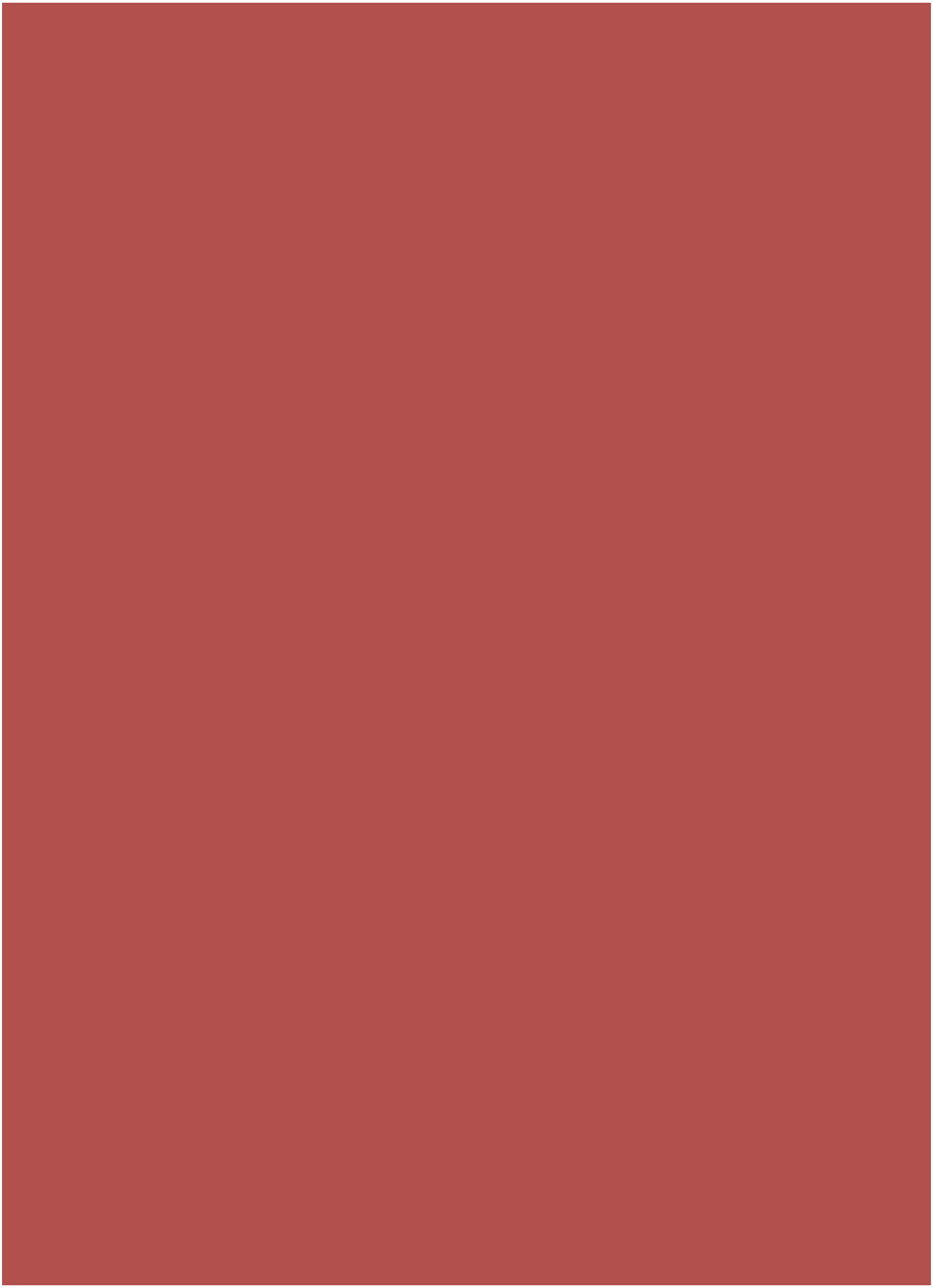
*PLUS, University of Salzburg, Salzburg, Austria

17:45-18:00

Replacing substances of concern: Green synthesized, antimicrobial nanoparticles as potent, safe and environmental friendly alternative

The contamination of work surfaces, medical equipment and devices poses significant risk to exposed individuals, in specific, those at compromised health, contributing to over 13.7 million worldwide deaths caused by infectious diseases in 2019. It is imperative to explore alternative approaches to combat the increasing resistance to commonly used broad-spectrum antibiotics. Consequently, there is a strong emphasis on the development of antimicrobial surfaces utilizing engineered nanomaterials. Certain nanoparticles are already widely used in various commercial applications, notably materials based on silver (Ag). However, the growing prevalence and oversaturation of Ag in the environment raises significant sustainability concerns. There is, therefore, a pressing need to reduce, refine, and replace its usage in accordance with the requirements of the Green Deal of the European Union, which aims for a pollutant-free environment, with strong reduction measures already by 2030. This study, therefore, aims to provide and assess more sustainable alternatives for Ag-based antimicrobial materials. Zinc oxide (ZnO) and cerium dioxide (CeO2)-based nanomaterials were chosen for developing green synthesis methods (reduced temperature and fossil-based reagents required) and compared to the well-established materials in respect to functional performance (i.e. similar/improved antimicrobial functions) vs. safety and sustainability measures (i.e. acceptable health profile and drastically reduced environmental effects). Subsequently, we evaluated the green engineered materials for their antimicrobial properties broadly using pseudoviral, bacterial and fungal representatives and quantitatively analyzed their respective mechanisms (ROS production, ion release, membrane disruption) to combat these pathogens. We developed a novel assay based on the interaction between these nanomaterials and microbes on dried surface. Microbial growth was monitored over time to determine the minimal inhibitory concentrations of the materials, where we observed complete inhibition or a significantly noticeable delay of microbial growth, for some of the candidate preparations. We further tested the dose-dependent antiviral efficacy utilizing an advanced air-liquid interface exposure chamber, simulating deep lung inhalation responses. Safety effects were tested for the most prominent exposure routes (for inhalation with human alveolar epithelial cell line and for dermal exposure with immortalized keratinocytes derived from healthy human donors). In line with the EU-SSbD Framework's multi-objective optimization procedures for replacement of substances of concern we herewith

present greener and more sustainable ZnO- and CeO2-based nanomaterials exhibiting similar/improved performance measures in terms of combined anti-viral, -bacterial and -fungal effects for surface application compared to the current benchmarks.



Session 2

10:30-11:30

HAZARD ASSESSMENT

Novel approaches and models for advanced in vitro testing

Valérie Forest

Mines Saint-Etienne, Inserm, U1059 Sainbiose, Saint-Etienne, France

Biomonitoring of nanoparticles in human biological samples to investigate the role of inhaled biopersistent nanoparticles in the etiology of respiratory diseases

In a context of health risk assessment, the biological monitoring (or biomonitoring) of nanoparticles in human broncho-alveolar lavages (BAL) coupled to their in vitro toxicity could be a particularly useful approach to get new insights into the role of inhaled biopersistent nanoparticles in the etiology/development of some respiratory diseases. Biomonitoring has been widely used in pulmonology as it can bring critical information on the relationship between exposure to a harmful substance and biological/pathological effects. Our objective was to perform mineralogical analyses to monitor the presence of nanoparticles in BAL from patients suffering from interstitial lung diseases (ILD) and investigate potential correlation with clinical data (especially relationship with idiopathic diseases).

We conducted a clinical trial on a cohort of 100 patients suffering from ILD (NanoPI clinical trial, ClinicalTrials.gov Identifier: NCT02549248). Thanks to innovative protocols we developed for this purpose, we separated micron-sized particles (>1 µm) from submicron (100 nm-1 µm) and nano-sized particles (<100 nm) contained in BAL. We then determined the metal load in each of these size-fractions. We evidenced a concentration of submicron silica particles higher in patients suffering from sarcoidosis than in patients suffering from other ILD, suggesting a potential role of these inhaled particles in the etiology and/or development of sarcoidosis.

To better understand the underlying mechanisms of toxicity, we then proposed to couple this biomonitoring of nanoparticles to their in vitro toxicity assessment. However, BAL obtained from regular clinical practice are conditioned with sodium hypochlorite solution (in a 50% v/v ratio), which is toxic for cells. We therefore developed a protocol to neutralize sodium hypochlorite, allowing to properly investigate the toxicity of the nanoparticles BAL contain. We first tried to neutralize chemically the sodium hypochlorite using H_2O_2, ascorbic acid or sodium ascorbate but this approach was unsuccessful. In addition, standard toxicology assays (MTT, LDH) could not be used because of interference with neutralizing solutions. We thus changed strategy and used ultracentrifugation to isolate nanoparticles from the sodium hypochlorite solution, with satisfactory extraction yields (88 to 100%). We then incubated the extracted nanoparticles with macrophages from the RAW264.7 cell line and assessed the cell viability and pro-inflammatory response. This study can be used as a proof-of-concept for further study of the biological impact of inhaled biopersistent nanoparticles. This approach paves the way for studies aiming at a better understanding of the etiology of some idiopathic diseases and underlying mechanisms.

Nádia Vital

National Institute of Health Dr. Ricardo Jorge, Department of Human Genetics, 1649-016, Lisbon, Portogallo

Hazard Assessment of two Innovative Cellulose Nanomaterials through a set of in vitro Genotoxicity Assays

Recently, nanocelluloses (CNMs), derived from natural sources, emerged as interesting materials being developed for multi-application purposes, particularly for food and food packaging. Considering envisaged applications, safety assessment at early-stages of material R&D, based solely on CNMs cytotoxicity may not be sufficiently informative. The identification of potential hazards that may affect human health later in life, such as genotoxicity, will give a more reliable support to decide whether CNMs should move forward to the next development stage, in a Safe-by-Design perspective.
This work aimed to investigate the genotoxicity of two micro/nanofibrillated celluloses (CMF/CNFs), prepared from *Eucalyptus globulus* kraft, utilizing different evaluation approaches: DNA damage, chromosomal damage, and mutation induction. Regarding the first, the alkaline comet assay was applied, with and without formamidopyrimidine DNA glycosylase (Fpg-modified) and, for chromosomal damage, the micronucleus (MN) assay (OCDE TG487) was used with Caco-2 and HT29-MTX-E12 human intestinal cells. Gene mutations were analyzed with the standard *Hprt* gene mutation test using V79 cells (OECD TG476). To account for the effect of the digestion process on the CNMs bioavailability and toxicological outcomes, a harmonized protocol for *in vitro* simulation of human digestion was added to the experimental design.
Preliminary testing did not show cytotoxicity (MTT and clonogenic Assays) from 3.1 to 200 µg/mL (undigested samples) or 3.1 to 50 µg/mL (digested samples), irrespectively of the cell line. After 3h and 24h exposure to each CNM, the comet assay revealed induction of DNA damage in Caco-2 with several concentrations for CMF-ENZ cells and in HT29-MTX-E12 cells with CNF-TEMPO, although no dose-response was observed. No chromosomal damage was detected in the two intestinal cell lines exposed (52h) to each CNMs, using the MN assay. After simulated digestion, only CNF-TEMPO increased the MNBC frequency after exposure to 3.1 µg/mL, in Caco-2 cells. Preliminary experiments did not show induction of gene mutations in V79 cells exposed to CNF-TEMPO.
In conclusion, this study suggests the occurrence of some biological effects caused by two different CNMs, raising concerns about their impact on human health. Cytotoxicity assessment by itself, which is commonly used to determine CNMs biocompatibility, is not sufficient to fully evaluate their safety. Hazard characterization using this test battery for the genotoxicity/mutagenicity assessment at early-stages of product development may contribute to re-design cellulose-based nanomaterials to have better safety profile, while reducing the use of more expensive, time-consuming, and ethically questionable *in vivo* assays.
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Kevin Hogeveen

ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Contaminant Toxicology Unit, Fougères, France

High Content Analysis-Based Approaches for Hazard Evaluation of Nanocellulose and Nanofibre Materials

Nanocellulose (NC) is an emerging material in the food sector with prospective applications in the domains of food packaging, novel foods, and food additives. However, the potential hazards of NC following oral exposure are not well characterized, and a nano-specific assessment focusing on local toxic effects, and their ability to affect and cross the intestinal epithelium is required. In the EFSA-funded NANOCELLUP project, a tiered approach was implemented for the hazard evaluation of a panel of NC samples including nanofibrillated cellulose (NFC), cellulose nanocrystals (CNC) and bacterial nanocellulose (BNC) materials.
In the first tier, a high content analysis-based approach was used to obtain a maximum amount of information on the cellular responses in intestinal and macrophage cell models following exposure to a panel of NFC, CNC and BNC materials. A series of endpoints including cytotoxicity, DNA damage response, oxidative stress, and the proinflammatory response was quantified following a 24 h treatment of human intestinal Caco-2 cells and differentiated THP-1 cells. A selection of NC materials demonstrating significant cytotoxic effects in Tier 1 studies were investigated further in Tier 2 studies where the uptake and crossing of the intestinal barrier was assessed, as well as inflammation and genotoxic potential. No cytotoxic effects on the panel of endpoints were observed in the intestinal Caco-2 model. However, in differentiated THP-1 cells, while only slight cytotoxic effects were observed, significant increases in pro-inflammatory responses (IL-8 secretion) were detected. No genotoxic effects were observed with Tier 2 materials using the γH2AX/pH3 assay. Moreover, no effects on mucus production in HT29-MTX cells was observed following periodic acid-Schiff (PAS)-Alcian Blue (AB) staining, whereas a significant increase in MUC3A expression was observed following treatment with NFC and CNC materials.

These studies are being followed up in Case Study 2 of Lot 3 of NAMS4NANO, which deals with nanofibers with focus on nanocellulose. Within this case study, ANSES will use HCA as a high throughput screening approach to generate a maximum amount of information on the cytotoxic responses of both intestinal and macrophage cell models to the investigated panel of fibre materials.

Johanna Samulin Erdem

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In vitro alveolar model for assessment of genotoxicity and inflammatory potential of nickel nanoparticles

Exposure to nickel (Ni) in the work environment has been associated with increased risk of nasal and lung cancer. Ni has known genotoxic and carcinogenic properties, however, the risks related to occupational exposure for metallic Ni nanoparticles (Ni-NPs) are still unknown. In the present study, an advanced in vitro alveolar model was used to assess the uptake, genotoxicity and inflammatory effects of Ni-NPs. The cells were exposed at air-liquid interface (ALI) to relevant occupational doses, 1 and 3.5 µg/cm2, of Ni-NPs. Cellular uptake was assessed by transmission electron microscopy (TEM). Following exposure, cell viability and expression of genes relevant to oxidative stress responses, DNA damage and repair, apoptosis and inflammation were assessed after 4h, 24h, 72h, 7 days and 14 days. In addition, the secretion of cytokines and chemokines, and level of 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) and 8-oxoguanine (8-oxoG) were investigated. Our data show that exposure to Ni-NPs resulted in a temporal regulation of genes involved in DNA damage repair (RAD51, DNMT3B) and oxidative stress response (HMOX1). The exposure did not affect cell viability and secretion of cytokines. Altogether, our data indicate that in vitro ALI inhalation models represent valuable tools to assess the cytotoxic and genotoxic effects of nanoparticles.

Yvonne Kohl

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Which in vitro liver model suits best for predictive toxicological research: A comparison

Due to its role in the detoxification and biotransformation of endogenous and exogenous substances, the liver is a key organ for the investigation of pharmaceutical and toxicological substances. With regard to the need for more precise predictive models for hazard assessment in vitro, human induced pluripotent stem cells (hipSC) are getting more and more in the focus of the discussion. These reprogrammed cells can differentiate into almost all cell types of the three germ layers and the germ line of an organism due to their pluripotent status.

In the present study, comparative studies were carried out using cell lines and hepatocyte-like cells differentiated from hipSC to study differences in cultivation behavior as well as metabolic and functional properties under two-dimensional (2D) and three-dimensional (3D) conditions. For this purpose, three immortalized liver cell lines (HepG2, HepaRG, Huh7) and one hiPSC line differentiated into hepatocyte-like cells were compared to identify which in vitro system is most suitable for predictive toxicological research.

All tested in vitro models were cultivated under both 2D and 3D conditions and their growth behavior, viability and functionality were compared. In addition, the expression level of various hepatocyte-specific markers was examined using qPCR.

The results, with a focus on the significant differences between the cell models and the 2D and 3D cultures, are presented and evaluated, with a view to their contribution to the development of a better understanding of the mechanisms of action of toxic agents or relevant test material.

Deborah Stanco

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AOPs Pave the Way: Deciphering Nanomaterial Impacts on Gut Health

Intestinal organoids (iHOs) derived from intestinal pluripotent stem cells (iPSCs) are three-dimensional in vitro structures that model the complexity and functionality of the in vivo human intestine. A promising aspect is to use such model as a new approach methodology (NAM) to analyse the toxicity of nanomaterials (NMs) in food. In this context, the European Food Safety Agency (EFSA) guidance recommends the use of Adverse Outcome Pathways (AOPs) to assess side effects associated to NMs in food. Indeed, AOPs can support the mechanistic-based hazard assessment for chemicals including NM. However, no AOPs for describing effects of NM in the gut are present so far. iHO models coupled with AOP frameworks can elucidate the cellular uptake and translocation of nanomaterials (NMs) within the intestine.. However, stringent quality criteria are required accompanied with well-defined acceptance values is paramount.
Drawing upon existing literature, Adverse Outcome Pathways (AOPs) were first established to elucidate the effects of nanomaterials (NMs) within the gastrointestinal tract.. Then, iPSC pluripotency and iHO formation were analysed by morphological observation in culture and by specific marker expression including SOX2, OCT4, NANOG, TRA-1-60, LGR5, VILLIN, MUCIN 2 at protein (IF staining) and gene level (qRT-PCR). Finally, the iHO model is used to assess the toxicity and the uptake of silver and nanocellulose NMs by LDH assay and confocal microscopy, respectively. Samples treated with a cancerogenic drug (NaCrO4) and polysterene NM are used as positive control for the toxicity and uptake testing, respectively. Cell-type-specific AOPs link NM uptake to intestinal toxicity. iHOs' tissue-specific markers and crypt-villus architecture confirm their biological relevance for in vitro testing. We could demonstrate that high-pluripotency iPSCs internalized polystyrene NMs, and iHOs displayed sensitivity to Na2CrO4. The study provided sights into the biological relevance of iPSC-derived iHO models for in vitro toxicity testing. Nevertheless, additional research is needed to establish readiness criteria of the in vitro tests that can inter alia demonstrate the toxicological relevance of iHOs for nanomaterial testing. Additionally, our AOPs will enable the integration of other pertinent NAMs with the appropriate key events, facilitating a mechanistic-based approach of risk assessment.

Juergen Schnekenburger

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Tomographic imaging and quantification of single cell nanoparticle uptake

Holotomography is a quantitative phase imaging technique, which facilitates label-free optical imaging of cells with high three-dimensional subcellular resolution based on refractive index (RI) differences. The study of nanoparticle-cell interactions is of central importance for the analysis of nanoparticle toxicity as many effects are caused by cell membrane binding and internalization. Holotomography allows both, detection and quantification of unlabeled nanoparticles in single cells through three-dimensional imaging of the RI distribution. However, this is limited by the optical resolution and requirement of a difference in RI between nanoparticles and surrounding cellular components. Therefore, the detection of organic polymeric nanoparticles is challenging. Labelling particles with dyes and coupling holotomography with fluorescence microscopy enables the investigation of uptake and distribution of polymeric nanoparticles. Here, cellular association and uptake of labeled NR668-loaded poly (alkyl cyanoacrylate) (PACA) nanoparticles and unloaded and lipophilic dye 3,3´-Dioctadecyloxa-carbocyanine perchlorate (DiO) -loaded chitosan nanocapsules (NC), in human A549 lung epithelial cells and mouse RAW 264.7 macrophages were analyzed. We further analyzed the pathways of particles cell entry. The use of inhibitors, Chlorpromazine, Cytochalasin D, and Filipin III, that prevent specific endocytotic uptake pathways allows to identify possible entry pathways. A549 cells and RAW 264.7 macrophages were incubated with endocytosis inhibitors, which was followed by NP treatment with unloaded and NR668-loaded poly (alkyl cyanoacrylate) (PACA) nanoparticles and unloaded and lipophilic dye 3,3´-Dioctadecyloxacarbocyanine perchlorate (DiO) -loaded chitosan nanocapsules (NC). Cellular association of particles was observed over six hours by locating the fluorescence signal of labeled nanoparticles in 2D images resulting from the three-dimensional cell imaging. The inhibitors had a clear and time dependent influence on nanoparticle uptake, with differnces depending from the inhibitor type, nanoparticle and cell type. Our results show that Holotomography in combination with fluorescence microscopy is a promising technique to detect labeled polymeric particles in single cells, which provides information on the interactions between nanoparticles and cells.

Kirsty Meldrum

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Implementing an alveolar in vitro co-culture model to predict the potential hazard of engineered nanomaterial (ENM) inhalation exposure using Adverse Outcome Pathways (AOPs)

The repetitive, long-term, low-dose inhalation exposure to engineered nanomaterials (ENMs) can potentially pose significant human health hazards. Through the European Union's Horizon 2020 research and innovation program – PATROLS (Grant No.760813, utilising established Key Events (KEs) from published Adverse Outcome Pathways (AOP) (i.e. AOP302 and AOP33) and the Good In Vitro Method Practices (GIVIMP) guidelines, we aimed to test the potential of a well characterised in vitro model to predict the human response to ENM exposures. An epithelial cell line - A549 (ATCC® CCL-185™) and macrophage-like (PMA-differentiated-THP-1 (dTHP-1) cell (ATCC® TIB-202™) co-culture was exposed to TiO2 (JRC NM105), ZnO (JRC NM111), Printex90 and MWCNT (Mitsui7) at the air-liquid-interface using the VitroCell Cloud12 exposure system for 6, 24, and 72-hours. Cells were exposed to 1.3, 5.2 and 10.4µg/cm2 (TiO2 and ZnO); extrapolated from in vivo exposures (IVIVE); and 3.9 and 7.84µg/cm2 (Printex90 and MWCNT). Specific genes of interest that were associated with the KEs identified in AOP302 and AOP33 (previously AOP173) were assessed via a targeted quantitative PCR (qPCR) array approach for TiO2 and ZnO only. Results identified increases in IL-33 (1.3 and 10.4µg/cm2) after 6 hours and in TLR4, CEBPB, CCL5, SOD2, CCL2, and IL-18 expression at 24 hours post-exposure of TiO2 (1.3 10.4µg/cm2). Increases in IL-10, IL-6 and SOD2 (1.3 and 10.4µg/cm2) were induced by ZnO after 72hours post-exposure only. Transcriptomics established that pending the ENM type, concentration applied, and time-point of analysis the fold-change (>1.5 or <-1.5) of differentially expressed genes (DEGs) was altered. At the highest concentration (10.4µg/cm2) of TiO2 after 6 hours there was the most significantly changed DEGs. ZnO had significant increases after 24 hours at the highest concentration (10.4µg/cm2). MWCNT induced the most changes in DEGs after 24 hours in the lower concentration (3.9µg/cm2). Printex90 had effects on the canonical pathways observed at every time point (6, 24 and 72 hours) and both concentrations (3.9 and 7.84µg/cm2). This project has indicated that IVIVE relevant concentrations of various ENMS may elicit responses in vitro as previously seen in vivo, and that are identified as KEs in published AOPs (i.e. AOP302 and AOP33). Therefore, in conclusion, advanced in vitro systems have the potential to indicate in vivo response trends when combined with relevant ENM concentrations and specific, next-level toxicological approaches.

Sian Brooks

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Investigating the mechanisms responsible for multi-component nanomaterial toxicity using advanced lung models at the air-liquid interface: a tiered testing approach

Multi-component nanomaterials (MCNMs) require strategic testing approaches to elucidate their potential hazard. The project aims were to identify mechanisms of toxicity and determine structure-activity relationships by examining specific key events regarding inhalation-related adverse outcome pathways relevant to the in vitro models used. A tiered testing approach was employed to assess the mechanistic toxicology observed for specific materials, by first screening with an A549 monoculture model prior to using either an A549/dTHP-1 bi-culture for materials inducing genotoxicity with/without a pro-inflammatory response, or a hAELVi/NCI-H441/dTHP-1 tri-culture model for materials that generated a pro-inflammatory, or oxidative stress response in the absence of genotoxicity. Models were seeded onto 12-well transwell membranes (0.3 µm pore) and cultured at the air-liquid interface before exposure to MCNMs via aerosol using the VitroCell Cloud 12. Monocultures were exposed to human-relevant NM concentrations (390, 780, 3100 ng/cm2) and bi-/tri-cultures were exposed to specific concentrations.
Results from the A549 monoculture screening indicate that exposure to Fe2O3 (NRCWE-019), ZnFe2O4 (NRCWE-021), LaCo0.5Ni0.05O3, and LaNiO3 induced genotoxicity, as evidenced by micronuclei formation (CBMn assay) in the absence of an IL-8 pro-inflammatory response and were subsequently tested with the bi-culture. Both genotoxicity and pro-inflammatory markers (IL-8) were observed for monocultures exposed to Printex90, NiZnFe4O8 (NRCWE-020), and LaCo0.25Ni0.75O3, and so these materials were also tested with the

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biculture. The tri-culture was utilised for further testing of Fe2O3 (NRCWE-018), LaCo0.475Ni0.475Pt0.05O3, and LaCo0.475Ni0.475Pd0.05O3 as exposure of the monoculture to these materials increased IL-8 release significantly ($p<0.05$). The monoculture results suggest that the stoichiometry of mixed metal oxides may have an effect as emphasised by the materials with differing proportions of nickel. Cultures were also assessed for morphology (via confocal microscopy), cytostasis, oxidative stress (GSH assay), and DNA strand-breaks (COMET assay). Preliminary experiments with Silica-based materials indicate no impact ($p>0.05$) on barrier integrity (Blue Dextran assay) or elevated pro-inflammatory response after exposure to Silica-REF-Std (reference) or Silica-ANIS-Std (anisotropic). Significant micronuclei formation was induced by Silica-REF-Std but not Silica-ANIS-Std, indicating that NM shape may play a role in the observed effect. Results from this monoculture assessment were used to determine which co-culture would be appropriate for further testing. Silica (Silica-NOSIL/SIL-M) and DQ12 (benchmark material) were also included for initial monoculture assessment.
By utilising various models of increasing complexity, this tiered testing approach will provide a thorough assessment of the specific mechanisms of MCNM-induced (inhalation) toxicity.
Funded by EU H2020 research and innovation programme, grant agreement No. 953183 (HARMLESS).

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Combination of New Approach Methodologies (NAMs) for Hazard Assessment of Synthetic Amorphous Silica (SAS) in the NAMS4NANO Project

The application of New Approach Methodologies (NAMs) is considered a step forward for establishing the toxicity of nanomaterials. This approach is supported by the European Food Safety Authority (EFSA) and the funded NAMS4NANO project. With a strategy based on the Guidance for nanomaterial risk assessment released by EFSA, this project investigates several materials used in various sectors such as food and feed additives, food contact materials, pesticides, nutrient sources, and novel foods. The overarching aim of this project is to explore the practical implementation of selected NAMs in integrated approaches to testing and assessment (IATA) to substantiate risk assessment and to better understand the remaining challenges and uncertainties. Within the family of silicon dioxide materials, synthetic amorphous silica (SAS) is a group of materials authorised as a food additive (E 551) that is used in the food industry as an anticaking agent, thickener and carrier of flavours. The objective of this case study is to provide new data on SAS using NAMs that could be integrated in risk assessment. The strategy to achieve this aim is presented. As test items, in addition to the two SAS materials NM 200 and NM 203 from the Joint Research Center repository, selected SAS materials were sourced on the EU market to improve the coverage of available SAS types and assess if changes in the manufacturing process and physicochemical properties affect the toxicological responses. Four testing workpackages are featured. The first one is devoted to advanced physico-chemical characterization (dispersion protocol, dissolution and characterization in cell media). The second uses in vitro degradation tests to check in vitro dissolution rate in intestinal conditions and stability in biological fluids. The third is dealing with toxicodynamics. Potential toxic effects on intestinal, liver and immune cells will be investigated using both simple and advanced models. A panel of toxicity endpoints (viability, apoptosis, oxidative stress, inflammation, barrier impairment) will be measured depending on the cell model. Moreover, mechanisms of action will be investigated by omics. The last workpackage covers toxicokinetics and investigates particle uptake and translocation across the intestinal barrier.

Daniela Hahn

Biomedical Technology Center, University of Muenster, Muenster, Germany

A random effects meta-analysis as a tool to identify suitable genotoxicity test systems for nanomaterials

biculture. The tri-culture was utilised for further testing of Fe2O3 (NRCWE-018), LaCo0.475Ni0.475Pt0.05O3, and LaCo0.475Ni0.475Pd0.05O3 as exposure of the monoculture to these materials increased IL-8 release significantly ($p<0.05$). The monoculture results suggest that the stoichiometry of mixed metal oxides may have an effect as emphasised by the materials with differing proportions of nickel. Cultures were also assessed for morphology (via confocal microscopy), cytostasis, oxidative stress (GSH assay), and DNA strand-breaks (COMET assay). Preliminary experiments with Silica-based materials indicate no impact ($p>0.05$) on barrier integrity (Blue Dextran assay) or elevated pro-inflammatory response after exposure to Silica-REF-Std (reference) or Silica-ANIS-Std (anisotropic). Significant micronuclei formation was induced by Silica-REF-Std but not Silica-ANIS-Std, indicating that NM shape may play a role in the observed effect. Results from this monoculture assessment were used to determine which co-culture would be appropriate for further testing. Silica (Silica-NOSIL/SIL-M) and DQ12 (benchmark material) were also included for initial monoculture assessment.
By utilising various models of increasing complexity, this tiered testing approach will provide a thorough assessment of the specific mechanisms of MCNM-induced (inhalation) toxicity.
Funded by EU H2020 research and innovation programme, grant agreement No. 953183 (HARMLESS).

Asia Saorin

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Characterization of graphene oxide in complex biological media

Graphene oxide (GO) is the oxidized form of graphene characterized by high dispersibility and easy production which make it the most studied form of graphene based materials. Indeed, it is widely used in different applications such as sensing, batteries, solar cells and as filler in polymers. In parallel with the spreading of its applications, the potential exposure of human and environment is also increasing, leading to the requirement of careful risk and hazard assessment. Therefore, the environmental fate and interactions with biological systems are required to be carefully evaluated. In this context, it is emerging the need to consider materials beyond their pristine characteristics namely considering the real properties after exposure to environmental and biological fluids.
For these reasons the present work focuses on the characterization of different samples of graphene oxide with different sizes and oxidation levels which were exposed to complex biological fluids from cell culture media to simulated lung surfactants and digestion fluids. Indeed, the purpose is the evaluation of the real particle characteristics after accidental exposure through inhalation or ingestion. In particular stability of GO dispersions in complex media was evaluated along with the influence and characterization of biomolecular corona, which is known to be a crucial parameter that has to be assessed in order to properly foresee particles fate.

Tommaso Serchi

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Beneficial effect of differently-coated Selenium Nanoparticles in 3D cell culture models mimicking the respiratory tract and intestinal epithelium

Selenium (Se) is an essential trace element that plays a crucial role in various physiological processes, including enhanced immunity (Avery and Hoffmann, 2018) and antioxidant defense (Bjørklund et al., 2022). Ingested as well as inhaled Se nanoparticles (SeNP) can be absorbed by epithelial and resident immune cells and being incorporated into selenoproteins that scavenge reactive oxygen species (ROS) and protect against oxidative stress-induced damage. Moreover, Se has been shown to modulate the release of inflammatory cytokines, and Se deficiency has been associated with impaired intestinal barrier function and increased susceptibility to intestinal infections and inflammation. Therefore, Se serves as a valuable micronutrient in protecting against oxidative stress and inflammation. Understanding the mechanisms of Se metabolism and its effects on intestinal and lung physiology is crucial for developing therapeutic strategies to combat gastrointestinal and respiratory disorders. However, the direct administration of Se as an antioxidant is not advised due to its narrow therapeutic window (Hosnedlova et al., 2018).

The objective of this study was to evaluate the toxicity as well as the antioxidant and anti-inflammatory capacity of SeNPs in 3D cell culture models. The respiratory tract can be resembled by an alveolar in vitro test system called ALIsens® (Chary et al., 2019) built on a microporous membrane of hanging inserts by seeding human alveolar type II epithelial cells (A549) and endothelial cells (EA.hy926), macrophage-like (Mφ-THP1) and dendritic-like cells (DC-THP1). The physiologically relevant architecture of the system favors the development of a tissue-like microenvironment and facilitates exposures at the air-liquid-interface (ALI). The in vitro intestinal epithelium is based on a tri-culture model consisting of human intestinal epithelial cells (Caco-2) and mucus-secreting HT29-MTX cells, as well as hematopoietic cells (Raji B) able to promote Caco-2 conversion in specialized microfold cells (M-cell) (Araújo et al., 2013; Schimpel et al., 2014). The treatment shows that the SeNPs are well tolerated in both cell culture systems without inducing neither a strong basal cytokine release nor increasing oxidative stress measured by ROS formation.

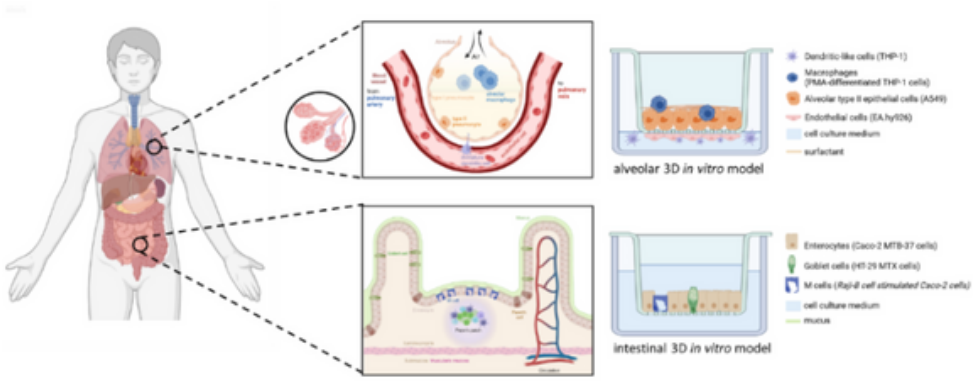

Figure 1: Representation of the human alveolus and small intestine and respective 3D in vitro model.

Ivan Rios Mondragon

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Exploring the Use of Cyclic Voltammetry to Assess Nanomaterial-Induced Oxidative Stress

The rapid advancements in nanotechnology have paved the way for the development of innovative nanomaterials with exceptional physicochemical properties, offering promising avenues for biomedical applications. However, the safety concerns associated with nanomaterials (NMs), including their potential to induce oxidative stress, must be addressed for their successful translation. Conventional colorimetric and fluorescence-based methods used to evaluate oxidative stress often suffer from NM-induced interferences. To address this problem, we monitored NM-mediated oxidation of L-ascorbic acid (AA) by using label-free cyclic voltammetry coupled with screen-printed electrodes. We observed size-, concentration-, time- and pH-dependent NM-driven oxidation of AA by different CeO2, TiO2 and Ag NMs in well-defined buffers and acellular conditions. The ranking of NMs based on AA consumption from high to low was: 20 nm Ag, 3,5 nm CeO2, 20 nm Ag, 50 nm Ag, Ag nanowires, 10 nm x 10 nm CeO2, 50 nm CeO2, 8 nm TiO2, TiO2 nanorods and 50 nm TiO2. CV analyses of AA in bovine serum incubated with NMs revealed similar findings. Thus, this method seems sensitive even in complex biological environments. Furthermore, we corroborated these findings by monitoring the generation of reactive oxygen species and cell proliferation in in-vitro cultures of lung-epithelial cancer cells exposed to the different NMs. We demonstrate that CV holds promise as a label-free and reliable technique for screening of potential oxidizing effects of NMs. Furthermore, implementation of disposable electrodes offers high-throughput scalability at low cost.

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hiPSC-based air-liquid interface model for inhalation studies in vitro

To assess health effects of inhaled substances such as drugs, chemicals or new materials, animal models are still a central tool in scientific and regulatory research. However, they are not the perfect model for predicting human responses. Therefore, the lung is an organ of interest in research and there is a great need for a reliable lung cell source for in vitro applications (Moreira et al. 2021). Ethical concerns also pushed forward the development of alternative methods in line with the 3R principles. In vitro models, simulating the physiology of the lung, especially the alveoli as the first target for inhaled substances, can serve as helpful alternative model for aerosol exposure studies. However, it is important to consider which cell source is used. In multiple studies, immortalized tumor cell lines, as e.g. A549, are used for air-liquid interface (ALI) model (Silva et al. 2023). Such cell lines are easy to handle, but do not represent the in vivo situation of a healthy person. On the other hand, human induced pluripotent stem cells (hiPSC) display the genetic background of each individual they are derived from. Due to their pluripotent status, hiPSC can differentiate into all cells of the human body. Therefore, they have high potential for novel and personalized cell models. To date, protocols for differentiating hiPSC into several lung cell types are available, but often focus on 3D organoid formation (Yamamoto et al. 2020, Müller et al. 2023). To date, they are not yet applicable protocols available for hiPSC differentiation and cultivation as ALI model for inhalation studies in toxicity and drug safety assessment. In the presented study, an ALI in vitro model of alveolar epithelial cells type 2 (AEC2) like cells from hiPSC was successfully developed and characterized. A workflow for the differentiation and the subsequent transfer and cultivation via Transwell™ technology was established. Cell viability, cell vitality as well as the expression level of various lung- and hiPSC-specific markers was examined using alamarBlue™ assay, Live/dead staining, immunhistochemistry and qPCR, respectively. The new model suits for aerosol exposure studies to assess health effects of inhaled substances such as drugs, chemicals or new materials. In addition to analyzing the transport of the test substance across the ALI barrier, different biological endpoints can be determined. In contrast to cell line based models, the developed alveolar hiPSC-based ALI system is physiological and presents an alternative model in line with the 3R principle.

Mohammed Almasaleekh

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Exhaust emission from marine diesel engines and aerosol aging effects on lung tissue models at the Air-Liquid Interface

Exhaust aerosols related to transport modes are important sources of air pollution and are associated to adverse health outcomes. However, current legislative control of particulate matter (PM) is based on mass concentrations and does not account for source dependent variation in PM toxicity or the impact of high numbers of nanoparticles (ultrafine particles; UFPs), which contribute little to PM mass. The specific aim of our study was to evaluate the toxicological responses of Air-Liquid Interface (ALI) lung tissue models to emissions generated by a ship diesel engine operated with fuels comprising low or high sulphur content, marine gas oil (MGO) and heavy fuel oil (HFO), respectively. Both fresh and photochemically aged UFP-containing aerosols were evaluated to unravel the toxicological effects of atmospheric photochemical aging on exhaust emission. Two different cell models were used including a monoculture of A549 alveolar epithelial cells and a triple culture of bronchial Calu-3 cells and THP-1 macrophages, grown on the apical side of a permeable membrane, with EA.hy926 endothelial cells on the basolateral side. Four-hour exposures were conducted in two ALI automated exposure stations running in parallel and supplied with different aerosol dilutions, and with or without electrostatic deposition enhancement. The role of gas-phase was explored by filtering the aerosols. Both fresh and aged MGO emissions induced a slight increase of LDH release with increasing aerosol exposure concentrations in the triple culture indicating cell membrane damage and cytotoxicity while only minor cytotoxic effects were noticed following exposures to fresh and aged HFO emissions. A significant decrease of the epithelial barrier integrity was associated to fresh MGO aerosol with enhanced deposition. The fresh MGO gas-phase contributed to the observed cytotoxicity in A549 cells. The molecular adverse effects were varying across fuels and exposure concentrations, with the tendency to differentiate fresh and aged aerosols based on their ability to induce oxidative stress and pro-inflammatory effects, respectively, as shown by measuring 180 metabolism and inflammation associated proteins. The

impact of photochemical aging in the toxicological context is yet to be clarified with further biological analyses being conducted. Ongoing genotoxicity and transcriptome analyses would further distinguish the possible different molecular mechanisms of actions of fresh and aged UFP-containing aerosols since the different chemical identity of UFP might play a pivotal role in determining their toxicological potential over the different physical-based dose metrics.

Figure: This project has received funding from the Europeaǹ Unionˋs Horizon under grant agreement No 955390

Session 2

10:30-11:30

HAZARD ASSESSMENT

Adverse Outcome Pathways (AOPs)

Rossella Daniela Bengalli

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In vitro hazard assessment of bio-based nanomaterials used in the production of polyurethane (PUR) foams

Bio-based nanomaterials (B-NMs), derived from biomass waste, has gained attention in the last years in the view of promoting the safety, sustainability and circularity principles in several industrial applications. In the BIOMAT project new B-NMs (Silica oxide – SiO2-, Lignin – Lig- and Crystal Nano-Cellulose – CNC- based nanoparticles) are proposed as innovative nanofillers for substituting petrochemicals compounds in the production of polyurethanes (PURs), also providing improved functional properties of the final product. However, few data are available about their safety, as well as proper approaches to test their potential toxicity on human health. Thus, a hazard testing strategy was designed considering as reference the safe and sustainable by design (SSbD) framework for new chemical and materials, in particular prioritizing the use of new approach methodologies (NAMs), such as in vitro testing, for generating data about the potential hazard of B-NMs and involving adverse outcome pathways (AOPs) approach. Thus, literature research in the database AOP-wiki was performed to identify the adverse outcomes (AOs) related to the selected B-NMs, focusing especially on lung diseases. All the AOPs investigated shared at least three key events (KEs): oxidative stress, inflammation and cytotoxicity. Therefore, after p-chem characterization of the BMs, the identified endpoints were investigated in vitro in A549 lung cells and THP-1 immune cells. According to the testing strategy and the methodologies proposed, all the tested B-NMs result not or moderately toxic toward human cells, testifying or their biocompatibility, also when compared to reference NMs of similar composition, but not of bio-origin (i.e. for SiO2 NMs). However, results showed that attention should be given to possible AOs deriving after specific functionalization of the B-NMs (i.e. addition of phenolic groups or phytic acid) that determined some cytotoxic, inflammatory and pro-oxidative effects. Moreover, the monitoring of long-term effects should be considered. This evidence suggests that a SSbD approach should be applied also after specific functionalization of non-toxic B-NMs to produce efficient new materials, while minimizing their hazardous impacts. Especially considering the lack of knowledge in this field, the nanotoxicity studies performed here represent a step forward in the state of the art of the safety assessment of innovative B-NMs.

Session 2

10:30-11:30

RISK ASSESSMENT AND MANAGEMENT

Dose-response, biodistribution

Marion Blayac

Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center (CPC-M), Germany, Member of the German Center for Lung Research (DZL), Munich, Germany

Surface area-based hazard ranking of aerosolized nanomaterials from epithelial lung cells cultured under air-liquid interface conditions: A meta-analysis

Respiratory toxicity of engineered nanomaterials has been extensively assessed with in vivo and cell-based submerged in vitro models. While in vivo testing is costly and time consuming, in vitro testing in submerged exposure conditions is neither representative of what occurs in the lung nor is it well controlled for cell-delivered dose. On the other hand, there is a substantial body of in vitro hazard data for air-liquid interface (ALI) cultured lung epithelial cells, which might overcome the limitations of submerged cell culture models. We conducted a meta-analysis searching the PubMed database to investigate the effects of nanomaterials on ALI lung epithelial cells. We retrieved more than 300 hits based on title and abstract, from which 57 studies were selected based on the following selection criteria: i) the study was conducted on human epithelial lung cells, ii) well-characterized pristine (i.e non-modified, non-coated) or standard reference nanomaterials were used, iii) accurate information on cell-delivered dose was available, iv) surface area was provided to be considered as the dose metric and v) cell viability (metabolic activity), LDH cytotoxicity and/or cytokine release were reported. Generating particle surface area-based dose-response curves, we investigated potential effects due to material type, time of exposure and post-exposure incubation time. Hazard ranking based on IC50 values for cell viability revealed zinc and copper oxide as the most toxic materials for A549 cells. Cerium and titanium dioxide were medium-level toxic materials, while black carbon and multi-walled carbon nanotubes (MWCNT) clustered in a low-toxicity regime. Culturing A549 cells in mono or co-culture conditions with differentiated d-THP-1 cells did not have any effect on the dose response pattern. On the other hand, bronchial epithelial cell lines or primary bronchial cells did not seem to respond substantially different to A549 alveolar cells. Moreover, dose-response seemed to be relatively independent of duration of exposure and post-exposure incubation except for copper nanomaterials for which longer exposure duration shifted IC50 to higher toxic values. Finally, comparison with selected in vivo data on acute pulmonary toxicity revealed good agreement between in vitro and in vivo hazard ranking for zinc oxide, copper oxide, titanium dioxide and carbon black materials. On the other hand, MWCNT in vitro data did not seem to be representative of in vivo response, but the in vitro data base is scarce. Overall, air-liquid interface cell culture models could be suitable for predicting acute in vivo pulmonary toxicity of at least some types of nanomaterials.

Muratov Viaceslav

University of Gdansk, Faculty of Chemistry, Gdańsk, Poland

Exploring the Role of Machine Learning in Identifying Potential Transcriptomic Biomarkers in Pulmonary Diseases Caused by Titanium Dioxide Nanoparticles

The widespread industrial use of Titanium Dioxide Nanoparticles (TiO2-NPs) and their hazardous ability to provoke pulmonary fibrosis and inflammation necessitate comprehensive safety evaluations to address their potential pulmonary toxicity. In this context, the utilization of omics-based biomarkers in risk assessment is crucial for understanding the mechanisms of pathology induced by TiO2-NPs, which result in diverse adverse outcomes following their exposure. Conversely, discerning the pivotal structural properties of materials capable of initiating cellular or tissue injury mechanisms holds significance, as it facilitates the prediction of

omics-based responses based solely on the known characteristics of the stressor-organism interactions. Thus, our study, aimed to answer the question: "How do the dose-time variations of differentially charged TiO2-NPs affect genes linked to lung inflammation, potentially serving as biomarkers for long-term exposure detection?" provides an advanced dose-time response modeling approach to elucidate the relationship between varying doses and post-exposure periods of TiO2-NPs and the transcriptomic changes in pulmonary tissues. This innovative approach leverages machine learning to predict alterations in gene expression being a result of NP exposure.
We have meticulously characterized the TiO2-NPs by dose and charge, accounting for their dynamic interactions with biological systems over the post-exposure periods. The investigation hinged upon analyzing transcriptomic data from mice exposed to these nanoparticles, identifying a set of genes whose expression levels were significantly modulated after the TiO2-NPs exposure. Thus, our machine learning model training procedure involved 618 genes, refining the prediction of their transcriptomic response to TiO2-NPs exposure based on their dosage, charge, and post-exposure periods. Based on our models, we identified that the dose of the TiO2-NPs is the most crucial factor affecting the transcriptomic response to the exposure. Our findings spotlight several genes, such as Arpc1b, Csf2, Ccl3, Ccl11, and Map3k6, delineating their significant roles in inflammation and suggesting their potential as transcriptomic biomarkers for TiO2-NPs exposure.

Session 2

10:30-11:30

RISK ASSESSMENT AND MANAGEMENT

Multicomponent nanomaterials and mixture effects

Dawid Falkowski

QSAR LAB, ul. Trzy Lipy 3, 80-172, Gdansk, Poland

A New Computational Approach for Toxicity Assessment of Binary Mixtures: Combining Dose-Response Curves with Mixture Toxicity Indexes

With the booming production of Innovative Advanced Materials (IAMs) and their inevitable release into the environment, assessing their toxicity right from the development stage has become crucial. While traditional lab tests often focus on single substances, the real world is far more complex. In environment, these substances mingle with a variety of other compounds, potentially altering their toxicity. That's why it's essential to evaluate a substance's toxicity within the broader context of its environmental interactions. Embracing the SSbD policy, we now lean towards computational toxicity assessment, a cutting-edge alternative that outshines traditional in vivo methods. In this work we propose a new combined approach to assess toxicity of the mixtures, before market launch. Based on this approach and a dataset containing nanomaterials-ions mixtures, we calculate several types of mixture toxicity indexes.

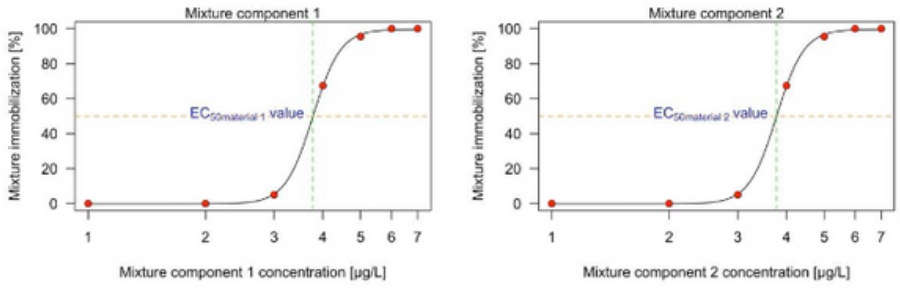

Figure 1 Graphical explanation of two DRC curves method

In our work, we propose a computational approach to support the prediction of the joint effects of AdvNMs and MCNMs at the early design phase, prior to synthesis. To address the lack of knowledge about the concentrations of individual components in EC50 mixture samples, we utilized the known concentrations of the individual components in the binary mixtures used to determine the EC50 dose and effect (e.g., immobilization). Based on these parameters, two dose-response curves were obtained for each component of the binary mixture. The individual concentration in the mixture samples was used as the dose, while the response of the binary mixture was used as the response. Using these curves, we determined the concentrations of the mixture components necessary for calculating mixture toxicity indexes.
Based on the compositions of binary mixtures of nanoparticles with metal ions available in the literature, the developed approach was utilized to obtain the values necessary for calculating toxicity indices. Four toxicity indices were calculated: STU, AI, MTI and MDR to assess modes of action (additivity, synergism, and antagonism) for specific samples. In the next step, the indices were compared and displayed graphically.
As a result, we obtained a set of 17 binary mixtures of nanoparticles (NPs) and metal ions, characterized by four toxicity indices: STU, AI, MTI and MDR. Primarily, the binary mixtures of nanoparticles with ions exhibited an antagonistic effect, but in four analyses, the effect was synergistic. The effects of toxicity as characterized by these indices were also compared. In 11 of the 17 samples, different types of indices yielded the same toxicity results

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Session 3

16:00-16:30
HAZARD ASSESSMENT
Ecotoxicity

Karsten Schlich

Fraunhofer IME, Ecotoxicology, Schmallenberg, Germany

Investigation of ecotoxicological effects of fibrous and platelet-shaped advanced materials for deriving adapted testing strategies - the project FaPlaN

Advanced materials are a very heterogeneous group of materials, and used in many applications. Responsible use of these materials also includes the identification of potential threats to people and the environment as quickly and as precisely as possible. Hence it is unclear to date, whether OECD test methods in the field of ecotoxicology (already established and adapted for testing nanomaterials) may also be applicable for this class of materials.
Due to their special properties, fibrous and platelet-shaped materials represent advanced materials for which a reliable assessment of a potential environmental hazard based on the available test methods is challenging. The project “FaPlaN” presented here therefore aims to obtain information on the mechanisms of action (MoA) and thus a mechanistic understanding of potential toxic effects exerted by fibrous and platelet-shaped advanced materials. Based on this, methods will be adapted which will allow to identify related effects. By this, ecotoxicological assessment will be facilitated in the long term.
This poster will present results from a literature review on the effects of fibrous and platelet-shaped advanced materials in aquatic organisms, with a focus on identifying MoA. In addition, an initial proposal for test systems that will potentially be part of the future test strategy will be presented.
However, the main aim of this poster is to exchange experiences with other (eco)toxicologists to learn more about possible mechanisms of action and to take these into account when adapting the testing strategies as the project progresses. There are three key questions, which are of main interest.
Key question 1: Which physical-chemical material properties must be considered?
Key question 2: Which MoA are relevant for fibrous and platelet-shaped advanced materials?
Key question 3: How need test methods/test strategies be adapted for fibrous and platelet-shaped advanced materials?

Maria Rivero

ITENE, Valencia, Spain

SSbD strategies validated for toxicity reduction applied to active nanomaterials with antibacterial and antiviral properties

A promising solution for reducing the risk of infection spread from dangerous pathogens on various high-traffic object surfaces is the development of coatings with antiviral and antimicrobial qualities based on active nanomaterials (ANM), such as bio-based active materials and inorganic nanoparticles (metal, plastic, and textiles). However, if the materials employed to create the coating are unsafe or perform poorly in terms of efficiency or durability, these advancements may influence the environment and human health. Therefore, a crucial component of ensuring their safety is the application of Safe- and Sustainable-by-Design (SSbD) techniques. Developing ANM safely, suited for the final applications when they will be used in coatings, with reduced cytotoxic and ecotoxic effects, has been one of the main goals of the HE SUSAAN Project. Zinc oxide nanoparticles are widely recognised for their antibacterial properties, but they also affect aquatic life and cells at the cellular level. Encapsulating these nanoparticles in silica has been one strategy used to reduce the risks associated with some of the ANMs created throughout the study. In vitro toxicological studies on human cell lines representative of the main entry routes into the organism (oral, dermal, and inhalation) have been conducted to evaluate the cytotoxic and dermal irritation effects. Additionally, bioassays using aquatic organisms in accordance with OECD procedures (201, Algae growth inhibition test; and 202, Daphnia sp., acute immobilisation test) have been carried out to validate the reduction regarding effects associates with environmental health. The results have shown a significant reduction of the toxic effect of ZnO nanoparticles encapsulated in silicon oxide on cell viability in the case of the three lines used (HaCaT, A549 and CaCo-2), as well as in aquatic organisms.

Acknowledgement:
The authors would like to acknowledge SUSAAN Project (SUStainable Antimicrobial and Antiviral Nanocoating) with Grant Agreement N° 101057988. Special acknowledgement to partner Lurederra, for developing and supplying the nanoparticles and their encapsulants.

Henk J. van Lingen

Wageningen University & Research, Laboratory of Systems & Synthetic Biology, Wageningen, Netherlands

Short and long term gene expression profiles in earthworms after exposure to ZnO and ZnO:Mn nanomaterials

Metal containing nanomaterials applied for purposes such as cosmetic products may be released into the environment during their use and be taken up by various organisms such as earthworms. These nanomaterials studied within the EU project DIAGONAL may exert toxic effects and affect gene regulation and metabolism in these earthworms. The aim of our study is to quantify gene expression in earthworms (Eisenia fetida) in response to ZnO and ZnO:Mn nanomaterial and MnCl2 exposure using bulk RNA-seq analysis. In a first experiment, earthworms were either exposed to clean reference soil or to soil contaminated with the ZnO:Mn nanomaterial. Earthworms from control and ZnO:Mn treated soil were collected after 14 days of exposure. A second experiment was performed in which the ZnO:Mn nanomaterial was replaced by MnCl2. Moreover, a third experiment was performed in which earthworms were harvested after only 2, 3, 4 and 7 days of exposure to control or ZnO contaminated soil. Whole earthworms from all three experiments were ground and subdivided into two equal amounts enabling both transcriptomics and metabolomics analysis. The present abstract focusses on the transcriptomics analysis and we refer to DIAGONAL project collaborators Zagana et al. for the metabolomics analysis. Depending on the experiment, extracted and sequenced RNA was assembled and annotated de novo or using a reference database, which resulted in read count number per gene and sampled earthworms. Read counts were normalized and analyzed for differential gene expression along with potential time-dependent effects in the third experiment. Functional enrichment of genes found differentially expressed in the obtained RNA-seq data identifies how these genes are involved in regulating the response to ZnO and ZnO:Mn nanomaterials and MnCl2 in earthworms.

June-Woo Park

Environmental Exposure & Toxicology Research Center, Korea Institute of Toxicology, Jinju 52834, Republic of Korea

Long-term aquatic exposure alters the toxic potential of multi-walled carbon nanotubes

Multi-walled carbon nanotubes (MWCNTs) are widely used in various industrial applications, but they are not readily biodegradable and therefore persist in environmental matrices, potentially serving as a source of toxicity to organisms. However, the effects of environmental weathering on the toxicity of MWCNTs remain unclear. To investigate this, we prepared aged-MWCNTs (a-CNTs) by incubating commercial pristine-MWCNTs (p-CNTs) for two years in water. Then compared their changes in physicochemical properties and toxic effects between a-CNTs and p-CNTs using zebrafish as a model organism. Characterization techniques, including transmission electron microscopy, X-ray photoelectron spectroscopy, Raman spectroscopy, and Fourier-transform infrared spectroscopy, revealed that a-CNTs had increased surface area, pore size, structural defects, and surface oxidation compared to p-CNTs. Zebrafish were exposed to 100 mg/L of both p-CNTs and a-CNTs for four days. The results showed that the mRNA expression of antioxidant enzymes, such as cat, gst, and sod, increased by 1.5 to 1.7-fold in the a-CNT group. This was accompanied by an upregulation of genes associated with inflammation (IL-8) and apoptosis (p53) compared to the control group. The difference in expression levels between p-CNT and a-CNT can be attributed to the increased oxidative potential resulting from the altered physicochemical properties. These findings offer new insights into the risk assessment and environmental management of MWCNTs in aquatic ecosystem. However, further testing is warranted using environmentally relevant doses, varied exposure durations, and diverse environmental weathering conditions to fully elucidate the implications of nanomaterial aging on their environmental impact.

16:00-16:30 **Session 3**

DATA

Data-driven modelling

Giorgios P. Gakis

NTUA, Research Lab of Advanced, Composite, Nano-Materials and Nanotechnology, Materials Science and Engineering Department, School of Chemical Engineering, Athens, Greece

Metal and metal oxide toxicity: Using structure-activity classification models for the extraction of toxicity information from larger datasets

The increasing intentional and unintentional exposure to engineered nanoparticles (NPs), due to the extended range of nanotechnology applications, has raised concerns over their safety. In recent years, the demanding nature of in vitro and in vivo methods has led to the emergence of in silico methods such as structure-activity relationship (SAR) models, aiming provide a more rapid nanomaterial toxicity screening. However, SAR models are usually developed using limited datasets, using complex descriptor combinations. The focus on the model predictivity over smaller datasets often hinders the interpretability of the results, deeming the extraction of scientific knowledge challenging, as the models are usually built for a specific case study, often referring to an individual set of experiments. In the present work, the focus is shifted towards the potential of using SAR models as a first step towards induction and extraction of valuable mechanistic information, using larger datasets and interpretable descriptors. For this reason, classification models are built for extensive and heterogeneous datasets consisting of toxicity measurements for pure and multicomponent nanomaterials consisting of metals and metal oxides. Although the classification is binary, restricting the quantitative prediction of toxicity, a more holistic understanding of metal and metal oxide NP toxicity can be obtained using the classification model, revealing key mechanisms of such NPs. The presented approach aims to trigger a discussion regarding the capability of SAR models to extract information from large datasets, in order to serve as a rapid first step for more detailed mechanistic investigation, revealing key nanomaterial properties that induce toxicity. Such a computational approach, combined with experimental

investigations in an iterative way, could pave the way towards a more knowledge-based risk assessment of NPs and guide researchers towards the synthesis of safe-by-design NPs.

Çiğdem Bilgi

İzmir Institute of Technology, İzmir Institute of Technology / Bioengineering Department, İzmir, Turkey

Machine Learning-Assisted Insights into Silver Nanoparticle Synthesis: The Role of Natural Reduction Sources

reen synthesis of nanoparticles involves utilizing natural resources (e.g., plants, microorganisms, or enzymes) as reduction agents instead of potentially toxic chemicals, offering an environmentally friendly and sustainable alternative to traditional methods, which often involve toxic reagents, solvents, and surfactants. Silver nanomaterials are utilized for diverse applications in medicine due to their potential therapeutic effects, including antimicrobial and cytotoxic activities. They can serve as drug delivery systems, imaging agents, and diagnostics with biosensing capabilities. Since almost half of the nano-enabled commercial products contain silver nanoparticles, researchers use green synthesis to develop new, green, and sustainable production techniques. Motivated by the rising interest in green-synthesized silver nanoparticles (GSSNs) and the recognition of inconsistencies in existing data, this study aims to investigate the influence of natural reduction sources on GSSN characteristics and bioactivity.
In this context, a cursory search of the Scopus scientific database was conducted using the keywords "cytotoxicity", and "green synthesis silver nanoparticle" and identified more than 1100 publications, including only research aritcles. A relatively large pool of data on cell viability from multiple assays was gathered, including characteristics of the nanoparticles (e.g., size, shape, zeta potential), and the details of the natural reduction sources, including botanical and phytochemical information such as plant family and secondary metabolite profiles (e.g., alkaloids, flavonoids, terpenoids, steroids, cardiac glycosides) associated with each source.
Employing different machine learning algorithms, we aim to develop a model that predicts the cytotoxicity potentials of GSSN based on these combined parameters. This approach could accelerate the development of safe and effective (with desired bioactivity) green-synthesized nanomaterials for biomedical applications.

16:00-16:30 **Session 3**

DATA

FAIR data and data management

Vesa Hongisto

Misvik Biology, Toxicology, Turku, Finland

Automatic workflow for in vitro high-throughput screening data fairification, preprocessing and scoring: A case study on nanomaterials

The field of toxicological research relies heavily on high-throughput screening (HTS) to evaluate the potential hazards of various chemical substances. With the advancement of technology, HTS has enabled the generation of vast amounts of data. In the realm of chemical safety and HTS data, The ToxPi software has gained popularity as a means of conveying risk prioritization and profile information to scientists, regulators and stakeholders. Tox5-Score is a novel concept for evaluating and prioritizing toxicity in vitro and is applied in two stages: (i) normalization of the HTS metrics for each time point and endpoint; (ii) combination of the normalized metric values to obtain final Tox5 endpoint scores.
Data management based on FAIR (Findability, Accessibility, Interoperability, and Reuse) guiding principles supports consistent machine-driven curation and reuse of the accumulated data by the nanosafety, cheminformatics and bioinformatics communities. We address the HTS FAIRification challenges – namely data pre-processing reproducibility and efficient data storage by 1) ToxPi score automation approach

implemented as add-on of the user friendly Orange Data Mining software 2) introducing well known and reusable binary format optimized for data matrices.
The automated Tox5-scorng is programmed in Python and follows exactly its original version implemented in Excel. It takes raw data files (containing results associated with each 384-well plate) and metadata file on input, where the metadata file is integrated in eNanoMapper Template Wizard for future reuse. To enhance accessibility for non-programmers, we have created an Orange Data Mining add-on for the Tox5-scorng system. The Orange Data Mining System https://orangedatamining.com/ is an open-source, visual programming tool designed primarily for data analysis and data mining. We demonstrate the workflow with two different HTS datasets, existing data from caLIBRAte project and new HTS dataset from HARMLESS project.
The TOX5 module and Tox5-score orange add-on extends a previously developed FAIRification workflow (the eNanoMapper workflow) towards application to HTS data. The HTS data is parsed and pre-processed with the TOX5 python module. The data structures are then converted into the eNanoMapper data model using pynanomapper library and stored as HDF5 file. The file has hierarchical structure with rich metadata and includes both raw, normalized and interpreted data (scores) in machine-readable format, which can be distributed as database independent archive and/or integrated into the eNanoMapper database and Nanosafety Data Interface.
Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 953183

16:00–16:30 Session 3
DATA
Multi-scale modelling

Roshan Shrestha

Molecular Microbiology and Structural Biochemistry (MMSB), UMR 5086 CNRS, University of Lyon, Lyon, France

Adsorption of Albumin on Graphene and Graphene Oxide: insight from molecular simulations

Graphene and graphene oxide-based nanomaterials (NMs) have been subject to extensive research for their use in both technological and biomedical applications. When incorporated into other bulk materials to form nanocomposites, they provide excellent mechanical, electrical, thermal, optical and chemical properties. However, they can potentially leak out and make contact with the environment and eventually with a physiological milieu. If incorporated into a biological environment, their interaction with proteins will govern the biological fate, uptake, and toxicity. Due to their high surface area / volume ratio, graphene and graphene oxide have strong adsorption capacities for these proteins. Coating by plasma proteins confers a new biological identity to the NMs, determining their biological fate. One of the major components of human blood plasma is albumin. Here, we performed coarse-grained (CG) molecular dynamics (MD) simulations to study the adsorption of albumin on graphene and graphene oxide with varying sizes and degrees of oxidation. In particular, we studied the formation of the so-called protein corona on these NMs and provide estimates for the strength of adsorption as a function of the degree of oxidation. Finally, we studied the interaction of these carbon-based NMs with membranes in the absence and in the presence of a protein corona. Our predictions can help interpreting experimental data on protein adsorption onto NMs.

16:00-16:30 Session 3

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Multicomponent nanomaterials and mixture effects

Elena R. Kisin

National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC), Morgantown, United States

Dermal Toxicity Following Acute and Sub-Chronic Co-Exposure to Spinel Ferrite Nanoparticles and Ultraviolet B In Vitro

The unique properties of spinel ferrite nanoparticles (SFNPs) have attracted significant attention and have led to utilization in various advanced applications such as sensors, catalysts, power electronics, spintronics, recording materials, drug delivery systems and more. However, there is limited or conflicting information available concerning the toxicity of SFNPs. It is also of importance to address the combined effect of SFNPs with ultraviolet B (UVB), one of the most significant environmental factors affecting human health and contributing to a number of cutaneous diseases. The aim of this study was to investigate the cytotoxicity, oxidative stress, DNA damage, inflammation and potential to induce cell transformation following exposure of primary human epidermal keratinocytes (HEK) to two SFNPs, NiFe2O4 or CoFe2O4, with or without UVB (1-2 kJ/cm2) pre-treatment. Acute (24-72h) co-exposure to SFNPs (0-20 µg/cm2) and UVB induced dose-dependent loss of viability, cell damage, intracellular ROS accumulation, release of inflammatory mediators, increases in oxidative stress markers and DNA damage. Based on the hierarchical clustering analysis of the inflammatory cytokine/chemokines responses, HEK co-exposed to SFNPs and UVB were segregated from UVB control and non-UVB exposed samples. Interleukin (IL)-8 and RANTES were upregulated in response to SFNPs alone exposure while IL-1β, IL-6 and tumor necrosis factor-α were significantly increased with UVB pre-treatment. Sub-chronic exposure for 9 weeks to sub-toxic dose of SFNPs resulted in alteration of cell morphology, significant oxidative modification of proteins, accumulation of lipid peroxidation products and induction of double-strand DNA damage. UVB pre-treatment induced marked amplification of the observed responses. Moreover, SFNPs alone induced significant changes in cells' invasion, migration, and anchorage-independent growth stimulated by UVB pre-treatment. Nickel NPs, known to cause cell transformation, were used as a positive control. These findings are further supported by observed levels of cytokines/chemokines/growth factors secretion related to inflammatory and TH2-type/regulatory immune responses. Altogether, these results clearly indicate that spinel ferrite NPs alone or combined with UVB pre-treatment could induce cytotoxicity, oxidative stress, and inflammation, and may potentially influence neoplastic-like transformation in HEK. Further studies focusing on understanding molecular mechanisms and likelihood to induce tumorigenic effects are necessary.

Franz Friebel

femtoG AG, femtoG AG, Zurich, Switzerland

Nanoparticle release from pigments - introducing the absolute (nano)particle concentration in a powder

The EU regulation on nanomaterials identifies a nanomaterial based on its relative content of particles with dimensions of less than 100 nm. Many pigment materials (TiO2 / titanium dioxide; FeOOH / iron oxyhydroxide) are coated with different components, which makes their analysis challenging. Whether or not the coating adheres to its core particle does affect the pigment's performance. More important, however, is that sheared-off coating particles can be smaller than 100 nm. Therefore, the material itself might have to be labelled as a nanomaterial. A simple number percentage does not represent the release of nanoparticles sufficiently well. Larger particles are simultaneously destroyed during the dispersion, release particles

(>100nm) and increase the absolute number of particles. Thus, nanoparticle concentration gets diluted. We introduce the absolute number of nanoparticles per gram of powder as a new unit to quantify the absolute amount of nanoparticles.
We adopted the newly developed Mass & Mobility Aerosol Spectrometer (M2AS) to record number-weighted distributions of the particle mass and diameter (el. Mobility) for the analysis of commercially available colour and white pigment as well as CNTs. Fig.1 shows a data set for an iron yellow pigment consisting of FeOOH particles coated with aluminium phosphate (AlPO4). The difference in the effective density (black dots, left axis) allows us to distinguish between both materials. The density of the AlPO4 particles is lower than that of the heavier FeOOH particles. By curve fitting and integration of the number-weighted mass distribution (turquoise, right axis), the number of particles per gram material and the total mass fraction of the two components can be determined. In total, one gram of material contains 1.5·1013 particles. 70% (number) of those are isolated AlPO4 particles, contributing only 10% of the total mass. This is a subset of the total AlPO4 content of 26% (mass) which the producer added. This implies that 40% of the coating material does not adhere to the FeOOH core. These isolated particles are light and small. In total, 9.4·1011 particles/g (6% by number) are smaller than 100 nm and have to be classified as nanoparticles. Similar results will be presented for TiO2 including data on the release of nanoparticles as a function of dispersion intensities.

Fabienne Testard

CEA, Universite Paris Saclay, CEA/CNRS_NIMBE, UMR 3685/LIONS, Gif-Sur-Yvette, France

Modified imogolite nanotubes: How inner functional group modification in multicomponent HARN impact the toxicity

The natural aluminosilicate imogolite clays1 have been used for different applications like catalysis, gas adsorption, membrane filtration, flam-retardant to cite a few.2,3 This large spectra comes from their specific properties (nanocavity, hydrophilicity, polarization, large band gap, etc..) intrinsically linked to the high aspect ratio nanotube morphology. However, innovative applications requires modification of the internal function of the nanotubes. Increasing the complexity of imogolite from natural one (imoOH, hydroxyl on the inner surface) to multicomponents hybrid imogolite (imoCH3, methyl on the inner surface)4,5 and Cu functionalized (on the outer surface) imoCH3 requires a knowledge on their potential hazard6,7 to develop a Safe and sustainable by design (SSbD) approach for these materials.
In HARMLESS (GA n° 953183), imogolites are studied as a case study in the field of plant protection. A general SSbD approach is under construction where in vivo studies are used as a dataset for validation. As a first step, in vivo studies were performed to determine the toxicity of imogolites (imoOH and imoCH3) in mice following intratracheal instillation into the lung. First results evidence a clear difference between the imoOH and imoCH3, where the latter induced lower inflammatory and acute phase responses suggesting a lower hazard potential. The inflammation were long-lasting and still significantly increased at 28 days for both imogolites and persisted at 3 months after exposure for imoOH only. These results will be discussed considering physico-chemical characterization of the materials and intended functionalities.

Karen Pillay

University of KwaZulu-Natal, School of Life Sciences, Durban, South Africa

Antibiotic activity of green synthesized nanoparticles

Antibiotic resistance is on the increase and this has a drastic impact on global public health. The incorrect prescription of antibiotics as well as their incorrect use has been a leading cause of antibiotic resistance; and even though good practice dictates that antibiotic treatment should be specific for the invading microorganism, broad-spectrum antibiotics against both Gram-positive and Gram-negative bacteria are often administered. This is often ineffective as bacteria have developed resistance to all classes of antibiotics commercially available. There is therefore an urgency to develop new therapeutic agents. Nanoparticles have been emerging as novel means to overcome the problem of antibiotic resistance due to their multi-pronged approach in effecting antibacterial activity. It has been seen that nanoparticles have the ability to generate reactive oxygen species that results in destruction of the bacterial cell membrane. Metallic nanoparticles have

also been theorized to result in slow release of metal ions which can disrupt electron transfer in bacterial cells thereby inducing a bactericidal effect. Due to the small size of nanoparticles, they are also able to easily penetrate the bacterial cell membrane and more importantly facilitate disruption of biofilms. Pathogenic bacteria are therefore unable to mutate rapidly enough to overcome all of the antibiotic mechanisms exerted by nanoparticles. Nanoparticles containing Ag, Au, Zn, Ti and Cu have been found to have good antimicrobial activity. Current physical and chemical techniques used for the production of nanoparticles have various disadvantages, one of which being the production of toxic by-products. There is thus an emergence of biological systems as synthetic agents and this study focused on the use of green strategies for metallic nanoparticle production. Both plant and bacterial species were evaluated. The plant species Aloe ferox was chosen as it contains phytochemicals that have demonstrated medicinal properties and the bacterial species Magnetospirillum magnetotacticum was chosen as it has an inherent ability to internalize metal and then package it into magnetosomes, which are nanoparticle-containing organelles. Both these species were found to have the ability to produce nanoparticles composed of either silver or gold. These nanoparticles were then evaluated for their antibacterial activity and the silver nanoparticles derived from A. ferox were found to show good potential as antibacterial agents.

Wendel Wohlleben

BASF SE, Dept. Analytical and Materials Science, Ludwigshafen, Germany

Assessing the dust released from inorganic aerogel mats: occupational release scenarios simulation and hazard implications in a SSbD context

Because of aerogel mats novelty in the global market, niche use and state as article, very little is known about their safety and sustainability along the life cycle. Due to their encapsulated use, these materials don't pose a threat to consumers nor the environment during use. Instead, mat installation by professionals, during which different mechanical treatments are applied, can release aerosols in the respirable range, leading to potential inhalation risks for workers. Screening approaches starting at early design phases can be valuable to inform Safe and Sustainable by design (SSbD) modifications.

Given that they don't consist of particles, we first simulated aerosol release in three realistic working scenarios for four Aerogel Mats intended to be used as thermal building insulators. The mats show different structural modifications, expected to enhance their performance. Release tests included cutting using an insulation knife, and a circular saw, both relevant for the construction process, and sanding, to mimic a tear-down scenario. The aerogel dust collected from each experiment was used to address possible hazard implications by using two in chimico assays established from the GRACIOUS IATAs, 1) surface reactivity on human blood serum using the Ferric reducing ability of serum (FRAS) assay, and 2) particle's dissolution kinetic under lung simulant conditions, to understand particle fate upon inhalation.

The four aerogel mats are compared with two conventional stone wool insulators for SSbD purposes. Conventional insulation materials such as stone wool show a thermal conductivity between 30-40 mWm-1k-1, whereas inorganic aerogel mats can go lower than 20 mWm-1k-1, hence more than 2-fold improvement. The improved performance is linked to higher benefits provided by aerogel mats in terms of energy savings. This aligns with the European Green Deal and positively impact some of the sustainable development goals (SDGs), however, the performance related benefits must be balanced with both safety and environmental sustainability aspects.

We provide guidance in the selection of criteria to be considered at the design phase of the product and, in the selection of screening methods, useful in a SSbD context, easily accessible to companies and start-ups for a safe and economically feasible innovation with advanced insulation materials.

16:00-16:30 Session 3

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Safe-and - Sustainable-by-Design strategies and case studies

Julia Voglhuber-Höller

BioNanoNet Forschungsgesellschaft mbH, (BNN), Graz, Austria

The Safe-and-Sustainable-by-Design approach for alternative metal-free wound dressings in NABIHEAL

Chronic wounds are characterized by persistence over more than 6 weeks despite active intervention and while showing no significant progression towards closure and healing. Such problematic wounds present in up to 2% of the population in industrialized countries alone where numbers are more easily available than in underdeveloped countries, with escalating concerns due to the aging demographic. This not only burdens individual patients but also strains public health systems, accounting for up to 4% of the total healthcare budget in Europe. Naturally, the gravest concern in regard to non-healing wounds is the high risk of infection with microbes. Current standard-of-care treatments rely heavily on silver-based agents, despite their limited efficacy, as proven by heterogenous outcomes of clinical trials, environmental impact, and costliness.
Addressing these challenges and the need for improved alternatives, NABIHEAL aims to develop metal-free biomaterials for novel wound dressings. A particular emphasis will be given to anti-microbial activities and wound healing properties. Alongside the scientific and technical development, a Safe-and-Sustainable-by-Design (SSbD) approach will ensure that the developed nano-based multicomponent materials, as well as the individual components, will demonstrate the least risk to humans and the environment with a special focus on nano-related issues. For this we will leverage the SSbD framework published in 2022 by the Joint Research Centre of the European Commission (JRC-EC) and rely on BNN's expertise in the area of advanced materials and their applications, especially in the biomedical field.
In a structured and tiered process, including multiple rounds of questionnaires and on-site visits at production sites, the concept generation has been started by gathering initial information and assessing the status quo of the available materials 1 year into the project. In a further stepwise approach to support the innovation process, recommendations and advice will be given in order to prevent late developmental failures due to a lack in safety or sustainability. Each round of questionnaires is followed by risk and safety assessments, and, ultimately, the formulation of recommendations to further improve the SSbD criteria of NABIHEAL will be provided. This iterative approach will not only monitor the progress of NABIHEAL, but steer the project in a desirable direction in terms of safety and sustainability. By implementing these actions, we contribute to the development of innovative SSbD wound dressings, alleviating the burden of chronic wounds on affected patients as well as healthcare systems.

Vamsi Kodali

National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, United States

Influence of impurities from manufacturing process on the toxicity profile of boron nitride nanotubes in vitro and in vivo

The toxicity of boron nitride nanotubes (BNNTs) has been the subject of conflicting reports, likely due to differences in the residuals and impurities that can make up to 30–60% of the material produced based on the manufacturing processes and purification employed. Four BNNTs manufactured by induction thermal plasma process (AP BNNT) with a gradient of BNNT purity levels achieved through sequential gas purification (BR BNNT), water and solvent washing (W1 & W2 BNNT), allowed assessing the influence of these residuals/impurities on the toxicity profile of BNNTs. Hexagonal boron nitride (h-BN, <100 nm in diameter)

was used as a control material for synthesis by-products. Extensive characterization including electron microscopy, infrared and X-ray spectroscopy, thermogravimetric analysis, size, charge, surface area, and density captured the alteration in physicochemical properties as the material went through sequential purification. BNNT were 1.68 and 0.017 μm in length and diameter. Agglomerate sizes in dispersion media (DM) ranged from ~285-350 nm. In vitro, screening was performed on differentiated THP-1 macrophages to evaluate toxicity, inflammation, mechanisms of toxicity, and macrophage function. In vivo, male C57BL/6 mice were exposed by oropharyngeal aspiration to 4 or 40 μg of sample/mouse in DM or DM alone. Mice were euthanized at 4 h, 1 d, 7 d, 1 m, and 3 m post-exposure, lung lavage was performed to evaluate lung injury and inflammation on one group of animals. Lungs from a second group were collected for histopathology. As the material increased in purity, there are more high-aspect-ratio particulates and a corresponding distinct increase in cytotoxicity, nuclear factor-κB activity, and inflammasome activation. There is no alteration in macrophage function after BNNT exposure with all purity grades in vitro. Lavage parameters of lung injury and inflammation were significantly increased by the high dose of BNNTs at the early time points with W2>BR>AP and persisted in the W2 group up to 1 m post-exposure. The hBN sample produced low to no toxicity in lavage parameters measured. In lung tissue, alveolar inflammation and microgranuloma formation were noted with hBN and BNNT exposures; however, severity was minimal to mild for all groups. Incidence and severity were greatest in the W2 group and began to resolve in all groups by 3 m post-exposure. Recovery was fastest in the hBN group. Both the in vitro as well as the in vivo data show that greater purity of BNNT corresponds to greater toxicity.

Andrea Brunelli

Ca' Foscari University of Venice, DAIS, Venice, Italy

Safe and sustainable by design assessment of a multi-component nanomaterial embedded into an LDPE film food packaging

Insect pests annually infect up to 40% of global crop production, ending up in huge loss of resources and money (each year, plant diseases cost the global economy over $220 billion). To overcome this challenge, innovative ecofriendly-based solutions are needed. Therefore, according to the European Green Deal, a safe and sustainable assessment of a multi-component nanomaterial (MCNM) (i.e., nanoclays encapsulating essential oils) embedded into a light density polyethylene (LDPE) film food packaging as anti-pest was carried out. This work falls within the H2020 SUNSHINE project, an industry-oriented project developing materials under the Safe-and-Sustainable-by-Design (SSbD) framework proposed by the European Commission. In detail, as first step of the framework, the material safety was assessed by merging information from the literature or generated experimentally when needed, on: i) the physico-chemical characterization of both individual and MCNM; ii) the precursors for the MCNM synthesis; iii) the corresponding (eco)toxicological data. Afterwards, in line with step 2 of the framework, an industrial hygiene survey to identify potential hotspots of particles emissions from MCNM production, handling, processing or maintenance and cleaning phases was developed to then carry out an occupational exposure assessment. Moreover, as indicated in the final application/use phase of step 3 of the framework and according to Regulation (EU) N°10/2011, the potential migration of the active ingredients and of the inorganic elements constituting the MCNM was investigated from both pristine LDPE films and those after accelerated weathering. In addition to the material safety and in agreement with the framework, environmental, social and economic sustainability aspects of this MCNM were also investigated with the aim to support the design and development of safe and sustainable innovative materials.

Cristina Fábregas-Ordóñez

LEITAT, Barcelona, Spain

Nanosafety assessment of NewSkin related nanoenable paints in case of potential exposure via inhalation

Development of Key Enabling Technologies (KET´s) will be crucial for a significant part of the services and goods that will arrive to the market in following decades. Nanoenable surfaces must be highlighted within KET's, as they present huge potential offering material solutions to address sustainable development goals, resulting in positive impacts for the society and key industrial sectors. The EU-funded project NewSkin pursues to create an Open Innovation Test Bed (OITB) to provide the Innovation Ecosystem to Accelerate the Industrial Uptake of Advanced Surface Nano-Technologies. As part of this action plan, a safety assessment must be considered following the guidelines from the safe and sustainable by design (SSbD) EC Framework. We applied the hazard assessment strategy developed in the EU-funded project SAbyNA to study and minimize health risks for workers related with the use of materials involved in the development of two new nanoenabled paints.
Worker exposure via inhalation during powder manipulation was considered as a potential worst-case scenario risk hotspot. Four different materials were evaluated: an already commercialized product, an usual additive in anticorrosive powder paints (AluC) and the two new nanoenable paints powders (NePPA and NePPB). First, a compilation of available hazard information from ECHA, safety data sheets and literature was performed. For in vitro testing nanomaterial's safety, we specifically focused on respiratory pathway, so we used a human leukemia monocytic cell line (THP1). Materials dissolution behavior in simulated biological fluids and hydrodynamic size in cell culture media were studied. Materials effect on cell viability was assessed using the Trypan blue exclusion method. To assess inflammatory events, the release of cytokines (IL-6) and factors (TNFα) in cell medium was determined by ELISA assay. Finally, we evaluated reactive oxygen species (ROS) production through a fluorometric detection kit.
Respiratory toxicity was described in ECHA for Al2O3 while there was not available information for the other materials. All the materials, except the commercial one, were inside the nano range of particle presenting a hydrodynamic diameter ≤ 1 µm when dispersed in H2O miliQ at 100 µg/ml. Cell viability results showed that all the compounds affected cell viability at the highest concentration tested (100 µg/ml) and they were classified attending to their obtained EC50 values: AluC (EC50=5.81), Commercial (EC50=9.75), NePPA (EC50=22.23) and NePPB (EC50=25.35). None of the materials induced ROS production.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 862100.

Elena Badetti

Ca' Foscari University of Venice, DAIS, Venice, Italy

Physical-chemical characterization of multi-composite nanomaterials for supporting (eco)toxicological testing

This work is framed within the EU funded H2020 SUNSHINE Project, aiming to develop/implement Safe and Sustainable by Design (S&SbD) strategies for products and materials incorporating multi-component nano materials (MCNMs). In this context, this research focus on the physico-chemical characterization of a core shell structure, both in the solid state and in dispersion, in order to support the realization of robust and reproducible (eco)toxicological testing and the interpretation of the outcomes. The case study is a mortar containing two different concentrations (1 and 5%) of a SiO2@ZnO MCNM for the photochemical removal of NO_x from air. Investigating the (eco)toxicity of the active ingredient (the MCNM) is challenging since it can exerted toxicity from the combination of the two components or from the individual components by themselves, or it can be subjected to different transformations when dispersed in environmental or biological medium. Therefore, information on the colloidal stability, surface charge and ion release of both single and multi-component nano materials (MCNMs) dispersed in different media relevant for (eco)toxicological testing were investigated, as well as the release of ions. From the combination of different analytical techniques, such as dynamic and electrophoretic light scattering (DLS and ELS), centrifugal separation analysis, Raman and infrared spectroscopy, transmission and scanning electron microscopy (TEM and SEM) and inductively coupled plasma mass spectrometry (ICP-MS), a comprehensive characterization of MCNMs have been performed. The obtained results allowed to estimate the qualitative and quantitative stability of the tested dispersions over time, and to gather further significant information for a better correlation with the results from (eco)toxicity studies.

Sattibabu Merugu

University of Gdansk, Faculty of Chemistry, Gdansk, Poland

Nanosafety Assessment through AOP-Anchored Consensus Nano-QSAR Model: Insights into MWCNT-Induced Lung Toxicity

As risk assessment shifts towards new approach methodologies (NAMs) and adopts the 'safe-and-sustainable-by-design' (SSbD) framework, integrating in vitro and in silico methods becomes crucial within the Next Generation Risk Assessment (NGRA). Adverse Outcome Pathways (AOPs) offer a means for toxicogenomic analysis, aiding in bridging the gap from genotype to phenotype realistically. Despite advancements, limitations persist in individual predictive (Nano)QSAR models, particularly in sparse data scenarios. To address this, we developed an AOP-anchored Consensus Nano-QSAR model, leveraging diverse predictive datasets and employing advanced computational techniques (KwLPR) to enhance our understanding of MWCNT-induced lung toxicity. Our findings reveal a relationship between transcriptomics pathway perturbation and physicochemical properties of MWCNTs, providing insight into lung toxicity at the molecular level. External validation and applicability domain assessment enhance model reliability. Comparative analysis demonstrates the superior performance of our model. Moreover, our study suggests future directions for nanosafety assessment, promoting the development of safer-by-design strategies and sustainable practices in nanomaterial research. This research contributes significantly to advancing nanosafety assessment methodologies.
Acknowledgment:
This research was funded by the Polish National Science Center under grant 2020/37/B/ST5/01894 (TransNANO project).

Eva Penssler

Yordas Group, Forchheim, Germany

Guidelines on Safe-by-Design integration in industry's innovation process

Safe-by-Design (SbD) is on everyone's lips. Nanomaterials have been proposed as excellent test case for SbD as both their hazard and exposure can be altered during the product development. SbD has now been incorporated into Safe and Sustainable by Design (SSbD), a key aspect of the Chemical Strategy for Sustainability. For it to achieve its goals, SbD needs to be adopted into the innovation process for the next generation of materials and products, meaning its cost effectiveness and simplicity need to be demonstrated to innovators and developers. The Sbd4Nano project has set itself the goal of designing SbD strategies for nanomaterials with the focus on making them realisable for the industrial sector. Since different actors with different expertise are involved the implementation of SbD approaches, the project has designed factsheets that represent various relevant SbD topics and their implementation in a concise and easily understandable form. The factsheets are aimed at nonexperts designed to give an introduction to why and how SbD should be integrated into product development. They show how the SbD4Nano project has put SbD into practice for nanomaterials and developed an e-infrastructure to allow others to do the same.

Lisa Kleon

University of Salzburg, Salzburg, Austria

How green zinc- and cerium-based nanomaterials relate to industrial benchmarks across safety and sustainability dimensions - identification of alternatives for potent antimicrobial agents introducing

Over the past years, the rising number of multidrug-resistant pathogens resulting from improper use of antibiotics has become a global problem. It is therefore of great importance to prevent the spread of these pathogens by treatment or smart design of commonly touched surfaces, devices, and instruments which serve as reservoirs for infectious microbes. Certain types of nanomaterials represent a novel approach

because of their distinct and advantageous antimicrobial capabilities and thus could be exploited as surface materials to actively inhibit contaminations. Due to their unique properties, such as high specific surface area, stability towards degradation, broad-band adverse effects or bioaccumulation in certain organisms and biological sinks, some nanomaterials have a negative effect on the environment, therefore, particular attention needs to be given to develop green synthesized nanomaterials and perform comprehensive and well balanced assessment of functional performance in relation to safety & sustainability dimensions, as recently advocated for by the Safe-and-Sustainable-by-Design concept. In this study, we evaluated functional performance in form of broad-band antimicrobial efficacy of green synthesized zinc- and cerium-based nanomaterials using a newly developed surface interaction assay. Tested pathogens were two relevant bacterial strains as representatives of gram-negative and positive-bacteria, namely Escherichia coli and Staphylococcus aureus. Further, two widely spread fungi, namely Saccharomyces cerevisiae and Trichophyton rubrum, were chosen to be investigated. The surface interaction assay allows for a proper interaction between nanoparticles and microbes, with properly trackable bacterial/fungal growth conditions. Here, many conditions can be investigated, such as varying light/UV exposure, nanomaterial dose/surface coverage and incubation times. For covering safety-related aspects and considering that the skin is the main area of exposure, we conducted cytotoxicity and inflammatory assessments in keratinocytes using viability and cytokine release assays, which will be further explored in a novel 3d skin cell culture model. Zinc- and cerium-based nanoparticles already showed promising results by inducing a growth delay and inhibition of the investigated pathogens, even at lower dosages when compared to the industrial silver nanoparticle benchmark. While nanomaterials offer a significant potential for innovative antimicrobial surface design, it is essential to comprehensively investigate their safety and sustainability profile in agreement with their functional performance measures. This will allow considerate evaluation of potential trade-offs across different relevant dimensions already during material selection, prioritizing the protection of the environment and human health. This study, thus, also contributes to methodological development of the current version of the EU-SSbD Framework.

Day 3
25 September 2024

Session 7A

9:30-10:30

SUSTAINABILITY ASSESSMENT

How to integrate sustainability aspects into safety assessments

Chairs: Lisa Pizzol & Sarah Devecchi

Lya G. Soeteman-Hernández*, Stella Stoycheva, Vrishali Subramanian, Agnes G. Oomen, Lisa Pizzol, Magda Blosi, Anna Costa, Shareen H. Doak, Vicki Stone, Arianna Livieri, Vikram Kestens, Hubert Rauscher, Neil Hunt, Willie Peijnenburg, Danail Hristozov

*National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

09:30-09:55

A Conceptual Framework for Applying Safe-and-Sustainable-by-Design as a New Product Development Tool for Environmentally Sustainable Innovations

New environmental and health policy ambitions such as those presented in the European Green Deal and the EU Chemicals Strategy for Sustainability (CSS) challenge traditional innovation management theories and new product development (NPD) processes. In addition, while consumers' interest towards more sustainable and environmentally friendly products continues to grow, prior research indicates that integrating safety and sustainability considerations in the NPD process may be risky, costly and time consuming. The Safe-and-Sustainable-by-Design (SSbD) concept is a central element of the CSS and demands a novel innovation approach by integrating innovation management with safety, sustainability and circularity of materials, products and processes without compromising their functionality or their technical and/or commercial viability. Importantly, adopting such an approach can also prevent regrettable substitutions, future liability and brand image issues for companies. Safety and sustainability need to be accounted for in all life cycle phases of chemicals, materials, products, and processes in the innovation management system. In this Perspective, we introduce SSbD as a NPD tool for environmentally sustainable innovations and develop a conceptual framework on how to integrate innovation management with SSbD using life cycle thinking principles. This work makes a number of contributions to various fields. At the practical level, it fills an important knowledge gap by providing a sound framework to support sustainable innovations.
At the methodological level, it advances technical, environmental and organization and management sciences by bridging innovation management with safety and sustainability theories and practice through an exploratory case study of multicomponent nanomaterials.
At the policy level, it contributes to an ever-increasing demand for knowledge to support the transition to a more sustainable future by providing a science-based conceptual framework that incorporates life cycle and SSbD thinking.
Finally, important recommendations are made to embed SSbD and life cycle thinking in newly developed training for professional designation for innovation management. Innovation managers can play a key role in bringing this transition into operationalization.
This work has been carried under the H2020 SUNSHINE project which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952924.

Massimo Perucca*, Andrea Garrone, Mattia Costamagna, Anna Luisa Costa, Magda Blosi, Rossella Bengalli, Paride Mantecca, Juliana Oliveira, Franco Belosi, Alessia Nicosia, Jesus M Lopez de Ipiña, Irini Furxhi

*Project HUB360, Torino, Italy

09:55-10:12
Towards a nano-specific, quantitative based and human centric-SSbD Approach in ASINA and INTEGRANO

The need of applying a Safe and sustainable by desing (SSbD) strategy to the development of new chemicals and materials finds its main reference guidelines in the EU SSbD Framework1. For nano-forms and nano-materials diverse SSbD implementation quantitative and semiquantitative approaches have been investigated within the nano-toxicology community.
Here, a quantitative, case-specific and human-centric methodology is proposed supported by the artificial intelligence algorithm implemented within ASINA and INTEGRANO projects, enabling the selection of SSbD solutions by simultaneosuly addressing multiple and composite KPIs related to the safety, environmental, economic and functional dimensions. The methodology requires generating an harmonised data set associated to a specified DoE matrix. The advantage is found in the inherent minimum number of necessary and sufficient specific tox and eco-tox F.A.I.R. primary data required, which implies minimising the experimantal burden, while reducing time and cost for developing each NM design case study.
Case studies for the development of antibacterial nano-coatings investigated within the ASINA project throught the ASINA-ES decision support system are presented addressing the synthesis and incorporation of NMs life cycle stages. The need to integrate the environmental and safety assessments through the development of nano-specific eco-toxicity and human-toxicity indicators addressed in the INTEGRANO project will be presented as an anabling approach for the exploitation of existing international standardised assessment methodologies with defined protocols and metrics.

*Figure SEQ Figure * ARABIC 1a Set of multi-optimal SSbD cases (red spots) provided by the ASINA-ES in the multi-performance space whose coordinates are the key performance indicators (KPIs) for environmental sustainability (GWP), Human toxicity (HTTP) and composite antibacterial functionality-cost KPI.*

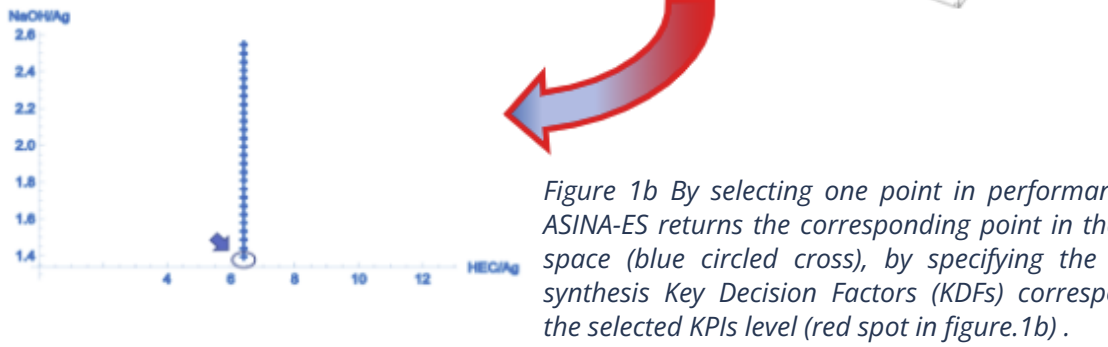

Figure 1b By selecting one point in performance space, ASINA-ES returns the corresponding point in the decision space (blue circled cross), by specifying the values of synthesis Key Decision Factors (KDFs) corresponding to the selected KPIs level (red spot in figure.1b) .

ASINA project received funding from the European Union's Horizon 2020 research and innovation programme under GA No 862444; INTEGRANO project has received funding from the European Union's Horizon Europe research and innovation programme under GA No 101138414

Caldeira, C., Farcal, R., Garmendia Aguirre, I., Mancini, L., Tosches, D., Amelio, A., Rasmussen, K., Rauscher, H., Riego Sintes, J. and Sala, S., Safe and sustainable by design chemicals and materials - Framework for the definition of criteria and evaluation procedure for chemicals and materials, ISBN 978-92-76-53264-4, doi:10.2760/487955, JRC128591

Jan Hildebrand*, Irmak Karakislak

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10:12-10:29

Public perceptions of nanomaterials & the role of trust – insights from the DIAGONAL project

application areas of nanomaterials, including cosmetics and electronics, are advancing to provide a more Safe-and-Sustainable-by-Design approach, public perceptions towards nanotechnology are sceptical. Within the DIAGONAL project, a multi-method approach was used in order to examine relevant factors on public perception. Firstly, a media analysis in Germany observed the societal discourse on nanomaterials, different actor groups and factors of public acceptance. This analysis included data from newspaper articles (N=272), semi-scientific magazine articles (N=99) and user comments from YouTube videos on nanotechnology advancements. Following the media analysis, a particular focus was on the public perceptions of nanomaterials in cosmetic sunscreens, which was one of the application areas of DIAGONAL demonstrators. Expert interviews and a pedestrian survey in Germany were conducted to prepare an expert workshop to discuss the potential and challenges of public trust towards nanomaterials in sunscreens.
Findings from the media analysis showed that the reporting of technology development was predominantly descriptive and did not discuss the social implications for the public. There was an ambiguity concerning the health and ecological risks of nanomaterials in the articles, which portrays the scientific uncertainty of this technology. Furthermore, social media users were opinionated towards nanotechnology, ranging from sceptical views of potential misuse to hopeful and curious outlooks on the future of applications. There tends to be disappointment in not receiving the promised advancements in nanoscience, shaped by its intangible nature and existing associations of the technology. Thus, there is a need for continued efforts to enhance public engagement by increasing and diversifying the range of topics in news reports and creating outreach activities from social media channels to disseminate information.
Following these results, an interdisciplinary expert workshop, which included 20 participants, covered the specific factors of public trust towards nanomaterials as well as connected institutions. Results indicate the role and responsibilities of actor groups in shaping public trust by providing transparency on products and procedures. By this means, potential outreach activities for creating awareness and providing differentiated knowledge should aim at increasing trust as a base for further communication. Overall, this study contributes to a nuanced understanding of nanotechnology and its societal implications.

Session 7B

9:30–10:30
RISK GOVERNANCE
Horizon scanning and foresight & SSbD (prevention-based governance)

Chairs: George Katalagarianakis & James Baker

Wilson Engelmann*, Raquel von Hohendorff

*University of Vale do Rio dos Sinos, Law School, São Leopoldo, Brazil

09:30-09:48

Safe by design as a tool for Responsible Research and Innovation (RRI), also concerned with Ethical, Social and Legal Aspects (ELSA) of nanotechnology innovation

Since 2000 or before, we have experienced the effects of the Fourth Industrial Revolution, where more and more technological innovations are consumed, many of them using nanotechnology.

Our Planet is overloaded and has shown numerous symptoms of depletion of reserves. It is still necessary to discuss the production and consumption patterns of the majority of Earth's inhabitants, behavior that has caused serious and irreversible consequences for human and environmental health. This work deals with nanotechnology risk management and highlights self-regulation based on safe by design, aligned with sustainable development objectives. It also considers the unlikelihood of intersystemic communication between Law and Science. The same characteristics that make nanomaterials different and beneficial also raise concerns about their behavior, especially in their interaction with the ecosystem. As a result of this new reality, caused by the rise of nanotechnology and its potential risks, the objective was to analyze the possibilities of the contribution of the safe by design tool as a form of coupling between the Legal System and the Science System. The connection is projected with the concept of Responsible Research and Innovation (RRI), which is also concerned with - Ethical, Social and Legal Aspects (ELSA), in the self-regulatory structuring of nanotechnology risk management, aiming at sustainability applied to innovation. The systemic-constructivist methodological perspective was used, considering reality as the construction of an observer, analyzing all the peculiarities involved in observation. It is a form of legal reflection on the conditions of meaning production, as well as the possibilities of understanding the multiple different communicative dynamics in a complex environment, such as that generated by nanotechnologies. In this way, safe by design can be understood as a possible structural coupling between the Systems of Science and Law, enhancing intersystemic communication about risk as a practical and creative way of applying the ideas of RRI and ELSA, by structuring a modality of regulated self-regulation of nanotechnology risk management, organizing and stabilizing expectations and inducing behaviors in search of sustainability in innovation. Thus, the immersion of the Legal System in observing nanotechnological risks is demonstrated to contribute a step towards the discussion of the possibilities and challenges that the use of nanotechnologies is already generating for present and future generations.

Cyrille Durand* , James Baker, Deven Joshi

*TEMAS Solutions GmbH, Hausen (AG), Switzerland

09:48-10:02

The SUNSHINE foresight framework within a trusted environment in support of sustainable innovation

Major industrial sectors such as energy, cosmetics, electronics, construction, food and healthcare are investing in research and technological development of advanced materials such as multi-component nanomaterials (MCNMs). These new materials offer unprecedented technological benefits as the integration of different components in a unique system can produce new or improved functionalities. However, MCNMs also pose significant challenges in terms of regulatory compliance and environmental, health and safety (EHS) concerns.

Innovations tend to develop rapidly and in a myriad of directions such that regulators often have difficulties to follow and assess the implications of innovation, and therefore to provide adequate regulatory cover. Regulatory Preparedness, ensuring regulations are fit for purpose and ready for implementation, requires that regulators are aware of upcoming trends and developments by industry in a timely manner. The Sunshine project aims to streamline and facilitate that process by developing and testing a Foresight Framework integrated into a Trusted Environment, which will host regulators, innovators, industry and key experts.

The creation of a Foresight Framework included in a Trusted Environment will benefit both SSbD and Regulatory Preparedness because when knowledge is openly shared between industry stakeholders and Regulators from the early stages of innovation, the time for Advanced Materials such as MCNMs-based materials and products to reach the market can be substantially reduced, while ensuring high levels of human and environmental safety.

The Foresight Framework, developed and piloted in the Sunshine project, comprises a number of steps by which various sources and databases are mined in order to identify weak signals or signs of upcoming industrial innovation and development. Refined processing will produce at regular intervals throughout a year a set of information which must be scrutinized and evaluated by the experts within the Trusted Environment. This selection of information will be the main output of the Foresight Framework.

Improving the anticipatory capabilities of regulators and (regulatory) risk assessors and to facilitate, where needed, timely adaptation of (safety) legislation, guidance guidelines and standards. Timely clarity on how regulators deal with novel materials such as MCNMs reduces uncertainty for industry about the information needed to comply with a regulation and how testing should be performed.

The implementation of the SUNSHINE Foresight Framework will support Regulatory Preparedness leading to a shorter time to market of MCNMs or MCNMs-enabled products.
The SUNSHINE project is funded under the European Union's Horizon 2020 Research and Innovation program, Grant Agreement 952924

Andrea Haase*, Julia Prinz, Gregor Nagel, Blanca Suarez Merino, Susan Dekkers, Eugene van Someren, Veronique Adam, Wendel Wohlleben, Veronique Di Battista, Michael Persson, Anders Baun, Otmar Schmid

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10:02-10:16
The HARMLESS Early Warning System

Advanced materials (AdMa) will provide important contributions to reach several of the global sustainability goals. AdMa comprise, however, heterogenous material classes and can be developed in multiple variants at increasing pace while regulatory implementations may lag behind. Therefore, tools and concepts addressing this discrepancy are urgently needed. Thus, the Safe-and-Sustainable-Innovation Approach becomes important, describing two elements: the Safe-and-Sustainable-by-design (SSbD) concept aiming at the innovators and regulatory preparedness aiming at the regulators, both demanding to systematically address issues concerning safety and sustainability as early as possible. The EU HARMLESS project developed an Early Warning System (EWS) to support regulatory preparedness, which is designed as a practical, easy-applicable tool that a) raises awareness for material-based concerns with little information/data taking into account risks, benefits/sustainability and regulatory applicability of existing frameworks and b) fosters communication between stakeholders.
The HARMLESS EWS is composed of two tiers. Tier 0 is based on the "Advanced Material Earliest Assessment" (AMEA) approach aiming at an initial material categorization. The outcome of Tier 0 defines how to proceed in Tier 1, e.g., suggesting to start with either an exposure or a hazard assessment. In addition, Tier 1 is available in two versions, a simple version using simple questions demanding yes/no answers only for the most important warning signals, intended for materials with little/no data (i.e., before market entry) and a more detailed version, where the same warning signals can be addressed in a more substantiated manner utilizing experimental data obtained by New Approach Methodologies (NAM), intended for materials with more data (i.e., materials on the market).
The HARMLESS EWS was already practically tested on two of the HARMLESS SSbD case studies, the aerogel case study and the perovskite case study. The outcome of both case studies demonstrates that the HARMLESS EWS is an easy applicable tool. Currently, our consortium works on a guidance document to facilitate its applicability.

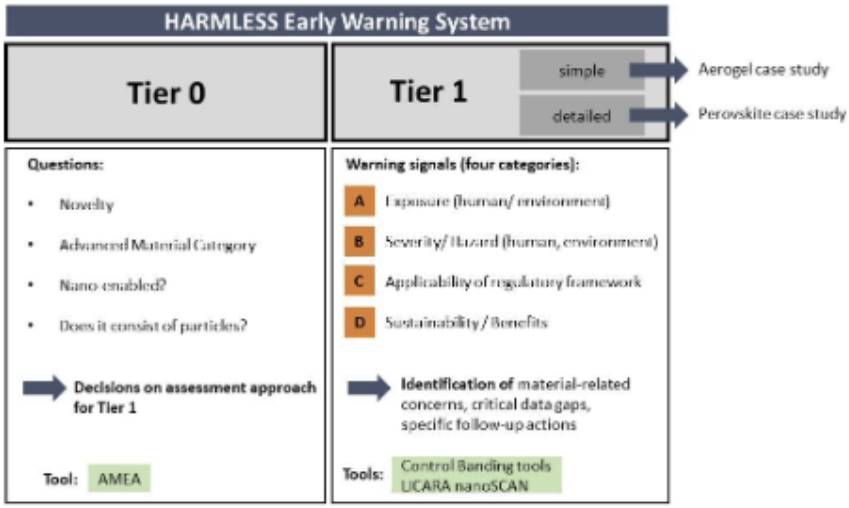

Figure: Overview of the HARMLESS EWS consisting of two tiers.

Ashi Rashid*, William Stokes, Eilidh Tonk, Ivana Burzic, Christoph Jocham, Aude Mezy, Nik Kapur - Andrew Nelson

*University of Leeds, Chemistry, Leeds, United Kingdom

10:16-10:30
Rapid screening of advanced coating materials and coatings developed within SSbD framework

This work as part of the BIO-SUSHY project focuses on the (bio)membrane activity assessment of the selected/developed PFAS free and biobased coating components/formulations and coatings. It uses the electrochemical model membrane sensing system (MMSS) and the innovative mini release accelerator (MRA) respectively following the safe and sustainable by design (SSbD) approach. The MRA has been employed for the release studies of the coatings under the conditions simulating natural environmental ageing varying the temperature, pH and exposure time. Leachates from release studies and the coating materials/formulations are subjected to MMSS screening to investigate the interaction of these materials with the model membrane. MMSS utilizes a self-assembled monolayer of dioleoyl phosphatidylcholine (DOPC) on a fabricated microHg-on-Pt chip electrode to generate characteristic rapid cyclic voltammograms (RCV) at 40 Vs-1. These RCVs contain current peaks due to underlying phase transitions in response to applied electric field. Changes in the RCV scan and associated capacitance peaks in the presence of (bio)membrane active substances are related to membrane disruption detailing the nature and extent of the interactions. Supernatants from the heterogeneous water dispersions are subjected to MMSS for screening and actual concentrations of soluble bioavailable species in the supernatants are determined from calibration curves acquired using high performance liquid chromatography (HPLC). Extraction of membrane affinity parameters from the data enables the estimation of structure activity relations (SARs) of materials with the sensing layer. These preliminary screenings will be intercalibrated with in vitro assays conducted by collaborating partners. The unique advanced material screening technology, the results and their analysis will be presented at this conference.

Keywords: PFAS, advanced material screening, MMSS, MRA, membrane disruption, leachate.

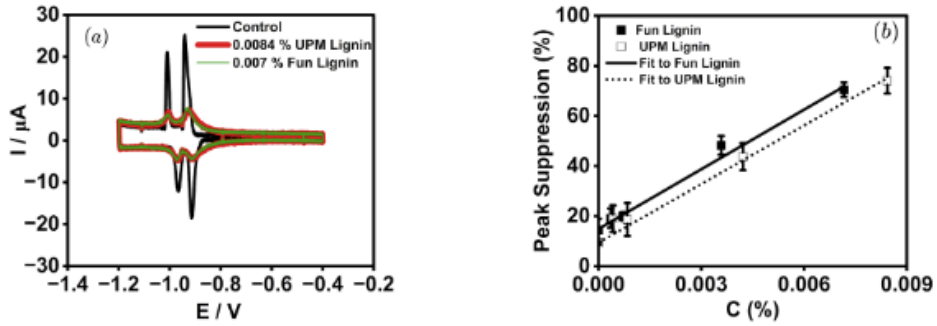

Figure: (a) RCV scans of DOPC monolayer in the absence (black) and presence of 0.0084 % UPM (red) and 0.007 % functionalized (Fun) lignin (green) in 0.15 % DMSO and 0.3 x 10-3 moldm-3 NaOH in water, acquired using MMSS at 40 Vs-1 (b) Capacitance peak suppression (%) versus concentration (%) plot of UPM lignin (□) with fit (dotted line) and Fun lignin (■) with fit (solid line).

The BIO-SUSHY project is funded by the European Union under the Grant Agreement Number 101091464. University Leeds is funded by the UKRI Horizon Europe Guarantee Fund: Grant Number 10056199. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA).

Session 7C

9:30-10:30

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Safe-and-Sustainable-by-Design strategies and case studies

Chairs: Steffi Friedrichs & Andrea Brunelli

Blanca Suarez Merino*, Beatrice Salieri, Wendel Wohlleben, Veronica di Battista, Eugene van Someren, Susan Dekkers, Veronique Adam

*TEMAS Solutions GmbH, Hausen AG, Switzerland

09:30-09:45

Safe and Sustainable by Design framework for advanced materials: the HARMLESS approach

The European Framework for 'safe and sustainable by design' chemicals and materials represents an important step towards the production of safer and more sustainable products. Yet, it requires resources not compatible with the expected commercial value at early innovation stages and recommends methods that are not applicable to emerging materials. To address such issues the HARMLESS project developed a SSdD framework that considers data availability and resources along the innovation process, is applicable to Advanced Materials (AdMa) and is cost-effective. It is aligned with the EU framework, complemented with a flexible stage-gate model and implements New Approach Methodologies (NAMs) tailored to AdMa.
The HARMLESS SSbD framework includes three innovation stages: ideation, laboratory scale and pilot scale. It starts with the categorization module of AMEA (Advanced Material Earliest Assessment) at the first innovation stage, testing the applicability of the Framework to the business case. At each innovation stage, five different modules enable the assessment of 1) intrinsic safety, 2) occupational and environmental safety at production, manufacturing and end-of-life, 3) consumer and environmental safety at use, 4) environmental sustainability at production and manufacturing and 5) environmental sustainability at use and end-of-life. Within each module, design principles are given to guide the user in making a product as safe and sustainable as possible, and methods and tools are suggested to facilitate the SSbD assessment. NAMs are prioritized to make the SSbD assessment as cost-effective as possible. After all modules are completed, a gate enables the user to balance the safety and sustainability of their product with cost and performance, assessing the relevance of going to the next innovation stage. This framework guides the creation of an online decision support system, which will be publicly available. The Framework has been further tested with industrial case studies from the HARMLESS project.

Lisa Pizzol*, Arianna Livieri, Alex Zabeo, Sarah Devecchi, Alberto Katsumiti, Andrea Brunelli, Michael Saidani, Konstantina Koutsiara, Carlos Fito, James Baker, Blanca Suarez Merino, Stella Stoycheva, Yasemin Ertugrul, Hubert Rauscher, Irantzu Garmendia Aguirre, Danail Hristozov

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09:45-10:00

The SUNRISE SSbD integrated impact assessment framework for advanced materials

The European Green Deal presents a roadmap for transforming the EU into a modern, resource-efficient and competitive economy by converting environmental, health and safety (EHS) and sustainability challenges into opportunities across all policy areas, including chemicals. In response to this, the European Commission published its recommendation for establishing a European assessment framework for the development of Safe and Sustainable by Design (SSbD) chemicals and materials (including advanced materials, AdMa), which is based on a holistic approach proposed by the EC's Joint Research Centre (EC-JRC). The EC-JRC framework highlights that assessing human, environmental, social, and economic impacts requires integrated approaches, able to address complex systems and to enable reproducible and transparent comparison of alternatives to select safer and more sustainable design options. To address the above challenges, the SUNRISE Horizon Europe project has been funded with the main goal of developing and testing an overarching Integrated Impact Assessment Framework (IIAF), based on lifecycle thinking and designed to support SSbD decision-making of AdMa and their products. The IIAF, fully aligned to the EC-JRC SSbD framework, is a 4-tiered approach with each tier corresponding to an integrated methodology, supported by a toolbox, for health, environmental, social and economic impacts assessment targeting relevant groups of users at different stages of the innovation process and requiring a different level of data and expertise. Tier 0 is a fast screening method based on a questionnaire composed of 18 key questions designed for situations when an innovator needs to prioritise from a high number of possible design alternatives (hundreds or thousands). Tier 1 is a further qualitative screening at the early R&D stages of the innovation process that aims to identify hotspots of possible safety and sustainability concerns along the lifecycle. Tier 2 is a semi-quantitative assessment based on a weight of evidence approach applied in the product optimisation phase when a mix of qualitative and quantitative data are already available. Finally, Tier 3 involves quantitative safety (regulatory risk assessment) and sustainability impact assessment (Life Cycle Assessment, Life Cycle Costing, and Social Life Cycle Assessment) for materials/products to be released on the market. Implementation of the IIAF will ensure better regulatory compliance for AdMa based products and a shorter time to market, thereby supporting the European Green Deal and the transformation of the EU into a modern, resource-efficient, competitive economy.

Figure 1. SUNRISE 4-tiered IIAF

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10:00-10:15
Safe-and-Sustainable-by-Design for Advanced Materials - A case study on the agricultural use of imogolites

Following the European Commission Recommendation for a European assessment framework for 'safe and sustainable by Design' (SSbD) chemicals and materials, the HARMLESS project built a SSbD framework tailored to Advanced Materials (AdMa). Based on this framework, an online Decision Support System, directed towards innovators, is being created. In this study, functionalised imogolites developed for agricultural purposes

(imogolite_OH, imogolite_CH3, Cu-doped imogolite_CH3) are taken through a flexible stage-gate innovation process and used to test the HARMLESS framework and decision support system. At the ideation and business case stage, the HARMLESS WASP module, composed of 14 questions to the user, raises potential concerns on safety and sustainability aspects of the different imogolites versions. At the second innovation stage (i.e. lab phase), key physchem, exposure, hazard and sustainability descriptors are measured to further define potential issues on the aspects that raised red flags. Differences among imogolite versions mainly come from increasing the complexity in terms of multi-component composition. Hazards could arise from components issued from the pristine materials such as gibbsite, alumina, silica, methyltrihydroxysilane and copper. Nevertheless, these AdMa show benefits towards sustainability as compared to benchmark materials (such as Cu salts), as they require less use of Cu. The contribution to several Sustainable Development Goals (SDGs) as defined by the United Nations, such as sustainable food production (SDG 2), efficient use of resources (SDG 12) or clean water and sanitation (SDG 6) will be discussed regarding their composition and potential functionalities. The quantitative SSbD assessment currently taking place at lab phase on physchem characteristics (e.g. size, composition, dissolution rate), toxicity (e.g. inflammation potential, cytotoxicity) and sustainability descriptors (e.g. use of critical raw materials, release of hazardous chemicals) feeds the discussion on the balance between risks, benefits, costs and performance of each imogolite version and helps the selection of the most appropriate version to take to pilot phase.

Ana Serrano Lotina*, Elvira Villaro, Mónica martínez, Miguel Angel Bañares, Julio Gómez, Magda Blosi, Simona Ortelli, Anna Costa, Andrea Brunelli, Elena Badetti, Alberto Martínez Sierra, Marco Monopoli, Angela Saccardo, Shareen Doak, Carlos Fito, Elena Barbero, Flemming Casee, Willie Peijnenburg, Hyunjoo Hong, Bernd Nowack, Sarah Devecchi, Arianna Livieri, Lisa Pizzol, Danail Hristozov

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10:15-10:30

Sustainable flame retardant additives: A Safe and Sustainable by Design approach

Increasing polymer usage has demanded functional additives that cope with plastics low fire performance, characterized by short ignition times and high heat release rates. Flame retardant additives are the most used fillers for plastic materials. While traditional FR, such as halogenated, phosphorus, and metal hydroxides, greatly reduce flammability and associated fire hazards, research has continually exposed a litany of health and environmental safety concerns. Hence, there is an urgent need for the development of sustainable FRs that reduce environmental footprints while simultaneously improving the fire performance of polymers. A multicomponent nanomaterial (MCNM) based on chitosan modified graphene oxide used as flame retardant additive for thermoplastic materials has been developed in an industrial environment and safety and sustainability concerns have been evaluated during the production process, the usage and its life cycle, following the Safe-and-Sustainable-by-Design (SSbD) framework proposed by the European Commission.

The physicochemical identity of MCNM was evaluated by several analytic techniques (XPS, TEM, SEM, XRD, Raman, spectroscopy...) and its toxicity was assessed through in vitro testing. Aerosol formation and particle release has been also studied to identify occupational exposure risks. Finally, the MCNM life-cycle release to the environment was also evaluated in order to reduce the impact on health and ecosystems.

This MCNM is melt-mixed with polyamide 6 (PA6) by twin screw extrussion and injected to prepare the nanoenable product. Polyamide 6 (PA6) is widely used engineered thermoplastic polymer and its graphene-based composites has attracted the interest due to their performance [1]. Different compositions were prepared, and their functionality (mechanical and rheological properties and its flame retardancy) was evaluated showing promising results compared to one of the benchmark materials (melamine cyanurate-PA6).

In a second stage, although chitosan is a relatively non-toxic, biocompatible material, care must be taken to ensure that it is pure, as protein, metal or other contaminants could potentially cause many deleterious effects both in derivative syntheses and in dosage forms.

Therefore, a SSbD Tier 2 action has been addressed to replace chitosan with whey protein (WP) or caseins. They are environmentally friendly FRs [2] and easily recoverable by waste or by-products of the cheese and milk industry. Functionality and physico-chemical properties were also measured and compared with GO-chitosan.

References

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[2] S. Ortelli, G. Malucelli, F. Cuttica, M. Blosi, I. Zanoni, A. L. Costa, Cellulose 25 (2018) 2755–2765

Session 8A

11:30–13:00

RISK ASSESSMENT AND MANAGEMENT

Implementation of NAMs for risk assessment

Chairs: Alicja Mikołajczyk & Danail Hristozov

Andrea Haase, Susanne Bremer Hoffmann, Valerie Fessard, Arno Gutleb, Jan Mast, Birgit Mertens, Agnes Oomen, Vera Ritz, Tommaso Serchi, Katherina Siewert, Deborah Stanco, Eveline Verleysen, Olimpia Vincentini, Shirin Usmani, Meike van der Zande, Francesco Cubbada

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11:30-11:55
Developing a NAMs qualification system to support regulatory acceptance

A large number of promising new approach methodologies (NAMs) resulted from many research projects but only a few have been formally validated. Despite this, many are meanwhile exploited in industrial settings. Hence, regulators are increasingly faced with data from non-guideline studies demanding for a complex and time-consuming case-by-case evaluation. Importantly, for nanomaterials (NMs) most NAMs are not yet formally validated. A qualification is an expert opinion (provided by an expert panel) on a method for a well-defined use in a specific context. Existing qualification systems operate in the context of Drug Development Tools (US FDA) and research and development into pharmaceuticals (EMA). Here we propose a NAM qualification system, developed within the EFSA-funded project NAMS4NANO. We considered all available information on existing qualification systems, published approaches to assess method readiness, guidance documents (e.g., EURL-ECVAM, OECD) as well as ongoing efforts (e.g., PARC) to propose a generic, fit-for-purpose qualification system for application in the food and feed sector. Furthermore, we propose readiness criteria as guidance covering different types of NAMs (e.g., QSAR, PBK, in chemico, in vitro). We have applied our approach for selected NAMs and further explore it, also in the context of five selected risk assessment case studies.Overall, a qualification system could significantly accelerate the identification of potentially promising NAMs and endorse those, which are demonstrated to be mature enough to facilitate their regulatory application for a specific purpose in a well-defined context.

Disclaimer: This research is performed under the NAMS4NANO action via EU funding through a grant of the European Food Safety Authority (agreement GP/EFSA/MESE/2022/01). This communication
reflects only the author's view and EFSA is not responsible for any use that may be made of the information it contains.

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11:55-12:10
Validation of NAMs for nanomaterials: the importance of big data and its FAIRification for accelerating risk assessment

Risk assessment of nanomaterials and nano-enabled products may occur during pre-market stages alongside innovation, or later during scale-up production due to legal requirements. We argue that materials' risk assessment would be accelerated by trustworthy and extensive FAIRified data sets, as it potentially enables the definition of broad applicability domains of New Approach Methodologies (NAMs), as well as saying goodbye to the traditional assessment concept of evaluating materials case by case. For rigorous regulatory risk assessment, the OECD Test Guidelines (TGs) are established and covered by the mutual acceptance of data agreement supporting a high level of legal certainty and reuse of data, saving costs and animal experimentations. However, development of OECD TGs is onerous and time-consuming, and today the speed of test method development outperforms the time required for validation, leaving many NAMs outside of regulatory risk assessment. In contrast, risk assessment at pre-market stages is less dependent on legal certainty, but nevertheless seeks scientific confidence in the NAMs applied, and strives, to the extent possible, to generate data that is later applicable for the regulatory requirements. These different focuses between NAMs and data applied in the pre-market stage, and those legally required for market application, cause discrepancies between how NAMs reach scientific confidence versus full OECD validation status. We present ideas for advancing OECD-level validation and regulatory acceptance of NAMs, taking the example of high throughput analyses and the Predictive Toxicogenomics Space for application to nano- and advanced materials' hazard assessments. We point to: i) the importance of widely available FAIR life science and reference toxicology data in the development of NAMs ii) the key role of FAIRifying the increasingly large data produced with NAMs within academic and industrial research; and iii) the cumulative, potentially synergistic, effect offered by the combination of big data and the rise of novel artificial intelligence (AI) approaches capable of supporting interpretation and applicability of data. These undertakings have extensive bearing to stage gate-driven Safety and Sustainability by Design and rich high throughput "omics" data generation to counteract uncertainty, providing overall more angles, explainable insight and legal certainty into AI toxicology and the risk assessment of nano- and advanced materials within the European chemicals' legislation.

This work is partly funded by project PINK (Horizon Europe, GA 101137809).

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12:10-12:25
NAMs for Genotoxicity Assessment of Metal Oxide Nanoparticles

Metal oxide nanoparticles (MONPs) induce DNA damage, which is influenced by their physicochemical properties. In this study, the high-throughput CometChip and micronucleus (Microflow) assays were used to investigate DNA and chromosomal damage in mouse lung epithelial cells induced by nano and bulk sizes of zinc oxide, copper oxide, manganese oxide, nickel oxide, aluminum oxide, cerium oxide, titanium dioxide and iron oxide. Ionic forms of MONPs were included, where available. The study evaluated the impact of solubility, surface coating, and particle size on response. Correlation analysis showed that solubility in cell culture medium was positively associated with response in both assays, with the nano form showing the same or higher response than larger particles, which was not correlated with the particle surface area. Surface coating of MONPs inconsistently affected genotoxicity. The observed difference in genotoxicity highlighted the mechanistic differences in MONP response, possibly influenced by both particle stability and chemical composition. The results highlight that combinations of properties influence response to MONPs, and that solubility alone, while playing an important role, is not enough to explain the observed toxicity. The results have implications on the potential application of read-across strategies in support of human health risk assessment of MONPs. The study also optimised high-throughput genotoxicity assays for the assessment of nanomaterials.

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*University of Genova, Department of Physics, Genova, Italy

12:25-12:40
Development of coarse-grained models of oxide surfaces to study bio-corona formation on the surface of silica nanoparticles

Nowadays, functionalized oxide nanoparticles are widely used as building blocks of multicomponent nanomaterials. However, the nanoparticles could get in contact with living organisms, and once in the biological environment, assessing if they are prone to being surrounded by a bio-corona is of fundamental importance. When investigating this process via molecular dynamics simulations, using CG models is mandatory, given the tipical system's size and complexity. The Martini 3 force field is a reasonable choice, being characterized by a degree of coarse graining that retains chemical specificity. However we actually lack good Martini 3 models for oxide surfaces.
Therefore, we started developing models and protocols to simulate oxide surfaces modeled within the framework of Martini 3. In our scheme, the surface hydrophobicity is tuned using the contact angle of water droplets as a target. The surface chemistry dictates the Martini bead choice, while the contact angle can be finely tuned by acting on the lattice constant of the solid surface. Furthermore, we use the adsorption of small molecules as an additional validation target.
We recently built a model for the Human Serum Albumin based on the most recent version of the Martini force field, Martini 3. Once the models of the surfaces will be ready, we will study the adsorption of Albumin on bare and functionalized silica surfaces, exploiting enhanced sampling methods to estimate how different functionalizations impact the adsorption.

Ewelina Wyrzykowska*, Maciej Stępnik, Kinga Nimz, Alicja Wojciechowska, Mateusz Balicki , Tomasz Puzyn

*QSAR Lab Ltd., -, Gdańsk, Poland

12:40-12:55
Addressing TiO2 Nanoparticles Safety Concerns: QSAR Modeling for Regulatory-Relevant Assessment

Titanium dioxide nanoparticles (nTiO2) are widely used in cosmetics, food additives, colorants, and photocatalytic environmental remediation systems. However, nTiO2 also raises concerns regarding its safe use. The International Agency for Research on Cancer (IARC) classified powdered nTiO2 as a possible carcinogen (inhaled) in 2006. In 2021, the European Food Safety Agency (EFSA) expressed concerns about the genotoxic potential of the food additive E171 (containing TiO2, including nanoforms).
Scientific committees such as EFSA (2011) and the Scientific Committee on Consumer Safety (SCCS, 2023) have established in vitro testing strategies for nanoparticles. These strategies focus on identifying gene mutations in mammalian cells and chromosomal aberrations or micronuclei. Although the Comet assay lacks formal regulatory authority, it is recognized as a DNA damage indicator test. However, these in vitro tests often require specialized facilities, trained personnel, and are costly and time-consuming.
Consequently, in silico methods are increasingly explored as preliminary screening tools for mutagenicity and genotoxicity assessment. Computational approaches, particularly Quantitative Structure-Activity Relationship (QSAR) modeling, offer the potential for accelerated research with reduced costs.
This study presents QSAR models developed to predict the mutagenicity and genotoxicity of nTiO2, based on results from the micronucleus test and Comet assay, respectively. Models' development involved a systematic literature review (2007-2022) with extracted data subjected to rigorous quality control, including these aligned with EFSA recommendations. Key selection criteria emphasized the relevance and reliability of genotoxicity tests, and completeness of reported physicochemical characterization of nanoforms. Data from each experiment was extracted, detailing nanoform and cell line characteristics, allowing the models to predict mutagenic and genotoxic effects for a variety of TiO2 nanoforms and diverse cell lines. Machine learning (including Logistic Principal Component Analysis (L-PCA) and supervised methods) helped to identify correlation

correlations between nanoform and cell line characteristics and the observed adverse effects.
These QSAR models are among the few capable of predicting regulatory-relevant endpoints for TiO2 nanoforms, positioning them as potential in silico New Approach Methodologies (NAMs).

Session 8B

11:30-13:00

RISK GOVERNANCE

Regulatory perspectives

Chairs: Laurence Deydier & Hubert Rauscher

Monique Groenewold*, Adrienne Sips, Eric Bleeker, Cornelle Noorlander, Rob Aitken, James Baker, Martine Bakker, Verónica Dumit, Elisabeth Heunisch, Keld Alstrup Jensen, Thomas Kuhlbusch, Andrea Porcari, Susanne Resch, Wouter Fransman, Danail Hristozov

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11:30-11:50
Future-proof approaches for risk governance of advanced (nano) materials

Gov4Nano joined forces with NANORIGO and RiskGONE (three H2020 NMBP-13 projects), to address the same goal: to ultimately ensure a sustainable and equitable nano risk governance infrastructure for Europe and beyond. The projects have gathered meaningful insights about challenges and issues in risk governance of nanomaterials, which are relevant for efficient and effective risk governance of advanced (nano)materials and chemicals. Approaches were developed for nanomaterials which are ready to be implemented for risk governance of new (advanced) materials and chemicals in scope of the Chemical Strategy for Sustainability.
Gov4Nano identified and established several key elements that were deemed essential to achieve coordinated risk governance of nanomaterials. Key objectives included (i) ensuring that quality data on safety of nanomaterials and their applications could be easily shared and reused (ii) perform research needed to develop harmonised test guidelines for characterising and testing nanomaterials, (iii) establish a Nano Risk Governance portal for all stakeholder information needs and (iv) provide a roadmap for setting up an organisation for Nano Risk Governance to coordinate (emerging) issues which require multi-stakeholder dialogue. Main result delivered included:

- GO FAIR Implementation Network to support the implementation of FAIR principles
- Advances in standardising guidance for characterising and testing nanomaterials.
- Web based Nano-Risk Governance Portal (NRGP) which gives access to governance tools
- The architecture of an organisation for Nano Risk Governance developed and tested with over 50 case studies involving over 500 stakeholders. The effectiveness of a 'House of nano risk governance' was proven through the successful co-creation of regulatory research roadmaps and governance briefs dealing with emerging issues.

Results and challenges were discussed with ~150 key stakeholders at OECD in Paris. The relevance of the outcomes for future governance challenges will be presented and positioned in the broader context of the Innovation Principle and current needs to support sustainable innovations for a sustainable future. Activities and projects addressing risk and sustainability governance can incentivise sustainable innovations, rather than forming a barrier. However, coordination actions, synergies and funding are urgently needed. First the goals and ambitions as laid down in the European Chemicals Strategy for Sustainability (CSS) and the Zero Pollution Action Plan, can become an integral part of various crucial innovation programmes dealing with advanced materials.

Camelia Constantin*, Diana Mestre, Laurence Deydier, Stephan Virginia Rodriguez Unamuno, Frank Le Curieux, Marianne Matzke

*European Chemicals Agency, Hazard Assessment Directorate, Helsinki, Finland

11:50-12:04
The use of NAMs to support regulatory safety assessment of nanomaterials under REACH

New Approach Methodologies (NAMs), covering a broad range from in vitro and in chemico methods, to in silico computational models, hold great potential to reduce the current reliance on animal toxicity tests as well as improving the science and predictivity of chemical safety testing.
While NAMs for chemical toxicological assessment are consistently gaining traction not many are available yet for assessing nanomaterial hazards1. To be used for standard information requirements under REACH regulation, NAMs should allow a conclusive outcome on the (lack of) hazardous properties for a given regulatory endpoint, i.e., NAMs should be able to reliably convert nominal concentrations measured or predicted into external doses that can be used to set safety levels which allow assessing the risks and if needed, can be used for classification purposes. Therefore, for those endpoints where a NAM can be a one-to-one replacement of a current animal study, the validation of such NAMs potentially leading to internationally accepted test guidelines will be crucial for their regulatory acceptance. Non-standardized NAMs may only be used to predict properties or build confidence in mechanistic hypotheses under adaptation possibilities such as read across, in weight of evidence approaches and to support the building of sets of nanoforms. Nevertheless, the data deriving from non-standardized NAMs should be scientifically sound, well documented, and widely accepted by the scientific community.
Substantial efforts are currently made by ECHA2 as well as OECD3, JRC4, and EFSA5 to map, develop and adapt NAMs available for the hazard assessment of nanomaterials. The current status of NAMs does not allow an immediate replacement of animal testing hence, a possible workaround is the use of integration of NAMs into DAs (Defined Approaches), as already practiced for the assessment of sensitization. This presentation will focus on how and where NAMs have the potential to support the risk assessment of nanomaterials under REACH.

Neil Hunt*, Hubert Rauscher, Lucian Farcal, Vikram Kestens, Lya G. Soeteman-Hernández

*Yordas Group, Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom

12:18-12:32
What is a multicomponent nanomaterial: a SUNSHINE perspective?

The term multicomponent nanomaterial (MCNM) is widely used across the three NMBP-16 sister projects (SUNSHINE, DIAGONAL and HARMLESS), but it is a term with no regulatory definition or description. It has become apparent that the understanding of what is meant by the term can vary significantly between stakeholders. This presentation examines the structures that could be described as a MCNM and how the choice of phrases used in any description or definition could have a significant impact on the types of structure that would be included or excluded from the term.

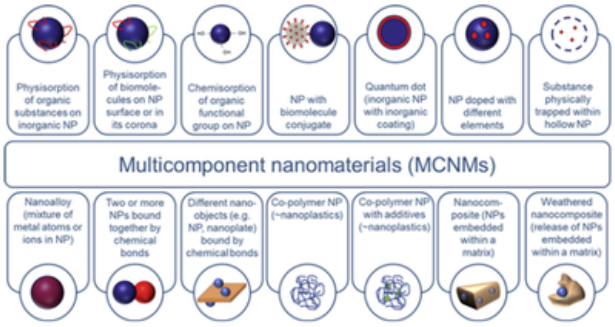

Figure: There are many generic structures that could be described as a multicomponent nanomaterial. Careful choice of the phrases used in a description is needed to include and exclude the structures as required.

Hubert Rauscher*, Neil Hunt, Vikram Kestens, Lucian Farcal, Lya Soeteman-Hernandez

*European Commission Joint Research Centre, Ispra, Italy

12:32-12:46
Regulatory preparedness of EU regulations for multicomponent nanomaterials: Case studies from SUNSHINE

Understanding regulatory requirements along the supply chain is essential for a product to progress from innovation to commercialisation. The uncertainties in how fundamental REACH concepts apply to multicomponent nanomaterials (MCNMs) have been introduced previously. This presentation examines four case studies from the SUNSHINE project and describe where regulations interact with their use. It highlights areas where the obligations from REACH and other EU regulations are currently uncertain for these novel materials with unique structures.

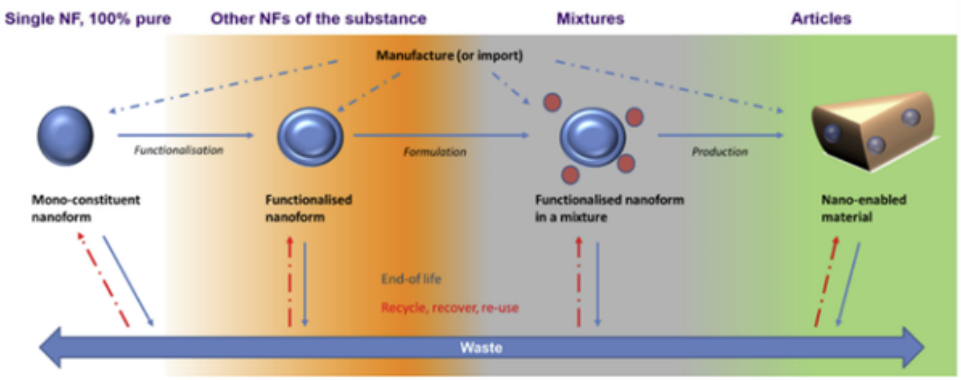

Figure: Different regulations apply to MCNM as they progress along their life-cycle and different definitions and obligations can apply at each stage.

Neil Hunt*, Hubert Rauscher, Vikram Kestens, Lucian Farcal, Lya Soeteman-Hernández

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12:46-13:00
Learning from SUNSHINE - Regulatory preparedness of REACH for multi-component nanomaterials

Multicomponent nanomaterials (MCNMs) may be regarded as advanced hybrid materials that contain more than one functional component conjugated by chemical bonds or other intermolecular mechanisms, where typically at least one component is a nano-object meeting the definition of a nanomaterial. MCNMs are being examined for use across many major industrial sectors. These new hybrid materials typically offer unprecedented technological benefits as the integration of different components in a unique system can even produce synergistic functionalities. However, they may pose substantial design challenges and might induce specific safety and sustainability concerns due to their complex interactions with biological and environmental systems, which the SUNSHINE project is attempting to better understand. Several case studies from SME industrial organisations are being used to elucidate these issues.
Regulatory preparedness is a tool that employs foresight as basis for anticipatory governance to ensure that regulations are adapted in a timely manner to cater for the specificities of advanced and innovative materials such as and products containing them. Understanding regulatory obligations depends largely on being able to confidently assign a product to the definitions used in a regulation. Where this is non straightforward, understanding even the most basic requirements can be difficult. This presentation will examine situations where it is difficult to align MCNMs to concepts fundamental to REACH and will discuss where clarification is needed in the regulation to allow the relevant toxicological properties of MCNMs to be identified and communicated to regulators and risk assessors.

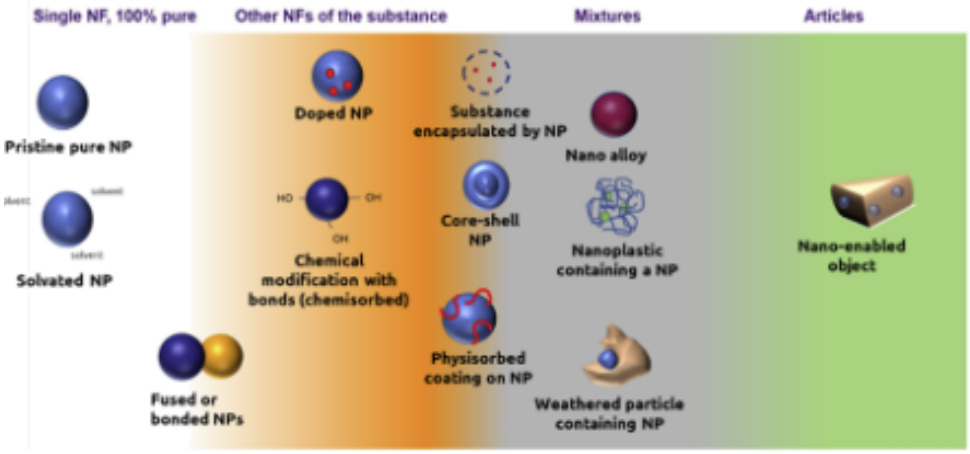

Figure: Different MCNM may be described by different definitions under REACH thus changing the regulatory obligations applicable.

Session 8C

11:30–13:00

ADVANCED MATERIALS: TOWARDS SAFE INNOVATION

Safe-and-Sustainable-by-Design strategies and case studies

Chairs: Arianna Livieri & Carlos Fito

Martin Himly*, Nico Watzek, Varun Giri, Karin Wiench, Iseult Lynch, Haralambos Sarimveis, Thomas Exner

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11:30-11:50
Models and computational integration approaches to satisfy industry needs in SSbD-guided research and innovation on advanced materials & chemicals

Safe-and-Sustainable-by-Design (SSbD) advanced materials and chemicals (AdMas&Chems) are a central requirement for reaching the ambitious goal of making Europe the first digitally-enabled circular, climate-neutral and sustainable economy. Novel AdMas&Chems need to provide the high functionality required for their advanced applications, whilst simultaneously exhibiting improved safety and sustainability performances that take into account the complete value chain and life cycle, as outlined in the SSbD framework proposed by the EU Joint Research Centre and adopted in the Commission Recommendation of 8 Dec. 2022. To facilitate adoption by industry and, by doing so, foster the twin green and digital transition of Europe's economy, the "PINK - Provision of Integrated Computational Approaches for Addressing New Markets Goals for the Introduction of Safe-and-Sustainable-by-Design Chemicals and Materials" Project aims to produce innovative modelling software and integrated workflows for the development of AdMas&Chems, which are combined into an industry-ready open innovation platform, the PINK In Silico Hub (PINKISH).

PINK takes a holistic "by-Design" approach targeting the primary goal to satisfy the needs of industry, as implementing SSbD presents the multi-objective optimisation problem to balance the four requirement categories functionality, cost-efficiency, safety and sustainability. In order to do so, PINK employs two industry-driven Developmental Case Studies establishing three scenarios that operate at different levels of detail representing the R&I stages of the AdMas&Chems' innovation pipeline:

- business opportunity stage (at Tier 1) where through inter-/extrapolation multiple candidates can be generated in silico and potentially later arising sustainability constraints are to be determined by predictive modelling in parallel to functional performance testing;
- innovation development stage (at Tier 2) defined by one lead structure identified and some information is available (performance and partially safety and sustainability) with in silico tool-based modifications going on and sustainability criteria being evaluated;
- later material design stages (at Tier 3) where more data for several dimensions becomes available.

Depending on Case Study requirements and Tier levels, PINK intends to address all five steps of the SSbD Framework continuously improving the confidence in the predictions over multiple design cycles by producing new knowledge on a constantly reduced set of better performing candidates for replacing substances of concern. Industry readiness shall be guaranteed by improving usability, practicability, user experience, data provenance, documentation, and security.

PINK has received funding from the European Union's Horizon Europe Research and Innovation programme under grant agreement No. 101137809.

Bulelwa Ntsendwana

Mintek, Nanotechnology Innovation Centre, Randburg, South Africa

11:50-12:04

Towards global research: Intergration of safe and sustainable by design approaches into the African Research space

Nanotechnology has revolutionized various industries ranging from transportation, materials, energy, electronics, medicine, agriculture, environmental science, consumer and household products. Nano-enabled products that are currently on the market remain few, due to health and environmental risks. This add a burden to the regulation system, since each material possess diverse physicochemical properties. Thus, researchers, engineers, toxicologist, technology developers, regulators collaborated on development of strategies for assessment, fate and end-of-life of nano-enabled products through the use of Safe and Sustainable by Design approach through the EU SHUNSHINE consortium. A prolific investment has made into the research and development of nanotechnology products, however, more practical materials with unique applications continue to evolve. It is therefore, critical that the Safe and Sustainable by Design approach transcend the confines of the EU consortium to other continents to solve the current nanotoxicity challenges, thereby promoting the internationalisation of nanotechnology-related research. Thus, this work package focuses on the formalization of African networks and strategic alliances into the EU consortium, through Nanotechnology innovation Centre, at Mintek, South Africa. These networks and alliances are viewed as vehicles for achieving academic, scientific, economic, technological objectives which cuts across continental sphere. Thus, a proposed international collaboration roadmap will be presented that highlights key strategic goals that are necessary to monitor the global impact of the networks. Additionally, an overview on participating African institutions and their activities to ensure functional internalization will be addressed, giving a well- structured African–EU networks. An efficient and effective stakeholders engagement approaches will be highlighted, which are the key catalysts in the internationalisation process.

Keywords: Nanomaterials, Nanotoxicity, Safe and Sustainable by Design approach, global research, international collaboration, EU-Africa Networks.

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12:04-12:18

Nano-enabled construction products: inventory, documentation, and marketing

Research and development regarding the use of engineered nanomaterials as additives in construction materials have shown promising advances for cleaner, smarter, and stronger constructions. However, knowledge gaps remain regarding where, how, and why engineered nanomaterials are utilized in current commercial applications. Identifying specific commercial products is the first step in identifying situations that may currently cause airborne exposure to engineered nanomaterials. In this work, nano-enabled products aimed for use in construction, renovation, and decoration of residential or commercial buildings on the Swedish market were identified. Products were included if they had 1) ascribed use of engineered nanomaterials (in literature or product inventories), 2) implicit use of engineered nanomaterials (having properties associated with nanotechnology such as 'self-cleaning') or 3) explicit use of engineered nanomaterials ('nano' mentioned in product name, marketing, or documentation). How the potential risks are communicated to the users was assessed through Information given in safety data sheets (SDS), and more general trends regarding the public perception of nanotechnology was studied by looking at phrases used in marketing such as product websites and product brochures.
In total, 63 products currently for sale could be identified, along with 17 discontinued products. The most common product types were surface treatments for concrete (n = 14) and for stone (n = 13), followed by reparation mortars (n = 8).
The most marketed properties were related to surfaces, such as water-repelling (n = 26), anti-microbial (n = 17) and self-cleaning (n = 13). Only 20% of products addressed the nano material content in their SDS and none of them had information about particle size, shape or concentrations. 27% of products utilized 'nano' in the product name but several products were found to have 'nano' removed from the product name in the last decade. More than half of all products did not provide enough information to identify the chemical form of the engineered nanomaterial.
In conclusion, there is a current commercial use of engineered nanomaterials in construction applications, primarily in the form of coatings with surface protective purposes. However, the use of nanotechnology is increasingly concealed in marketing and documentation. This makes it more and more difficult for researchers, safety professionals, workers and the general public to assess the risk and make informed choices regarding safe use of nano-enabled construction materials.

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12:18-12:32

Safe and Sustainable by Design strategies applied to multicomponent nanomaterials developed for the building industry: a case study

The concrete industry is a major contributor of the worldwide CO2 emissions. Enhancing the mechanical strength of building materials may reduce the amount of structural concrete required for edifices, thereby decreasing the environmental impact of their construction. In addition, the industrial sector contributes to air pollution (i.e., particulate matter, ozone and NOx gases) with an estimated cost of 2-3 % of the EU GDP.

NOx gases have detrimental effects on human health, even at low concentrations; therefore, reducing their presence in the air can benefit both economy and environmental health. This calls for the development of innovative materials that are safe and sustainable and can help reduce the carbon footprint and air pollution of the concrete industry. CIAC developed two different multicomponent nanomaterials (MCNMs); the first is SiO2-APTES, which enhances the mechanical properties of building materials. This MCNM can act as hydration nuclei to improve cement hydration and enhance specific material properties (i.e., durability, mechanical). The second multicomponent nanomaterial is SiO2-ZnO for photocatalytic NOx gases removal. NO and NO2 gases are oxidised to NO3- thanks to the nano-enabled product and are thus removed from the air. This material was developed as an alternative to commercial mortar with TiO2, a first step in the Safe and Sustainable by Design (SSbD) approach since TiO2 has been under much scrutiny due to its toxicity. In addition, CIAC implemented some SSbD strategies with a) the use of renewable energy in its facility, b) a high production yield for both materials and c) liquid precursors to obtain the SiO2-APTES to reduce potential occupational hazards. Such an approach was then extended to synthesising SiO2-ZnO as an additional SSbD step, maintaining the same properties of the original MCNM. A synergistic work between physicochemical characterisation, human and ecotoxicological hazard testing and life-cycle analysis supported the decision-making. In addition, CIAC conducted numerous tests on the MCNMs to assess their performance in time. The results indicate that the SSbD alternative to SiO2-ZnO, obtained with liquid precursors, performs well, and its production can be implemented. SiO2-APTES nano-enabled concrete increased both its flexural and compressive strength. However, the increased strength does not justify the production price: the less-performing mortar with silica fumes is a cheaper solution, proving how challenging the balance between functionality, safety and sustainability can be.
This research has received funding from the European Union's Horizon 2020 research and innovation programme for the SUNSHINE project under grant agreement No 952924.

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12:32-12:46
SSbD Industrial Case Study: Anti-Stick Coatings for Aluminum Molds in Bakery Applications

In the production of aluminum molds for bakery applications, PTFE coatings have traditionally been the preferred choice due to their anti-stick properties. However, EU Reach regulations are imposing stringent limits on PTFE quality and composition due to concerns about the potential
release of PFAS compounds, which are persistent, toxic, and widespread in the environment. Laurentia Technologies has developed a sol-gel coating based on a SiC@TiO2 core-shell nanostructure, a multicomponent nanomaterial (MCNM), proposed within the SUNSHINE EU project to replace PTFE. This represents a first step towards a Safe and Sustainable by Design (SSbD - Tier 1) approach. The advantage of applying SiC-based nanoparticles lies in the improved thermal conductivity, which contributes to reducing cooking times and temperatures, promoting longer durability, and ensuring anti-stick functionality. TiO2 is added to improve SiC compatibility and prevent agglomeration once embedded into the silicone paint. However, recent EFSA opinions raise concerns about the use of TiO2 NPs in food applications. Therefore, a second SSbD alternative (SSbD - Tier 2) has been developed during the project, replacing the TiO2 shell with amorphous SiO2 (SiC@SiO2), characterized by the same hydrophilic character and capability to be hetero-nucleated on SiC seeds through a sol-gel route. A series of actions were implemented to characterize and assess the Safety and Sustainability profile of SiC@TiO2 (SSbD - Tier 1) and SiC@SiO2 (SSbD – Tier 2). A multistep characterization approach was employed to gather data covering a three-dimensional domain of

safety/ functionality/ sustainability along the life cycle stages: synthesis, manufacturing, use, and end of life. The main physicochemical properties (size, shape, surface charge, composition, dissolution, crystalline phase) and their evolution when exposed to relevant media were monitored. For both SSbD alternatives (SiC@TiO2 and SiC@SiO2), the technical functions of the coatings (wettability, abrasion resistance, durability, migration in simulant food) were measured, and a life cycle assessment of the entire process was implemented. Exposure and hazard testing campaigns, related to manufacturing and use stages, contributed to evaluating the (eco)toxicological impact of the MCNM. The results suggest that both SSbD alternatives, SiC@TiO2 and SiC@SiO2, are promising substitutes for PTFE coatings, preserving technical functions with reduced (eco)toxicological and overall environmental impacts. Particularly SiC@SiO2 showed significantly lower impacts in both environmental and economic assessments, despite an increased inflammasome activation effect that is still under investigation.

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12:46-13:00

The SUNRISE approach for defining the methodological basis for assessing environmental, health and safety (EHS) impacts

The SUNRISE project aims to develop an overarching Integrated Impact Assessment Framework (IIAF), based on lifecycle thinking and designed to support Safe and Sustainable by Design (SSbD) decision-making along supply chains of advanced materials (AdMa) and their products. The IIAF will be designed as a 3-tiered approach with each tier corresponding to an integrated methodology (supported by a toolbox) for health, environmental, social and economic impact assessment targeting different groups of users at different stages of the innovation process and requiring a different level of data and expertise. For environmental, health and safety (EHS), SUNRISE will address the methodologies including hazard and exposure. Here, we present the SUNRISE approach for defining methodological basis for assessing EHS impacts in the 3 tiers of the IIAF. To this end, we identified potential EHS-related building blocks: tools, model, databases and methodologies, including NAMs (in vitro, in chemico and in silico methods) that are evaluated for their applicability in the different tiers and aligned to Steps 1, 2 and 3 of the EC-JRC SSbD framework. These building blocks include existing methods/tools to measure the indicators pertaining to 'what they are' (intrinsic physicochemical identity), 'where they go' (lifecycle release, environmental fate, biodistribution and transformation by-products exposure), and 'what they do' (human health and environmental effects). In addition to that, needs to adapt existing NAMs for AdMa hazard assessment or develop new ones in line with the three Rs for regulatory purposes were identified. This will be one of the aims of SUNRISE in the following months, finally leading to the development and application of Integrated Approaches to Testing and Assessment (IATA) based on New Approach Methods (NAMs) to support the generation of robust non-animal data for a next generation risk assessment (NGRA).

Session 4

10:30-11:30

SUSTAINABILITY ASSESSMENT

Environmental sustainability, LCA

Veronique Adam

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Life Cycle Assessment of Advanced Materials – A case study of graphene oxide in drinking water filters

The European Commission, in its Recommendation for the implementation of a framework for safe and sustainable by design (Caldeira et a. 2022), advises to use Life Cycle Assessment (LCA) to evaluate the environmental sustainability of chemicals and materials. LCA is a standardised methodology (ISO 14040, 14044) to assess the environmental impacts of a product lifecycle across various impact categories. However, its applicability to Advanced Materials (AdMa) can be challenging due to lack of data, pertaining to the novelty of these materials and the products in which they are embedded.
Graphene oxide (GO) is used in drinking water filters to enhance their functionality and enable the removal of molecular-level emerging contaminants such as PFAS, metal ions and antibiotics. This case study is investigated with an analysis of the contributions of the flows and unit processes of the filters production to various environmental impacts, including climate change, resource use and ecotoxicity.
The lifecycle inventory was built on exchanges of information with the filters producer, enabling the use of first-hand foreground data, but several data gaps still needed to be covered. The Lifecycle Impact Assessment shows the contributions of different flows and unit processes to environmental impacts; the need for a GO-specific characterisation factor is discussed. The potential implications of these results on the design of the industrial process and on the environment are discussed.
The filter use and end-of-life will be investigated in continuation of this work to obtain a cradle-to-gate LCA, and the lifecycle impacts of these filters will be compared to the benchmark product (i.e. same filter without GO). This work contributes to the sustainability assessment of AdMa; it will be considered in the GO drinking filter production in the aim of making it as sustainable as possible.

Session 4

10:30-11:30

SUSTAINABILITY ASSESSMENT

How to integrate sustainability aspects into safety assessments

Harald F. Krug

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From EHS-Aspects to Sustainability: Advanced Materials in DaNa and MANTRA

In addition to environmental health and safety (EHS), more general issues relating to the sustainability of new innovative materials are playing an increasingly important role in the overall assessment of risk and benefits. The DaNa project has undergone this change over the past 14 years. From a pure safety assessment of nanomaterials at the beginning, advanced materials were increasingly included in the evaluation. Shortly before the end of the project, sustainability aspects were also added. The knowledge base can still be accessed at www.nanoobjects.info.
However, the evaluation in a new project goes well beyond this stage. The MANTRA project, funded by the Federal Ministry of Education and Research (BMBF) in Germany, will apply both safety and sustainability indicators to new and innovative materials in order to assess their impact on society, economy, human health

nd the environment. The development of new materials with enhanced properties e.g. for catalysis is undergoing a tremendous transformation. Simply by using AI, new possibilities can be discovered that previously seemed impossible. However, these are associated with significant limitations when the entire life cycle will be considered, from the extraction of raw materials to processing and integration into new applications down to deposition or recycling. If we want to do this in a safe way for all, the climate, the environment, and the society, we need to take a more holistic approach that allows an assessment based on comprehensible indicators in order to promote sustainable developments.
The DaNa project has already developed criteria for the safety aspects that can be used to ensure that the studies on material toxicology are of a sufficiently high quality. For sustainability, these criteria will be developed in the new MANTRA project and will jointly lead in this just started new activity to a holistic assessment of safety and sustainability.

Beatrice Negrini

University of Milano Bicocca; Project Hub-360, Department of Biotechnology and Biosciences; Department of Earth and Environmental Sciences; Sustainability & Innovation Consultancy Company, Milano, Italy

Safe and sustainable nano-enabled antimicrobials to reduce the presence of contaminants of emerging concern in the aquatic environments: An integration perspective

Contaminants of emerging concern (CECs) from intensive fish farming, including antibiotics and antimicrobial resistant (AMR) bacteria, pose threats to environmental and human health. Nanoparticles (NPs) and nano-enabled products (NEPs) emerged as a promising antimicrobial solution against AMR bacteria. However, assessing nanomaterials (NMs) safety and sustainability at the early design phase is imperative due to the uncertainties highlighted by toxicological and ecotoxicological studies. Furthermore, their potential emissions to environmental compartments, including fresh and marine waters, pose risks to non-target species.
The AMROCE project (GA nª869178) aims at facing the spread of AMR bacteria in aquatic ecosystems through the development of innovative nano-antimicrobial products alternative to antibiotics. This includes the synthesis of water-based nano-copper oxide (n-wCuO) and its incorporation by polymers coating or into bulk polymeric matrices, to finally obtain antimicrobial/antibiofilm fish cage nets and water filtration membranes NEPs. Additionally, bio-based nano-formulated antimicrobial actives and antibiofilm enzymes are explored as possible alternatives to conventional antibiotics for fish feed supplements.
To ensure the safety and sustainability of the biocide-NEPs since their early design stage, a multifaceted approach is proposed by integrating both in vivo and in silico methodologies to evaluate efficacy and to assess risks in exploitation scenarios.
Characterization and assessment of NPs emission to selected environmental compartments is addressed by TEM, DLS, and ICP-OES of NPs suspensions and leachates from polymeric materials. Eco-toxicity assessments of the novel NEPs are conducted using Danio rerio as a model organism, employing the Fish Embryo acute Toxicity test to assess acute toxicity and morphometric and behavioural analyses to investigate sub-lethal effects.
By utilizing primary data provided by AMROCE partners and secondary data from the Ecoinvent 3.7 database, LCA studies were performed following ISO14040-140044 standard methodology by applying CML 2001 method and using OpenLCA software to assess midpoint impacts of n-wCuO synthesis and incorporation to obtain antimicrobials NEPs in fish farming contexts. Nevertheless, mere impact assessment of NMs synthesis and incorporation would be incomplete if not considering direct endpoint effects of NMs on the environment.
Data on NMs emission sampling, combined with nano-eco-toxicity endpoints investigating potential damages to organisms suggests generating nano-specific eco-toxicity characterisation factors able to integrate multiple end-point effects peculiar to nanomaterials. This challenge is addressed within the INTEGRANO project (GA nª101138414) including the generation of nano-specific datasets, which offers the opportunity of exploiting the already standardised LCA methodological framework to support decision-making since the NMs early design stage for attaining safe and sustainable NEPs.

Session 4

10:30–11:30

SUSTAINABILITY ASSESSMENT

Socio-economic sustainability, societal acceptance of innovation

Lisa Pizzol

GreenDecision Srl, Venice, Italy

Socio-Economic Life Cycle-based Framework for Safe and Sustainable by Design of Advanced Materials

This study describes an innovative approach to socio-economic assessment of (advanced) engineered nanomaterials and nano-enabled products (NEPs) to support safe-and-sustainable-by-design (SSbD) decision making by industries in the early stages of product development. This semi-quantitative methodology is developed based on a sound conceptual framework grounded in the combination of social life cycle analysis and multi-criteria decision analysis methods and supports decision making based upon socio-economic impacts assessed over the full life cycle of a product. To facilitate its application by industries, the methodology was developed as an excel-based tool and is currently being transferred to a web-based self-assessment tool. This easy-to-use, cost- and time-efficient tool can guide users through their SSbD decision making regarding newly developed nanomaterials and NEPs and can also be applied to re-evaluate existing materials and NEPs in order to improve their sustainability from a socio-economic perspective. The relatively low requirements of this tool regarding the level of efforts and expert knowledge needed for its application make it a good sttarting point for initial assessment to highlight socio-economic issues in the value chain. As a steppingstone for a more holistic assessment, the S-LCA Framework is currently being used in the H2020 SUNSHINE Project Tier 1 Assessment and will be integrated as a mid-level sustainability assessment in the Horizon Europe SUNRISE project e-infrastructure for SSbD of advanced materials. Currently, testing and refinement of the tool in real case studies is being conducted including but not limited to photocatalytic ZnO/Silica complexes used in scratch and abrasion-resistant coatings for the construction sector, core–shell silicon carbide (SiC)–titania (TiO_2) anti-stick coatings for use in consumer products and graphene oxide-based materials for electrodes and energy storage (batteries).

Session 4

10:30-11:30

RISK GOVERNANCE

Harmonization & Standardization

Adriana Scattareggia Marchese

European Food Safety Authority (EFSA), Parma, Italy

Risk assessment of nanomaterials: EFSA`s nano guidance documents

In the EU context of regulated food and feed products, the European Food Safety Authority (EFSA) carries out scientific assessments to evaluate their safety to support risk managers in the decision-making. When a dossier submitted to EFSA contains a material that meets the definition of engineered nanomaterial set out in Regulation (EU) 2015/2283, this must follow the EFSA Scientific Committee Guidance on Risk Assessment of Nanomaterials, published in 2018 and updated in 2021.

This Guidance covers the application areas within EFSA's remit, including novel foods, food contact materials, food/feed additives, and pesticides, in the context of human and animal health risk assessment. The Guidance proposes a structured pathway for carrying out the safety assessment of nanomaterial and provides practical suggestions for the type of testing needed and the methods that can be used. However, nano-specific considerations for risk assessment may be required also for conventional materials that contain a fraction of small particles, but do not meet the definition of engineered nanomaterial. For this reason, following a mandate from the European Commission, EFSA has developed and published in 2021 its new Guidance on Technical Requirements to establish the presence of small particles including nanoparticles. This Guidance sets out the information requirements for applications in the regulated food and feed product areas and establishes criteria for assessing the presence of a fraction of small particles, including particles requiring specific assessment at the nanoscale, in conventional materials which do not meet the definition of engineered nanomaterial. Both Nano Guidances should be considered as complementary, and their application should be integrated into the risk assessment of relevant sectoral frameworks. Since their publication, the EFSA cross-cutting Working Group on Nanotechnologies has been working to support EFSA Panels in the smooth and harmonised implementation of both Nano Guidances. In light of the experience gained from practical cases and ad hoc surveys targeted to various stakeholders, possible elements for improvement were collected to consider future actions to further support applicants and risk assessors.

Soyeon Jean

Dong-A University, Busan, Korea, Republic Of

Bronchoalveolar Lavage Fluid Analysis Method for the Inhalation Toxicity Studies of Nanomaterials

The safety of nanomaterials has become a global agenda due to the rapid industrialization of nanotechnology, leading to a heightened focus on Environmental, Health, and Safety (EHS) studies and regulations. Consequently, the analysis of bronchoalveolar lavage fluids (BALF) and lung burden in inhalation toxicity studies of nanomaterials has become more important.

However, the absence of standardization in BALF analysis methods has led to potential laboratory discrepancies. Therefore, we evaluated various collection and analysis methods in BALF using nickel oxide nanoparticles, zinc oxide nanoparticles, and graphene oxide nanoparticles. The process of BALF collection had crucial factors, including the number of collection times, flushing, and massage. The total cells, lactate dehydrogenase (LDH), and total protein in inflammation analysis of BALFs decreased as the number of lavage fluid collection times increased regardless of nanoparticle instillation. Moreover, a group that did flush and massage (wFwM) showed an increase in total cells, LDH, and total protein compared to the other group that did not flush and massage (woFwoM). However, the proportion of inflammatory cells in the wFwM group gradually decreased as the number of collected lavage fluid increased. These results could be interpreted overly in analyzing lung inflammation caused by nanoparticles. The PBS with fetal bovine serum (FBS) was excellent as the re-suspension manner for cells after centrifugation of BALF, and the 4 °C was better than 37 °C as storage temperature because other manner showed cell death with the frustrated cell membrane. Lastly, we compared the manual and automatic methods of counting total cells using a hemocytometer and Nucleocounter. When the number of total cells was high, the manual method tended to count far more cells than the automatic method. These results can contribute to the standardization of processes by providing a method for collecting and analyzing BALF, thereby enhancing the reliability and comparability of research in this field.

10:30-11:30 Session 4

RISK GOVERNANCE

Regulatory perspectives

Wilson Engelmann

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The regulatory challenges of the NanoWorld

Regulating new technology is always a delicate matter because two choices must be addressed: regulate it right away or wait for a more fully developed information framework. If the implemented regulatory framework is too restrictive, the progress of the technology itself may be hampered. David Collingridge (1980) characterizes the two faces of this technological dilemma as a pair of seemingly opposing problems: a) an information problem resulting from a lack of knowledge about how to structure a regulatory framework or scientific questions about the risks of some nanoparticles. This is strictly an issue of nanotechnology; b) a power problem: even when nanotechnologies are fully established, with adequate information for regulation, it may still be difficult to set limits or other legal constraints. The dilemma lies in the challenge of finding a regulatory formula that can provide legal certainty without curbing research and technological innovation. To design an appropriate path toward solving this dilemma, one must consider the advantages that new technology may bring to people's lives in society, as well as the potential risks and damages it may pose to the environment and healthy living beings. Respect for fundamental rights and guarantees (internal sphere) and the safeguarding of human rights (external sphere) must be valued. These two guidelines regulating nanotechnology should be designed by a diverse, standard-setting group of stakeholders and experts in technology, in compliance with the so-called "Regulatory Science". This includes the development of new tools, standards, and approaches that efficiently and consistently assess the safety, efficacy, quality, and performance of products. Data from regulatory scientific research supports education by sharing best practices through the mentoring of national and international peers, regulatory decision making, the development of scientifically sound reference materials, reviewed decisions, marketing authorization decisions, regulations, consumer recommendations, labeling.

This set of tools and actions for building the regulatory architecture of the disruptive innovation that is typical of progressive nanotechnology will need an incremental, reflexive and cooperative regulatory approach, using the creation of both public and private rules. Guided by the drivers of adaptation, co-evolution, and agility, combined public and private efforts can lay the foundation for the effective structural reform of technology regulation, and its innovations. The regulatory sector must also go through the different formats and paces of the innovative process to standardize these novelties. Across the globe, several standard-setting bodies or agencies develop and publish "normative standards" and present different regulatory options for nanotechnology.

Session 4

10:30-11:30

RISK GOVERNANCE

Safe & Sustainable by Design (prevention-based governance)

Natalia Fernández Pampín

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Application of the SSbD framework to alternative Plating on Plastics (PoP) processes hexavalent chromium (Cr6+) and palladium free

The European Commission (EC) has recently developed a framework for the definition of criteria for SSbD chemicals and materials to transform the EU's current economy into a greener and more sustainable one (EC, 2019), aiming to promote the substitution or minimization of the production and use of substances of concern, as well as reducing hazard, risks, environmental and socio-economic impacts. In the present work, the plating on plastics (PoP) process was selected in the context of FreeMe project to evaluate the application of the SSbD guidelines during the first stages of its life cycle, including design, development, production, and use of chemicals and materials. Pating on plastics (PoP) technology has been experiencing significant growth in recent years due to its wide range of applications: automotive, aerospace and home appliances industry. However, the conventional process for PoP presents some significant disadvantages related to environmental sustainability, occupational safety, and quality of coatings i.e the use of hexavalent chromium (Cr6+) in the etching process and the use of palladium in the activation step.

FreeMe project provides a safer and more sustainability alternative to the conventional PoP process, proposing two revolutionary approaches for the metallization of polymeric (plastic or resins) surfaces without the use of hexavalent chromium (Cr6+) and palladium. The first approach is based on the use of resins (e.g. epoxy-acrylate resins) with the additions of suitable nickel-based precursors while the second approach is based on a Cr6+ and Pd free pre-treatment of the polymers surface.

Following the SSbD framework, the two alternative approaches proposed were evaluated in order to establish comparisons with the conventional method. Both approaches show a better performance in terms of safety, environmental and social sustainability. Therefore, the FreeMe technology contributes to enhance the safety and sustainability of the PoP industry.

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Practical guidance and uptake of SSbD strategies and concepts by the industry

Safe and sustainable by design (SSbD) can be described as an approach that focuses on providing functional materials/products while avoiding damage to human health or the environment because of their fabrication process, use, or disposal. Although this concept has been adopted recently by the nanosafety community as a means to dampen human health and environmental concerns, the SSbD principles are still in their infancy when applied to more complex nanomaterials such as multi-component nanomaterials (MCNMs) or advanced materials (AdMa). Integration into product development may be hampered by a lack of quality data on the performance, sustainability, hazards, and release potential of the great variety of MCNMs in use.

The H2020 SUNSHINE project (grant agreement No 952924) aims to address this problem by developing an approach to operationalize the concept of SSbD focusing on MCNMs and promoting the dialogue and collaboration of all stakeholders involved in the supply chain. As practical guidance for the implementation and uptake of SSbD concepts that apply to the specific case of MCNMs, a series of factsheets are being developed within the project framework. These user-friendly factsheets are aimed at introducing how and why SSbD should be integrated into product development within the whole value chain, involving all the relevant actors and stakeholders.

The factsheets include aspects such as how to implement SSbD strategies in real industrial case studies, the impact of surface or chemical modification of the MCNM on product functionality, safety and sustainability. In addition, the factsheets reflect how SSbD is understood within the supply chain and how the current regulatory framework is aligned with the SSbD approach.
These practical guides show how the SUNSHINE project has implemented SSbD approaches for real industrial case studies dealing with MCNMs from the early stages of innovation and has developed the SIA infrastructure for decision-making support, allowing other users to uptake this valuable knowledge. In addition, these factsheets will promote the dissemination of the different SSbD approaches and the overall results derived from the project.

(1) Caldeira, C., Farcal, R., Garmendia Aguirre, I., Mancini, L., Tosches, D., Amelio, A., Rasmussen, K., Rauscher, H., Riego Sintes, J. and Sala, S., Safe and sustainable by design chemicals and materials - Framework for the definition of criteria and evaluation procedure for chemicals and materials, EUR 31100 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-53264-4, doi:10.2760/487955, JRC128591.

Session 4

10:30-11:30

RISK ASSESSMENT AND MANAGEMENT

Implementation of NAMs for risk assessment

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THE EFSA NAMS4NANO PROJECT: Review of NAMs for application in Risk Assessment of Nanoparticles in the Food and Feed Sector

New Approach Methodologies (NAMs) show great potential in advancing risk assessment (RA). However, their regulatory implementation is lagging behind compared to their scientific development. The EFSA Guidance on RA of nanomaterials suggests nano-specific RA is best achieved through Integrated Approaches to Testing and Assessment (IATA) with NAMs as the first choice to generate new information. Integrating NAMs in RA promises several advantages such as a better human focus, a stronger emphasis on molecular mechanisms and a higher efficacy. However, applying NAMs to nanomaterials (NMs) also poses considerable challenges such as issues related to dispersion stability and dosimetry. Significant efforts are being undertaken to establish nano-specific NAMs. Within the EFSA-funded project NAMS4NANO we have conducted a review of NAMs which are potentially useful for application in RA of NMs in the food and feed sector.
Our review covers nano-specific NAM-frameworks and individual NAMs. Specifically, for NMs we emphasize physicochemical characterization methods for their inclusion as NAM based approaches. A particular focus was on initial screening methods according to the EFSA framework, i.e., on NM degradation/dissolution, genotoxicity, cytotoxicity, oxidative stress, inflammation, and barrier integrity. In total, we identified 242 relevant individual NAMs, including 24 NAMs for genotoxicity, 27 for cytotoxicity/ cell viability, 16 for reactivity/ oxidative stress and 8 for inflammation and barrier integrity. In addition, 37 NAMs for physicochemical characterization and 23 for dispersion are included in the review. In summary, various nano-specific NAMs could be relatively mature and therefore very promising to be further explored for RA, especially in integrated approaches, along with conventional animal and human data (where existing).
To further advance the regulatory application of NAMs two general recommendations are proposed. Firstly, it is considered important to discuss selected NAMs that are deemed to be mature with regulatory and validation experts. Secondly, such NAMs need to be practically tested in nano-specific RA case studies to better explore their potential along with remaining challenges and uncertainties. Several of the identified issues are currently addressed within the umbrella of the EFSA-funded NAMS4NANO projects.

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Use of a suite of advanced human intestinal models for assessing particle uptake and crossing in the EFSA NAMS4NANO Project

Obtaining reliable evidence on the uptake and potential crossing of the human intestinal epithelium by particles plays a crucial in role in assessing the risks of food-relevant nanomaterials as well as particulate materials with a nanofraction. If following ingestion particles reach the human intestine as such and translocate across the intestinal barrier, systemic exposure to particles has to be taken into account in risk assessment.
The EFSA-funded action NAMS4NANO 'Integration of New Approach Methodologies" aims to promote the use of NAMs in nanospecific risk assessment of food-related applications, covering both nanomaterials and conventional materials containing a fraction of small particles. Within this project, a suite of advanced human intestinal models is used at ISS for the assessment of internalization and possible translocation of particles. A co-culture of three human-derived cell lines, i.e. Caco-2 cells, HT29-MTX cells, and Raji B lymphocytes, is used as an advanced absorbitive model for featuring mucus secreting cells and microfold (M) cells to study the oral exposure to nanomaterials with enhanced physiological relevance. The same model will be used in dynamic (instead of static) conditions resorting to a fluidic system. The array of models will be completed by a reconstructed tissue system. The performance of the static triculture will be used as benchmark for the latter two models. Uptake and crossing studies will be performed both on pristine materials and 'aged' materials, i.e. on samples obtained applying procedures to simulate the physicochemical changes caused by transit in the gastrointestinal tract. Materials showing complete dissolution in gastric conditions will be excluded from uptake and crossing studies. Several real-world particulate materials and nanomaterials used in food application, sourced in the market, will be investigated along with model nanomaterials or reference materials from the JRC repository. For inorganic nanomaterials, i.e. metals (Ag) or oxides (ZnO, Fe and Cu oxides, SiO2), the characterization and quantification of the particles taken up or translocated, as well as of any soluble chemical species originating from them, will be achieved by ultra-sensitive, state-of-the-art triple quadrupole ICP-MS, either alone or in combination with ultrafiltration, and by single particle ICP-MS with microsecond dwell-times. Control experiments with ionic counterparts (e.g. soluble Zn species representative of dietary zinc in the ZnO case study) will be carried out.

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The EFSA NAMS4NANO Project: Designing and Conducting Methodological Case Studies

The EFSA-funded action NAMS4NANO 'Integration of New Approach Methodologies results in chemical risk assessments: Case studies addressing nanoscale considerations' is a multiannual project including several individual projects. The overall aim is to promote the use of NAMs in nanospecific risk assessment, covering both nanomaterials and conventional materials (i.e. not engineered at the nanoscale) containing a fraction of small particles. According to the framework on risk assessment of nanoparticles in applications related to food and feed falling within EFSA's remit (nutrients and nutrient sources, novel foods, food contact materials, food additives, food flavourings, feed additives, and pesticides), nanoscale specificities are integrated in the risk assessment process as nanoscale-based hypotheses. NAMs are the first choice to generate information for addressing these hypotheses and improve mechanistic understanding of processes at the nanoscale. IATAs are used for the integration of human, animal and NAMs-derived evidence.
NAMS4NANO Lot 3 is a 4-year project focused on designing and conducting a set of five proof of concept methodological and cross-cutting case studies (CS), which develop NAM-based tools and procedures of high relevance for nanospecific risk assessment within the EFSA remit. Examples are the development of tools and methods to cover nanoscale considerations in a particular phase of the risk assessment, e.g. for the characterisation of the material or for assessing cellular internalisation, gastrointestinal uptake and barrier crossing.

The first two CS focus on the assessment of complex nanomaterials, i.e. a nanocarrier loaded with a pesticide active substance and food-relevant nanofibres. CS 3 aims at developing a procedure for simulating transformations in the GI tract to obtain relevant materials for in vitro testing. CS 4 deals with the development of diseased models for the intestinal barrier to generate information on potential risks in vulnerable populations. Finally, CS 5 focuses on simple whole organism models – i.e. flatworms (Schmidtea mediterranea), nematodes (Caenorhabditis elegans), and zebrafish (Danio rerio) - representing the bridge between in vitro models and in vivo models in nanoparticle toxicity assessment. The implementation of these case studies will contribute to methodological progress and update of EFSA guidance documents.

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A new approach methodology for inhalation risk assessment of new nanomaterials

New Approach Methodologies (NAMs) are valuable tools collecting toxicological data crucial for next generation risk assessment while reducing the use and the number of animals. Nowadays, nanomaterials (NMs) find extensive application across various commercial products, sparking concerns regarding potential hazards to human health. Here we introduce a NAM-centered framework designed to identify and evaluate the hazard associated with new silver (Ag) and titania (TiO2) based NMs with different coatings. The use of Ag and TiO2 nanoparticles (NPs) comes from the former's antimicrobial properties and the latter's photocatalytic activity. To assess the potential hazard of these NMs, we determined the most likely retained lung dose of a worker exposed during the production of Ag and TiO2 nano-enabled products using monitoring campaign data collected in a manufacturing site. The use of relevant doses of exposure to assess the hazard is becoming increasingly important in the risk assessment framework. The average alveolar retained dose was calculated considering a working shift of 8 hours for 5 days and the exposure doses used in this study (in the order of ng/cm2) represent a chronic human exposure of 1 month, 6 month and a year. A cell contact co-cultures (A549 and THP-1 cells differentiated in macrophages) was used as in vitro model representative of the alveolar space, cultured and exposed at the air-liquid-interface (ALI). Exposure to aerosolized NPs was performed by the Vitrocell® Cloud Alpha 12 system. The deposition efficiency (DE) of each NP was preliminary determined by using a quartz crystal microbalance available with the system. Our results show different DE depending on NPs physico-chemical (p-chem) characteristics. This emphasize the importance of DE assessment for an accurate definition of the concentration of NPs to be nebulized to achieve the desired exposure dose. The p-chem properties of the NPs were evaluated by TEM and DLS. The effects on the biological model in terms of cell viability and cytokine release were evaluated 24 hours after the exposure. Whole-Genome transcriptomics changes were also studied to determine the relevant pathways induced by NPs. Altogether, the preliminary results suggest the lack of hazard at doses representative of a chronic inhalation exposure, confirming the safety of the developed NPs.

Aleksandra Nowak

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Calculations for band gap of Ni-doped LaCoO3 (LaCoxNi1-xO3). A DFT+U approach

Perovskite oxides ReTO3 (Re – rare or alkaline earth cations, T – transition metal cations) attract a lot of attention due to their interesting properties, such as superconductivity or catalytic activity. Furthermore, their functional properties can be tuned by doping with different transition metals ions, characterized by different properties. For example, LaCoO3 at its ground state is a non-magnetic insulator, however, partial substitution of Co ions with Ni ions induces ferromagnetic behavior.
In this study, we performed calculations for band structure of Ni-doped LaCoO3 and observed how the concentration of Ni influence the band gap width. Understanding band gap structure of perovskites is crucial, as it's directly related to their electronic properties and is often correlated with toxicity – wider band gap typically indicates lower toxicity.
It is known that density functional theory (DFT) approach can give incorrect results for materials that contain transition metals (1). Therefore, we performed DFT+U calculations, in which standard DFT energy functional (EDFT) is corrected by an additional term (EU) that uses Hubbard model in order to describe on-site correlations between electrons.
In many DFT+U calculations U values are chosen to be constant, however it has been demonstrated that these values can vary in the same material in relation to such quantities as spin state of transition metals cations or cell volume (2). Thus, in order to obtain more accurate results, we performed linear-response calculations for Hubbard U. In this approach DFT+U is rendered ab initio, so the need for any semi-empirical evaluations in the corrective functional is eliminated.
The calculations were performed with the Quantum Espresso package. Computed band gap values can be further used as descriptors in computational models for toxicity prediction.

Session 4

10:30-11:30

RISK ASSESSMENT AND MANAGEMENT

Tools beyond nano

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CHIASMA, INSIGHT and PINK: an European approach to animal-free Safe and Sustainable by Design

The three newly funded, complementary HorizonEurope Projects CHIASMA - Accessible Innovative Methods for the Safety & Sustainability Assessment of Chemicals & Materials (1), INSIGHT - Integrated Models for the Development and Assessment of High Impact Chemicals and Materials (2), and PINK - Provision of Integrated Computational Approaches for Addressing New Market Goals for the Introduction of Safe-and-Sustainable-by-Design Chemicals and Materials (3) will address the lack of validated alternative and standardised methods, insufficient stakeholders' coordination, and limited access to relevant data that have hitherto hindered the full adoption of a more ethical and sustainable toxicology and hampered the emergence of a comprehensive approach to safe and sustainable by design (SSbD) of chemicals and advanced/nano-materials, which is crucial to address the challenges of modern society. Traditionally, safety assessment heavily relies on the use of animal models, which are costly, unethical and often generate unreliable results, which are difficult to translate to humans. With the Green Deal and the One Health programs, the EU

Commission has set ambitious goals for a better and more sustainable environment and society, where available resources are used in a more ethical and sustainable way. The three projects will aid the reaching of these goals by generating new conceptual approaches to integrate NAMs and data for the next generation of hazard- & risk assessment of chemicals and materials. This will include integration of multi-tiered "in knowledge", AI enabled in chemico/in silico and in vitro approaches answering specific industry and regulatory needs. The projects were developed and funded to work in a collaborative and synergetic manner (incl. the sharing of industrial case studies); they started on the 1st January 2024, and will run for 48 months, during which 37 individual research institutions from all over the world (i.e. 13 EU members states, United Kingdom, Switzerland, Korea, USA, Brazil and Australia) are driving innovation through a combined budget of over 23.2 Million € (17 from the EU, and 5.8 from the funding agencies of the associated partners).

10:30-11:30 Session 4
Other

Franz Friebel

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The M2AS - Mass and Mobility Aerosol Spectrometer

We describe the M2AS, a new system for measuring aerosol particle masses and its application for the characterization of airborne nanoparticles.
For non-spherical particles, diameter measurements are dependent on both the particle size and its shape whereas the mass is always well-defined.
Different diameter metrics (e.g. mobility diameter, aerodynamic diameter) measured by different instruments are differently dependent on density, which is often not well known for real-world aerosols.
In many areas such as health effects and pharmaceuticals, the mass of material in the particle is of primary importance on its effects on the human body. The M2AS consists of a unipolar charger feeding a CPMA with the selected aerosol measured by a CPC and a new instrument, the MSE Mobility Separator - Electrometer. The aerosol mass distribution is measured in a single scan of the CPMA. The high concentration unipolar ion charging produces a charge distribution which is effectively continuous and this allows the charge state to be calculated from the ratio of electrometer and CPC counts rather than requiring a prior assumption. Thus the particle mass at any point in the scan can be unambiguously calculated from the CPMA selected mass : charge ratio. The Mobility Separator is a classifier which divides the aerosol output from the CPMA into two streams according to their electrical mobility: each stream is fed to a separate electrometer detector.
The classification voltage required to maintain a given ratio of these two electrometer signals is directly proportional to the median electrical mobility of the aerosol. From this and the charge state the mechanical mobility is thus obtained. This allows the CPMA transfer function width to be calculated, which is required to accurately determine the input aerosol concentration from the CPC measurement. The mobility diameter is also calculated from the mobility, and thus the particle density is also calculated.
This new device was applied to characterize diesel soots and soot surrogates. The latter are frequently used in research to avoid experimental challenges that come with generating freah diesel soot. We present data that highlight significant differences between both material classes and raises the question whether or not freshly produced diesel soot can be substituted or not.

10:30-11:30
Session 4
Data-driven modelling

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Unveiling Graphene Nanoparticles: First Principles Atomistic Insights and Toxicity Predictions Using QSAR/QSPR Models

In this study, we investigate the material properties, biological effects and potential toxicity of graphene nanoparticles (GNPs) through a comprehensive atomistic modelling approach. A total of 100 graphene nanoflakes were constructed (considering size, shape and edge diversity) and optimized using first-principles calculations. Detailed analysis of electronic and structural properties was conducted, providing insights into their behavior at the atomic level. Key nanodescriptors, reflecting crucial characteristics were computed for each nanoflake and curated using Principal Component Analysis (PCA) to ensure robustness and relevance. The properties of these nanoparticles were examined with a focus on their geometric features, revealing significant variations in behavior and interactions based on their specific configurations. Interaction studies were performed to evaluate the binding affinities of the GNPs with 100 different human target proteins (considering different toxicological routes), as well as model lipid bilayers, which are essential components of cellular membranes. These interactions are critical in understanding the potential biomedical applications and toxicological profiles of the nanoparticles. Quantitative Structure-Activity Relationship (QSAR) models were developed to predict the properties and biological activities of the GNPs. These models integrated the nanodescriptors and interaction data, allowing for the in silico prediction of nanoparticle properties, including potential toxicity. The QSAR models demonstrated high predictive power, offering a valuable tool for assessing the safety and efficacy of graphene nanoparticles in various biological contexts. This study provides a detailed atomistic-level understanding of GNPs, highlighting the importance of geometric features in determining their biological interactions and potential toxic effects. The combination of first-principles optimization, nanodescriptor calculation, and QSAR modeling offers a robust framework for predicting the behavior of graphene nanoparticles, paving the way for safer and more effective applications in nanomedicine and other fields.