European Commission | Horizon 2020 European Union funding for Research & Innovation

SmartNanoTox
Smart Tools for Gauging Nano Hazards

SMART TOOLS FOR GAUGING NANO HAZARDS

NMP-29-2015: Increasing the capacity to perform nano-safety assessment

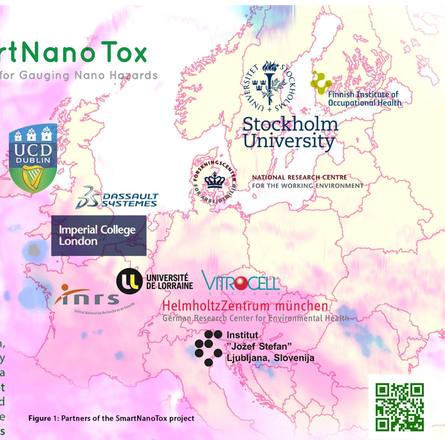

Figure 1: Partners of the SmartNanoTox project

Background and Motivation:

In this project, using a comprehensive self-consistent study, which includes *in vivo*, *in vitro* and *in silico* research, we address **main respiratory adverse outcome pathways** (**AOP**) for representative set of nanomaterials, identify the **mechanistic key events** (**KE**) of the pathways, and **relate** them to **interactions at the bionano interface** via careful post-uptake nanomaterial characterisation and molecular modelling. Our goal is to **formulate a novel set of toxicological mechanism-aware end-points** that can be assessed by means of economic and straightforward tests. Using the exhaustive list of end-points and pathways for the selected nanomaterial and exposure routes, we attempt to relate the **AOPs** to the properties of the material via **quantitative structure-activity relationships** (**QSAR**). This will lead to grouping of nanomaterials based on their ability to trigger the pathway, and will enable an identification of properties of concern for new nanomaterials.
The SmartNanoTox predictive model for gauging the toxicological and biological impacts of nanomaterials will be based on the mechanistic approach, which makes use of detailed understanding of the response of the organism to exposure to nanomaterials from the initial contact to the adverse outcome.

Figure 2: Conceptual diagram of the novel paradigm for NM toxicity assessment: The new approach relates the NM properties to KE/MIE rather than adverse outcome or *in vitro* toxicity endpoint.

Project structure:

- **WP1** deals with in vivo exposure supplemented with in vitro experiments: inhalation, instillation of rodents, analysis of the gene expression and proteomics and supplies the data about the NM entry routes and endpoints for **WP2** and **WP3**
- **WP2** contains in vitro experiments, either complementing the in vivo studies of **WP1** or standalone and aims at characterising the NM state after entering the organism, or specific molecular mechanisms of the uptake, transport or toxicity
- **WP3** is devoted to analysis and integration of the gene expression and/or proteomics data as obtained in and **WP2**, data mining and statistical modelling aiming to establish the adverse outcome pathways and identify the MIE/KEs
- **WP4** is purely computational and deals with modelling biomolecules in contact with nanoparticles and building a database of bionano interactions
- **WP5** assesses the candidate MIE/KEs as extracted in **WP3** either in silico, using the models from **WP4**, or in vitro and validates the identified pathways and MIE/KE by modifying either the NM or the target system
- In **WP6** we finally construct a simplified in vitro or in silico test (iQSAR) for the identified AOPs using the database from **WP4** combined with findings of **WP5**
- **WP7** contains dissemination and exploitation tasks, while **WP8** accumulates the management tasks

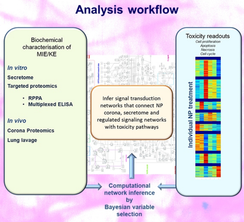

Figure 4: Scheme of determination of the toxicity pathways

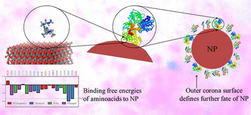

Figure 5: Scheme of the multiscale simulation technique for measuring the protein adsorption energies and testing the likelihood of molecular events

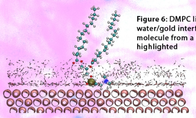

Figure 6: DMPC lipid at an water/gold interface, one molecule from a bilayer highlighted

Partners and their core activities:

- **In vivo experiments - inhalation and instillation**
  HMGU, ***NRCWE***, and ***INRS*** perform *in vivo* experiments on mice. Histology from all in vivo experiments will be analyzed by ***FIOH***.
- **Omics and statistical modeling:**
  UCD and ***UL*** teams use data obtained *in vivo* by ***HMGU***, ***NRCWE*** and ***INRS***, to develop statistical models, which link the corona composition, secretome and altered signaling pathways to the cell fate e.g. apoptosis, proliferation or cell cycle arrest
- **In vitro experiments: Nanoparticle uptake and nanoparticle-biomolecule interactions**
  JSI design particle labelling techniques and fluorescence and superresolution micro(spectro)scopy (FMS & STED) to track the NMs within lung epithelial cells and macrophages. ***Vitrocell*** delivers an air-liquid interface aerosol exposure system for realistic cell line exposure. ***UL*** and ***HMGU*** are involved in *in vitro experiments* (cell culture in a rat/mouse models). ***NRCWE*** measures the dependence of lipid monolayer surface tension after exposure to NMs.
- **Multiscale modelling of nanoparticle-biomolecule interaction:**
  UCD , ***SU***, and ***Imperial*** use molecular simulation to provide quantitative descriptors of NMs before and after initial contact with living organisms and quantitatively address MIE/KE, the events at bionanointerface involving the nanoparticle that can steer the toxicity pathways.
- **Nanoparticle descriptors:**
  UCD , ***SU***, ***Imperial*** , ***Biovia*** calculate NM descriptors such as water adsorption energy, adsorption energies of amino acids and lipids, width of conduction band gap, aqueous solubility, etc.
- **Databases and QSARs:**
  We deliver a database of physicochemical characterization of NMs containing newly developed descriptors and of *in vivo* and *in vitro* toxicity assessment results, which will be made public after completion of the project.

Nanomaterials

The project plans to use 61 NM available at the ***NRCWE***, ***JSI*** and other partners, including TiO_2, SiO_2, CNTs, asbestos, ZnO, Fe_2O_3, etc.

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SmartNanoTox objectives:

- To **identify** main **pulmonary adverse outcomes** induced by common nanomaterials, and identify associated **MIE**, **KEs** and **toxicity pathways** leading to adverse outcome.
- To **establish relationships** between physicochemical properties of nanomaterials and KEs steering the toxicity pathways leading to adverse outcome, and suggest descriptors for grouping of NMs according to their toxicological mode-of-action.
- To **create a database of bionano interactions** that will enable development of read-across and **QSAR tools for the toxicity assessment** of new nanomaterials.
- To **develop a smart screening approach**, where predictions of toxicity of a nanomaterial can be made on the basis of purely computational or limited *in vitro* screening tests focused on crucial bionano interactions.

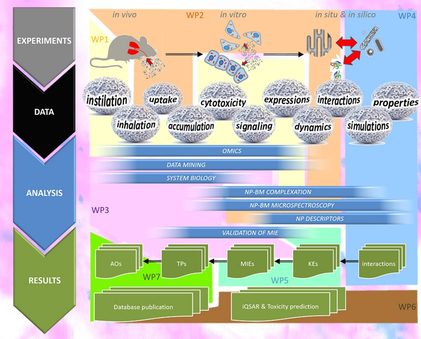

Figure 3: Outline of the concept and approach with Work packages and their interconnections

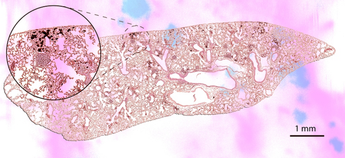

Figure 7 : We aim to identify and analyse *in vivo* pulmonary toxicity by histopathology for a variety of engineered nanomaterials

SmartNanoTox projected outcomes:

- **Mechanism-aware AOP-oriented QSARs** for toxicity prediction.
- **Toxicity assessment strategy** based on *in vivo* study of the acute and chronic toxic effects, with relation to the AO and in-depth an alysis of the pathologies.
- **Analysis of post-uptake state of NMs**: biomolecular corona of the NMs, NM tracking inside the biological fluids and identification of molecules involved in bionano interactions.
- **Identification of generic properties responsible for pulmonary toxicity** for carbonaceous materials, non-metal oxides.

with expected impacts on NMPB Programme and the call (*Increasing capacity to perform nanosafety assessment*):

- **New screening tools** to enhance the efficiency of end-rate at which NM hazard profiling can be performed
- **"Safer by design" approaches**, tailored to stakeholders' needs (modelers, industry and regulators)
- Data in a recognized and **accessible database** for use beyond the lifetime of the project
- **Solutions to the long-term challenges of nanosafety and nanoregulation**

Figure 8 (background of the poster): Nanomaterial (blue) tracked within living lung epithelial cells with labeled membrane structures (purple) as seen by superresolution STED microscopy (scale is on the right)

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