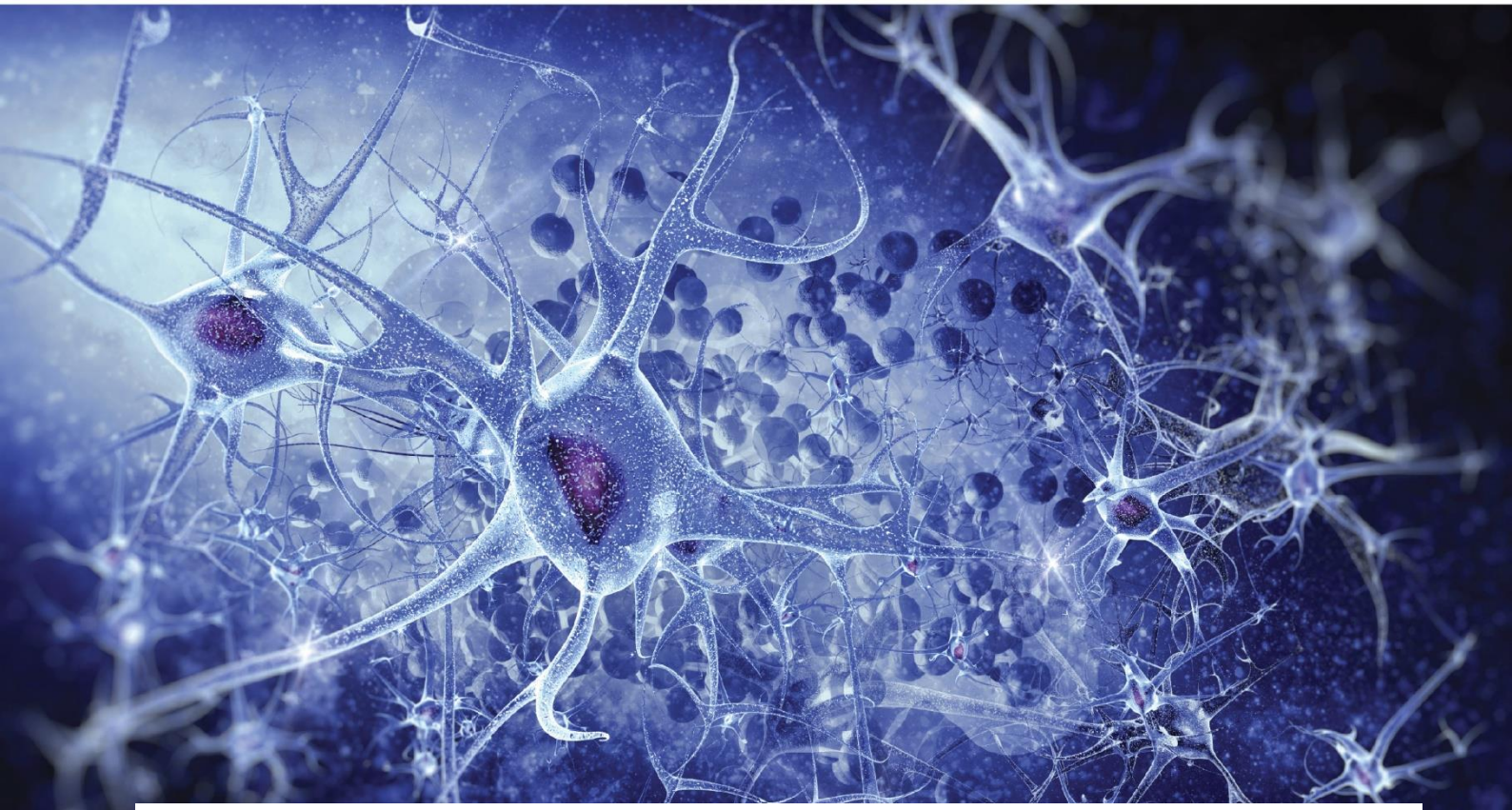
BILAT USA 4.0



EU-US PRIORITIES IN NANOSAFETY

IDENTIFIED DURING THE BILAT US 4.0 EVENT
"FOSTERING EU-US COOPERATION IN NANOSAFETY"
MARCH 5-6, 2019, HARVARD UNIVERSITY

Table of content

CONTEXT

The event on **Fostering EU-US Cooperation in Nanosafety** took place in the context of the BILAT USA 4.0 project which was funded by the European Union, and initiated on 1 February 2016. This initiative aims at enhancing and developing science, technology, and innovation (STI) partnerships between the U.S. and Europe. A particular focus of the project activities is on intensification of interactions between EU and US researchers and innovators, the support for the improvement of research and innovation framework conditions, the provision of analyses delivering a sound base for political decision making and an enhanced coordination and synergies between different European and US policies and programs. The workshop on Nanotechnology/Nanosafety is one of six workshops designed to boost STI collaboration activities in several established priority areas (health, marine and arctic, NMP, transport) for EU-US research and innovation cooperation.

Nanotechnology offers great potential of innovation in several sectors and global research investments in this area are growing exponentially. This potential can be implemented only if concerns about risks are adequately addressed. For this reason, a number of nanosafety research programs have been established in both the USA and EU that devote considerable resources to hazard and exposure assessment and safe-by-design work. Cooperation between these efforts has existed for years, in the shape of seven Communities of Research having annual face-to-face meetings, and this ought to continue and extend.

Starting with presentations of the ongoing policies, initiatives and projects on nanosafety in Europe and the US, and basing the discussion on the state of implementation of the roadmap "Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations", and current nanosafety activities coordinated by participating in US National Nanotechnology Initiative (NNI) federal agencies, this workshop aimed to answer two main questions:

1. What should be the future research priorities in nanosafety and other advanced materials?

2. What are the opportunities for EU-US cooperation priorities in nanosafety?

The answer to these questions lead the more than 30 participants from academia, industry and policy to the drafting of recommendations to be shared with both the European Commission and the relevant US funding agencies in view of the upcoming Horizon Europe.

EVENT SUMMARY

After an initial introduction with an overview on 15 years of cooperation in nanosafety between the EU and the USA, the first day started with an overview of the European and US perspective on Governance of technologies and current programs in Nanosafety from the European Commission and US National Nanotechnology Coordination Office.

The second session in day 1, titled Current US and EU running projects and next actions in Nanosafety, introduced relevant projects and initiatives and state-of-the-art in nanotechnology research and policies.

In the third session in day 1, focusing on Reporting from current research projects and consortia, defining scope of cooperation, the participants had the chance to share knowledge between EU and US projects in Nanosafety, focusing on Nanosafety cooperation to next generations of nanomaterials.

The second day was designed with the intention to help the group converge on concrete ideas and craft priority areas together for future research cooperation between EU and US. An initial session set the scene with a panel discussion on implementation, research priorities, and future orientations.

The introduction session set the scene for a structured discussion by highlighting existing priorities identified on both the EU and US roadmaps on nanotechnologies. It was followed by a roundtable discussion where panelists and the audience were asked to reflect on what has been implemented and what needs to still be a priority?

Using Nominal Group Technique through a facilitated and structured dialogue, then lead to the identification of common EU-US research priorities and ways to convey them to the relevant policy-makers and funders.

IDENTIFICATION OF RESEARCH PRIORITIES

As a result of the exercise 7 main research priorities have been identified:

1. Environment and human hazards
2. Emerging nanomaterials and potential risks
3. Social and natural science research to support balanced risk governance of emerging materials
4. Nanoinformatics
5. Exposure assessment at both environment and human population levels
6. Standard methodologies , reference materials and harmonization
7. Life cycle/transformation/value chain/stewardship

The research priorities are developed in the following section of this document.

In addition, during the discussion some potential instruments for advancing the EU-US cooperation in the topic were highlighted:

- Twinning of existing projects
- Exchange of young scientists and mobility of researchers
- Participation of US to H2020 and future Horizon Europe. EPA, NIEHS, NSF and other federal agencies could fund the US participation in EU initiated nanosafety programs.
- Establishment of joint EU-US research programs
- Promotion of the Malta initiative and opening to the US
- Future potential COST-like projects[1]
- Inducement prizes[2]

[1] COST (European Cooperation in Science and Technology) is a funding organisation for research and innovation networks. It helps connect research initiatives across Europe and beyond and enable researchers and innovators to grow their ideas together in any science and technology field by sharing them with their peers. COST Actions are bottom-up networks with a duration of four years that boost research, innovation and careers.

[2] In Horizon 2020, the European Union Framework Programme for Research and Innovation, Horizon Prizes are challenge prizes (also known as inducement prizes) offering a cash reward to whoever can most effectively meet a defined challenge. The aim is to stimulate innovation and come up with solutions to problems that matter to European citizens.

ENVIRONMENT AND HUMAN HAZARDS

In order to achieve a more predictive, better focused and more realistic hazard assessment for humans and the environment, different aspects need to be considered:

We need a stronger focus on the development of high throughput, predictive screening assays and functional assays (including acellular tests). In addition, better models have to be developed for how to do *in vitro*, *ex vivo* and *in vivo* assays. In particular, for *in vitro* assays we need new and advanced models that better reflect the *in vivo* situation and that also can be used for long term exposure. In addition, for environmental hazards we need a broader spectrum of relevant tests, including multi-generational studies.

This has to be combined with the choice of relevant endpoints. In some cases, novel endpoints should be established (e.g., from a better mechanistic understanding or for assessing low dose or chronic endpoints). This also includes areas that are currently not researched, such as the microbiome.

Furthermore, we need a more mechanistic-based hazard assessment that is not just focused on classic toxicity endpoints. This then may lead to the development of adverse outcome pathways (AOPs) that also help to better link *in vitro* to *in vivo* situations and that eventually will achieve regulatory acceptance. Also, this work certainly will allow for a more precise and better focused in vitro testing (based on underlying mechanisms of action [MoAs]).

For putting categorization into practice, we need to establish clear linkages between pattern of similarities in physical and chemical properties and toxicological outcome or fate. In particular, grouping strategies already exist for human hazards (although they mainly focus on inhalation), but are largely absent for the environment.

Finally, individual assays have to be combined into IATAs consisting of *in silico*, tier 1 *in vitro* (screening or functional assays), tier 2 *in vitro* (more complex *in vitro* or 3-D models), and *in vivo* systems. This should include decision points and cut-off criteria within the toxicity assessment.

Assays

- Develop functional assays that are predictive and high throughput
- New and advanced models (in vitro, in vivo, ex vivo)
- Develop in vitro models that better mimic in vivo conditions
- Broader spectrum of relevant and accurate environmental tests

Relevant Endpoints

- address chronic realistic low dose
- novel endpoints such as microbiome assessment

Mechanistic Knowledge

- Develop Adverse Outcome Pathways (AOPs) to link in vitro to in vivo and risk assessment
- Greater focus on mechanisms of action (not just classic endpoints)
- This focus may lead to new assays and new endpoints

Categorization

- Establish how similarities in physical and chemical properties are linked to toxicological outcome or fate
- Grouping strategies exist for human scenarios but strategies are lacking for environmental scenarios
- More case studies to achieve regulatory acceptance

Integrated Approaches to Toxicity Assessment (IATA) for human and environmental systems

- Establish integrated approaches that consist of in silico, tier 1 in vitro (screening or functional assays), tier 2 in vitro (more complex in vitro or 3-D models), and in vivo systems
- Establish decision points and cut-off criteria within the toxicity assessment
- Consider potential phys-chem transformations of ENMs in complex biological media
- Include dosimetry in IATA approaches
- Use exposure data to assess relevant doses for IATA testing

EMERGING MATERIALS

Emerging advanced (nano)materials present novel properties that are not exhibited by their bulk or more conventional nanoscaled counterparts. These may be carbon-based, metals, metal oxides, nitrides, carbides, dichalcogenides…). Many emerging materials are bi-dimensional or composite. In addition, there is growing debate about incidental nanomaterials such as nanoplastics.

- **Bidimensional** novel materials, such as graphene or MXenes, which maximize the surface-to-volume ratio, even reaching monolayer thickness. This creates unique sites, with new properties.

- **Anisotropic biopolymer ENMs such as nanocellulose currently explored in agri-food applications and beyond**

- **Composite nanomaterials** (including bidimensional components) result in new chemistry associated with unique entanglement of elements, which interaction lead to new structures, properties and reactivity.
- **Incidental nanoplastics** are also increasingly present in the environment and ultimately reaching human body.

Identifying these emerging materials is necessary to understand their (eco)toxicity, aging, transport and fate. There is a need for adequate detection, identification and quantification in complex matrices.

Characterisation of these emerging materials should steer their categorization based on their aspect ratio, composition, bulk structure, area, surface structure and surface reactivity. These will be instrumental to explain the nature of interaction among them in different boundary conditions (environment or body) and their aging. Composite materials bring an additional complexity for they may blend nano and non-nano components. This calls for technologies to identify the presence of nanoscaled materials in composite matrices, along with their quantification.

Modelling of these emerging materials should bring the rationale of their reactivity and stability, accounting for the nature of their interaction with environment and body and conditions where it may dissolve.

SOCIAL AND NATURAL SCIENCE RESEARCH TO SUPPORT BALANCED RISK GOVERNANCE OF EMERGING MATERIALS

Emerging materials and technologies (including bi-nanomaterials [incl. nanoplastics, bidimensional and composites etc.], biomaterials, synthetic biology and related technologies) drive rapid technology innovation but the knowledge on associated risk and benefits may be lagging. Risk Governance requires explicit tradeoffs under significant uncertainty across multiple metrics of risk, benefits, societal importance, manufacturing priorities and cost. In line with on-going risk governance projects and the MALTA initiatives focusing on nanotechnology, this research area will support integration of top-down coordinated social science research with bottom-up data and knowledge generated in the field of human and environmental hazard, exposure and risk characterization of emerging materials to guide policy decision making. Formal decision-analytics tools (including decision analysis, value of information analysis, machine learning and artificial intelligence) can be utilized to sustain this objective. Custom-driven research will start with visualization of stakeholder needs and societal concerns. The outcome of this research will provide direction and tools for selecting policy alternatives and assessment of associated regulatory readiness level, implementable on a global scale.

NANOINFORMATICS

Nano-informatics is identified as a research priority between EU and US. This involves building data bases, data curation, innovative data analysis, and modeling. Data curation involves sharing data between EU and US (frameworks & tools) and putting existing or forthcoming data into linked and commonly available databases around key focal areas e.g. characteristics, exposure/fate, toxicity, etc. Data curation needs to ensure standardization of ontology, quality of data and ensure updates to cover emerging technologies. Databases should use common architectures and metadata formats, e.g. ISA-Tab. Automated data curation approaches should be implemented or if necessary developed. Existing tools from related fields should be reviewed.

Data analysis and relevant modeling will then be carried out to integrate the information to derive results that can be used for research and regulatory purposes. This aspect is quite broad but needs to be considered upfront in the design of the informatic tools and frameworks as it dictates the type and presentation of data. Due to the different types of data from many studies there will need to be parallel development of tools to enhance data use such as improved methods for *in vitro* to *in vivo* extrapolation and dosimetry. One output could be a guidance document around standardized data collection and curation approaches for nanosafety research.

It is recommended that discussions around nanoinformatics take a tripartite approach involving academics, regulatory agencies and industry to ensure the widest coverage and harmonization of data sources. The creation of a globally accessible database (e.g., European Open Science Cloud) for all stakeholders will help to facilitate and harmonize research priorities.

EXPOSURE ASSESSMENT

Summary

- What are people exposed to and does this match with the way the toxicity testing is performed?
    - Life cycle analyses (LCA) to understand the fate and transformation of ENMs in nano-enabled products and potential release dynamics
        - In the environment/ environmental species
        - In the human body (biodistribution as input modelling)
    - Low level, long term exposure (move away from acute exposure testing)

- Aggregate exposure i.e. combined exposures via various routes or from various sources and mixtures of pollutants
- Generate exposure data that can be used to link with human health surveillance data (epidemiology)

Rationale

Exposure of humans and the environment is a result of many sequential or concurrent processes. These facts have emerged from research related to ENM production, NM characterization, aging of products containing ENM, human and environmental induced release of NM into the environment, transport, transformation, degradation and possibly accumulation of ENM in the environment or along the food chain. Only a limited number of research reports have addressed LCA of nanotechnology-based materials and products.

At present, there is very little integration of kinetic and toxicological effects testing for ENMs; as for instance OECD Test Guidelines for health effects testing substances do not require biokinetics. Biokinetics is not only important to increase our understanding on how (well) ENMs are distributed across the body, but the information is also used for interspecies extrapolations as well as for the design of follow-up longer term exposure studies and *in vitro* studies. Lack of integration leaves many questions open such as whether the data obtained during kinetics testing do apply to the results as obtained in the effects testing studies that is pivotal for reliable risk assessment. Coating/surface layers have been shown to influence both ENM biokinetics and toxicity at the level of a cell, a tissue and organism. Coating/surface layer has been show to influence both ENM biokinetics and toxicity at the level of a cell, a tissue or an organism.

Exposure characterization should feed the hazard assessment making sure that the exposures in toxicity test resemble real life exposure rather than using rather artificial exposures that are currently by and large driven by the desire to get good reproducible and stable suspensions in either air or fluids.

(text also based on NSC Research Roadmap 2015-2025)

STANDARD METHODS, METHODOLOGIES, REFERENCE MATERIAL AND HARMONIZATION

Documentary standards

Good reproducible science is critical for technology advancement and product development. Standard methods allow for comparison of results from different laboratories. They include standardized protocols for nanomaterial sample preparation, physico-chemical characterization, *in vitro* biocompatibility assessment and *in vivo* safety and efficacy assessment. Documentary standards developed through consensus with stakeholder involvement helps in harmonization based on good science. Standard test methods developed through pre- and post-standardization processes through inter-laboratory studies provide precision and bias in measurements. Standard guides are equally critical where precision is not easy to achieve but nevertheless used for regulatory purposes. Once developed, these documentary standards can be recognized by regulatory agencies to facilitate faster regulatory review, advancing the product development.

Reference material

Whether they are reference material, standard reference material or certified reference material, these standards can only be developed by national metrology laboratories, and used for instrument qualification and calibration of assays. There are very few reference material that are produced in Nanomaterial – including gold, silver, silica, carbonaceous material standards. In the US, there is reference ENM repository for nanotoxicology research established as part of NIH/NIEHS NHIR consortium which includes extensive number of well characterized and property controlled metal and metal oxide ENMs as well as emerging anisotropic materials such as 2D and nanocellulose materials. Reference materials are specific for an intended purpose. They can be for analytical assessment or as controls (positive or negative controls) for in vitro assays. In cases where a number of such standards cannot be developed “benchmark material” can be utilized in a consortium model for material assessment. Nevertheless, availability of reference material enables quality assurance, quality control, and technology advancement to assure responsible development of nanotechnology.

It will already be a major step forward if labs have access to benchmark materials to compare results among laboratories or to use these as positive or negative controls. This would greatly enhance the impact and usefulness of the findings. For example, the EU JRC as well as Harvard have repositories with nanomaterials that can be distributed to various users

LIFE CYCLE/TRANSFORMATION/VALUE CHAIN/STEWARDSHIP

The focus of this priority is to gain a more complete understanding of life cycle of ENM to support effective risk governance. A prime objective of this research track is to provide a framework to coordinate information from other projects. It is critical to understand these key components of the product/ material life-cycle. Research issues cover occupational, consumer and environmental/public health exposure concerns. Included are transformations of the nanomaterials as they move through the life cycle and the resultant changes in their potential hazard. This project provides a coordinating role between exposure research and epidemiological studies. Research is also needed to develop safe by design practices and to document that those practices are effective.

Bullets:

- Accurate data on volumes and types of nanomaterials entering commerce
- Simple assays for
    - release
    - fate and transport
    - transformation and aging
    - exposure
- Evidence for changing hazard or risk characteristics along the Life-cycle
- Evidence that “safer – by – design” principles are working