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Fourth National Conference

Nanotechnologies and nanomaterials in the food sector and their safety assessment

Istituto Superiore di Sanità
Rome, October 26, 2023

Edited by
De Battistis F., Ferraris F., Prota V.,
Raggi A., Vincentini O. and Cubadda F.

ISTITUTO SUPERIORE DI SANITÀ

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ABSTRACT BOOK

Edited by
Francesca De Battistis, Francesca Ferraris, Valentina Prota,
Andrea Raggi, Olimpia Vincentini and Francesco Cubadda

Department of Food Safety, Nutrition and Veterinary Public Health

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This volume gathers the abstracts of the contributions presented at the "Fourth National Conference on nanotechnologies and nanomaterials in the food sector and their safety assessment" organized by the Department of Food Safety, Nutrition and Veterinary Public Health of the Italian National Institute of Health. The volume provides an overview of the applications, regulation, analytical determination, toxicology and risk assessment of nanomaterials in food and agri-food production. The main theme of this edition is the use of New Approach Methodologies (NAM) in the safety assessment of nanomaterials, since NAM appear particularly promising tools for addressing nanoscale specificities in the risk assessment process, avoiding the need for additional animal studies.

Key words: Nanomaterials; Nanoparticles; Food safety; Toxicology; New Approach Methodologies; Risk assessment

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Parole chiave: Nanomateriali; Nanoparticelle; Sicurezza alimentare; Tossicologia; New Approach Methodologies; Valutazione del rischio

Conference Chair: Francesco Cubadda

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Andrea Raggi — *Istituto Superiore di Sanità, Rome, Italy*

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PREFACE

Developments in the fields of nanosciences and nanotechnologies in the past few decades have opened up new ways of controlling and manipulating material properties through reduction of particle size down to the nanoscale, i.e. approximately 1 to 100 nm. This has led to the development of a number of novel or improved products and applications for a wide range of sectors, including the agricultural and food sector. Nanotechnology is recognized as one of the six Key Enabling Technologies (KETs).

Aside all the projected benefits, the use of nanomaterials has raised concerns that the same nanoscale features that make them desirable for a number of applications may also render them harmful to human health. These concerns originate from the changes in the physicochemical properties of materials that occur when particles are manufactured in the nanoscale, which may also alter their biological fate and behaviour and lead to different or new adverse effects compared to conventional bulk equivalents. In particular, nanoparticles may be able to cross biological membrane barriers that normally prevent larger particulate materials from entering cells and tissues.

Compared to conventional chemical substances, physicochemical characterisation and toxicological testing of nanomaterials is more challenging. Although the current risk assessment paradigm has been broadly considered to be also applicable to nanomaterials, adaptations in certain methods are necessary to take account of the nanoscale particulate nature of nanomaterials. In this context, the European Food Safety Authority (EFSA) has provided in 2021 two guidance documents on risk assessment of regulated food and feed products to be placed on the European Union (EU) market. The first one outlines the scientific risk assessment and appropriate safety testing of nanomaterials to ensure consumer protection and updates the previous guidance published in 2018. The other guidance presents the technical requirements to establish the presence of (a fraction of) small particles in conventional products, i.e. not engineered at the nanoscale, and how to assess any associated risks. Both guidance documents cover the application areas within EFSA's remit, including novel foods, food contact materials, food and feed additives, and pesticides, in the context of human (and animal, for feed additives) health risk assessment.

According to the structured pathway outlined by the two guidance documents, risk assessment of nanomaterials (or conventional materials containing a fraction of small particles and requiring a specific assessment at the nanoscale) is best achieved through integrative approaches. Nanoscale specificities are integrated in the risk assessment process as nanoscale-based hypotheses. New Approach Methodologies (NAMs), avoiding animal testing, are the first choice to generate information for addressing these hypotheses and improve mechanistic understanding of toxicokinetic and toxicodynamic processes at the nanoscale. Integrated Approaches to Testing and Assessment (IATAs) are then used for the integration of human, animal and NAMs-derived evidence. Such framework is oriented towards Next Generation Risk Assessment (NGRA) to harness exposure-led, hypothesis-driven risk assessment approaches.

A relevant application of the framework is provided by the first engineered nanomaterial proposed in the EU for direct use as human food, namely iron hydroxide adipate tartrate (IHAT), recently assessed by EFSA (2021) as a novel food and source of the nutrient iron,

intended to be used as food supplement. In the IHAT dossier, the applicant presented evidence demonstrating that this nanosized iron source, albeit taken up as particles by the enterocytes, enters the intracellular iron pool and behaves similarly to dietary iron, i.e. it does not bypass homeostatic control leading to possible bioaccumulation and adverse effects. NAM-based data were essential, along with targeted *in vivo* data, for concluding the safety assessment. Following the EFSA assessment, IHAT was authorised to be placed on the market within the EU in 2022.

The Nanosafety Team of the Department of Food Safety, Nutrition and Veterinary Public Health operates within this context and is active in the following areas:

- physicochemical characterization of nanomaterials and other particulate materials and their analytical detection in food and in biological specimens;
- toxicological assessment, with special focus on the use on NAMs for generating evidence, e.g., by *in chemico* (solubility, dissolution/degradation rate, simulated human GI digestion, lysosomal degradation) and *in vitro* (advanced models of the intestinal barrier for particle uptake and crossing, cell models addressing tissue and organ toxicity) methods;
- risk assessment of nanomaterials and other particulate materials either at the national level or in support of the activities carried out by EFSA, i.e. (i) Cross-cutting working group Nanotechnology and other working groups dealing with the assessment of novel foods, food additives, feed additives, food contact materials, pesticides, (ii) EFSA Network for Risk Assessment of Nanotechnologies in Food and Feed.

Analytical activities are performed in a unique clean room facility, featuring state-of-the-art equipment and analytical instrumentation, such as single particle-ICP-MS and AF4-UV-MALS-DLS-ICP-MS. This structure is the National Reference Laboratory (NRL) for nanomaterials in food and is one of the six European expert laboratories supporting the European Commission Joint Research Centre in the area of regulatory analytical method development and harmonisation.

In the area of nanotoxicology, the Team established collaborations with national and international partners, participated in several national and European projects and coordinates EFSA-funded European projects in the area on NAMs, such as NANOCELLUP and NAMS4NANO (Lot 3).

This volume gathers the abstracts of the contributions presented at the “Fourth National Conference on nanotechnologies and nanomaterials in the food sector and their safety assessment” organized by the Department of Food Safety, Nutrition and Veterinary Public Health of the Italian National Institute of Health. Following the first three editions organized in 2013, 2016, 2019, this fourth edition provides an overview of the applications, analytical determination, toxicology and risk assessment of nanomaterials in food and agri-food production. In addition, a regulatory update focusing on the revised European Commission's definition of nanomaterial of 2022 is given. This definition is expected to improve the clarity of the regulatory framework concerning nanomaterials in food. Presently, the definition of engineered nanomaterial in the food legislation, as it appears in the Novel Food Regulation (EU) 2015/22837 and in Regulation (EU) 1169/2011 on the Provision of Food Information to Consumers, is not aligned with the general European Commission's definition of nanomaterial. The appearance of the revised version of the latter is expected to produce such an alignment and, according to the Novel Food regulation, will lead to a single definition of engineered nanomaterial in the area of food law, required for consistency and coherence purposes. This

will also support enforcement activities, e.g., official control of engineered nanomaterials for food labelling purposes as established by Regulation (EU) 1169/2011.

Similarly to the previous editions, the meeting is an occasion for the experts of the Department, along with experts from the European Commission (DG Joint Research Centre, Ispra), EFSA, and other relevant institutions to offer an up-to-date overview of the rapidly growing area of nanomaterials in food from the standpoint of food safety. The contributed oral and poster presentations show that the meeting stimulated interest from researchers and is expected to promote discussion on cutting-edge topics for science and risk assessment related to the applications of nanoscience and nanotechnologies in the agri-food system.

Francesco Cubadda

Conference chairman
Member of the Cross-cutting Working Group Nanotechnologies
of the EFSA Scientific Committee
National scientific expert in the EFSA Network for Risk Assessment
of Nanotechnologies in Food and Feed

NOTE FOR THE READER

This volume gathers all the contributions presented at the conference. Abstracts are divided into oral and poster presentations. For easy consultation, oral presentations are listed in the order of the programme.

Posters are listed after the oral presentations. The abstracts of the contributed oral presentations are numbered with a code including the letter “O” followed by a progressive number. The poster abstracts are numbered with a code including the letter “P” followed by a progressive number.

At the end of the volume, the authors’ index is provided for the reader’s convenience.

Oral session 1

Chairpersons
Alberto Mantovani, Marco Silano

RISK ASSESSMENT OF NANOMATERIALS IN FOOD: EFSA'S NANO GUIDANCE DOCUMENTS

Maria Chiara Astuto
European Food Safety Authority (EFSA), Parma, Italy

In the EU context of regulated food and feed products, when an application concerns a material that meets the definition of engineered nanomaterial set out in Regulation (EU) 2015/2283, the application must follow the EFSA Scientific Committee Guidance on Risk Assessment of Nanomaterials (Guidance on Nano-Risk Assessment), published in 2018 and updated in 2021 (doi: 10.2903/j.efsa.2021.6768). 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 on Nano-Risk Assessment proposes a structured pathway for carrying out the safety assessment of nanomaterial and provides practical suggestions for the types 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 Nanoparticles (Guidance on Particle-Technical Requirements) (doi: 10.2903/j.efsa.2021.6769). 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. This presentation will focus on the structure and main provisions prescribed by the EFSA Nano Guidances, with some mention of recent experience and possible future developments.

THE EUROPEAN COMMISSION'S UPDATED DEFINITION OF NANOMATERIAL AND RELATED GUIDANCE

Hubert Rauscher
European Commission, Joint Research Centre (JRC), Ispra (VA), Italy

The Recommendation on a definition of the term 'nanomaterial' by the European Commission (EC) provides a general basis for regulatory instruments across all areas of European Union policy. A new Recommendation on the definition of nanomaterial (2022/C 229/01) was adopted by the European Commission in 2022 to serve different policy, legislative and research purposes when addressing nanomaterials or issues concerning products of nanotechnologies. It is broadly applicable across a wide variety of fields. The update takes into account technical and scientific progress in the field and aims at a clearer understanding of the key terms. The presentation introduces the new definition and discusses the changes introduced with respect to the old definition. A guidance, prepared by the European Commission's Joint Research Centre, supports the implementation of the new nanomaterial definition. That guidance gives an overview of the key terminology and concepts, provides a decision tree to identify nanomaterials and addresses identification of nanomaterials through measurements. The presentation will highlight some important new elements of the guidance. Finally, a brief overview of the regulatory situation for nanomaterials in the EU, including the food sector, and an outlook towards possible regulatory convergence in the legislation addressing nanomaterials is provided.

Oral session 2

Chairpersons
Alberto Mantovani, Marco Silano

NAM-BASED HAZARD ASSESSMENT OF NANOCELLULOSE ORAL EXPOSURE: THE NANOCELLUP PROJECT

Olimpia Vincentini
Department Food Safety, Nutrition and Veterinary Public Health, National Institute of Health, Rome, Italy

Nanocellulose (NC) is an emerging material in the food sector with several application areas, including prospective use as a novel food, with one application already received and under evaluation by the European Food Safety Authority (EFSA), or as 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. As an example, the morphological features of the different NC types are quite different. Although all NC materials typically have a high aspect ratio, CNC usually consists in rod-shaped crystals, whereas NFC consists in fibrils composed of fibres with a length up to 2-3 μm; nanofibers are even longer in BNC and organized in networks. For all the NC types, the diameter can be very small (as low as 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 by NC, (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 NC samples belonging to the three NC types, plus a comparator in the micro-range, were selected as study materials and submitted to a thorough physicochemical characterisation. All experimental studies were performed to ensure relevant and reliable results in the perspective of their use for regulatory risk assessment. As the first prerequisite, this entailed a detailed physicochemical characterisation and the development of proper dispersion protocols, in line with the EFSA SC Guidance on Nano - Risk Assessment ("Guidance" hereunder). In fact, one key element when testing nanomaterials *in vitro* is the potential for agglomeration as well as stability in different media. This element is important also in relation to the selection of the concentrations/doses to be used. On the one hand, the tested concentrations/doses should be high enough to enable the detection of relevant effects. On the other hand, agglomeration is expected to increase with the concentration/dose, leading to a reduction in actual exposure levels to NC at high doses. Therefore, a specific SOP detailing the protocol for NC dispersion was developed to ensure that a similar level of dispersion was achieved through the full dose/concentration range. The maximum concentration applicable in *in vitro* studies according to this SOP was 30 μg/mL. It was considered that exceeding this concentration, which is lower than the 100 μg/mL mentioned in the Guidance for nanomaterials in general, would not be relevant from the biological and toxicological point of view, taking into account the carbonaceous nature of NC and the fact that the resulting particle number concentrations are most likely comparable to those achieved at 100 μg/mL by inorganic nanomaterials (such as metal- and oxide-based particles). For *in vitro* testing, in absence of 'validated' *in vitro* methods, "valid" methods covering the different endpoints were used, taking into account the recommendations from international bodies (e.g. OECD, EURL ECVAM), from the literature and in the guidance. Accordingly, 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 controls, and (v) replication of key studies in different laboratories. A battery of *in vitro* tests was used to provide insight into NC hazard and mode of action according to a tiered approach, which lead to selection of three materials belonging to the three main NC types for in depth-testing. Cell uptake of these materials was demonstrated, and such uptake was greater in a triculture model, which better simulates the barrier properties of the human intestinal epithelium, as compared to Caco-2 monolayers. Uptake was the greatest in repeated exposure conditions, in which intestinal barrier crossing was demonstrated for CNC. Pro-inflammatory responses accompanied by

massive NC uptake in macrophages, indicative for potential immunotoxicological effects, and barrier function impairment were observed, whereas no indications for genotoxicity were obtained. Finally, no formation of smaller particles following colonic fermentation of NC was observed.

This research received funding from the European Union through a grant of the European Food Safety Authority (agreement GP/EFSA/SCER/2020/04). 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.

THE NAMS4NANO PROJECT: DEVELOPING A QUALIFICATION SYSTEM FOR NAMS (LOT 1) AND CONDUCTING RISK ASSESSMENT CASE STUDIES (LOT 2)

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

NAMs are usually understood in a broad context to include in silico, in chemico and *in vitro* methods. They offer many advantages such as higher efficiency, being human-focused and providing detailed mechanistic insights. Regulatory application of NAMs is challenging as method validation to demonstrate reproducibility and predictivity is lagging behind. For Nanomaterials, (NMs), NAMs appear particularly useful to cope with the large number of variants. EFSA guidance suggests risk assessment is best achieved through integrative approaches whereby nanoscale specificities are integrated as nanoscale-based hypotheses. NAMs are highlighted as the first choice to generate information for addressing these hypotheses and improve mechanistic understanding of processes at the nanoscale. However, NAMs for NMs face several additional challenges, e.g. issues with dispersion stability, dosimetry, agglomeration, dissolution, transformations in biological environments and potential assay interferences. This talk will provide an overview on two of the three EFSA-funded projects under the NAMS4NANO umbrella. These four-year projects have been started in April 2023 involving nine European and two international organizations. Lot 1 and 2 are coordinated by the German Federal Institute for Risk Assessment (BfR). Within the first Lot, our consortium has already reviewed the currently available NAMs for NMs and is currently working on a qualification system to assess their regulatory maturity. In parallel, we are exploring the practical implementation of selected NAMs in integrated approaches to testing and assessment (IATA) for providing additional information to substantiate nano risk assessments within five selected case studies (i.e. zinc oxide, silicon dioxide, silver, iron oxides and copper oxides). Furthermore, five additional methodological case studies are conducted in Lot 3 addressing selected methodological challenges, which will be presented in a separate talk.

This research is performed under the NAMS4NANO action. NAMS4NANO has received funding from the European Union 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.

THE NAMS4NANO PROJECT: DESIGNING AND CONDUCTING METHODOLOGICAL CASE STUDIES (LOT 3)

Francesco Cubadda
Department Food Safety, Nutrition and Veterinary Public Health, National Institute of Health, Rome, Italy

The EFSA-funded action NAMS4NANO "Integration of New Approach Methodologies results in chemical risk assessments: Case studies addressing nanoscale considerations" (www.iss.it/en/nams4nano) is a multiannual project including several individual projects funded as separate lots. 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. Integrated Approaches to Testing and Assessment (IATAs) are used for the integration of human, animal and NAMs-derived evidence. NAMS4NANO Lot 3 is a four-year project coordinated by the Italian National Institute of Health and involving other 9 organizations from Europe and one from New Zealand. It focuses on designing and conducting a set of five proof of concept methodological and cross-cutting case studies, which develop NAM-based tools and procedures of high relevance for nanospecific risk assessment within the EFSA remit. The development and implementation of these methodological case studies aim to ultimately demonstrate that the combination of existing *in vivo* information and NAM-based data can provide better information for safety assessments than new animal studies. 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 case studies focus on the assessment of complex nanomaterials. Case study 1 concerns a nanocarrier loaded with a pesticide active substance, whereas case study 2 focuses on nanocellulose and other food-relevant nanofibres. Case study 3 aims at developing a procedure for simulating transformations in the GI tract to obtain relevant materials for *in vitro* testing. Case study 4 deals with the development of diseased models for the intestinal barrier to generate information on potential risks in vulnerable populations. Finally, case study 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. These case studies will be developed following an overarching and coordinated approach with Lots 1 and 2 of the NAMS4NANO action, addressed by the same consortium. The implementation of these case studies will contribute to methodological progress and update of EFSA guidance documents.

This research is performed under the NAMS4NANO action. NAMS4NANO has received funding from the European Union 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.

Contributed oral presentations

Agri-food applications and analytical determination

Chairpersons

Alberto Mantovani, Marco Silano

O1 NANOMATERIALS IN FOOD: A COORDINATED ACTION TO INCREASE THE ANALYTICAL CAPACITY IN EU MEMBER STATES

Josefa Barrero Moreno, Otmar Geiss, Ivana Bianchi, Hind El Hadri
European Commission, Joint Research Centre (JRC), Ispra (VA), Italy

Nanotechnology has found multitude of applications in food manufacturing, processing, and packaging. Beside the benefits of its use, some concerns due to the occurrence of engineered nanomaterials in food, the ingestion and the potential health effects have been raised. The European Union (EU) has an internationally acknowledged reputation on food safety based on clear rules, ensuring the protection of the health and interests of European citizens while fostering the well-functioning of the single market and the EU competitiveness. The legislative requirements of EU food legislation related to (engineered) nanomaterials are set up in the Novel Food Regulation that includes a definition of engineered nanomaterials that is also directly applicable to other EU food legislation (e.g. Regulation (EC) No. 1333/2008 on food additives and Regulation (EU) No. 1169/2011 on food information to consumers). To address potential health concerns surrounding nanoscale materials in food and feed, the European Food Safety Authority (EFSA) has developed comprehensive guidance on risk assessment. However, the identification and characterization of materials in the nanoscale in foods is a major bottleneck due to the lack of (certified) reference materials, validated/standardised methods, and in some cases insufficient technical/analytical capacities and knowledge of the official control laboratories in charge of compliance testing. To overcome these limitations, the European Commission Joint Research Centre (JRC) and the Directorate-General for Health and Food Safety (DG SANTE) are collaborating to address the aforementioned issues and support competent authorities in Member States with the implementation and enforcement of current food legislation pertaining to nanomaterials. In this context the JRC is coordinating an action involving the identification and exploitation of synergies with other national/international activities to address the challenges of nanomaterial analysis in food. A variety of activities such as design and organisation of training sessions for official control laboratories and joint efforts for development and validation of analytical methods are part of the JRC work. This presentation offers a general overview of the above activities and the related analytical methodologies used for nanoparticle analysis.

O2 CIRCULAR ECONOMY AND SUSTAINABLE AGRICULTURE: HYDROXYAPATITE FROM BIOWASTES AS SMART NANOFERTILIZER - PRIN 2022 CLEOPATRA

Luca Marchiol
Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy

Nanotechnology has the potential to become the driver of a new technological revolution in agriculture. Nano-enabled agriculture could play essential roles in increasing crop yield and Nutrient Use Efficiency (NUE), lowering environmental impacts, and improving agroecosystem resilience. So far, most studies have analyzed the properties of the nanofertilizers and assessed plant responses mainly at the greenhouse scale without any measure of NUE, which is a fundamental aspect concerning plant nutrition and crop fertilization. The PRIN 2022 CLEOPATRA project has set out to systematically investigate the potential of nano-Hydroxyapatite (*n*HAP) derived from biowastes and its resulting nanohybrids, with the aim of producing effective and environmentally friendly nanofertilizers. Recent studies have shown that *n*HAP - alone or combined with other elements (such as N and micronutrients) or molecules - can improve plant nutrition, protection, and yield quality when compared to traditional fertilizers. However, they produced conflicting results and positive outcomes lack mechanistic evidence. Furthermore, they have been carried out using synthetic apatite. This will be the first comprehensive investigation carried out using a nanohybrid of biological origin from circular economy chains as innovative fertilizer. CLEOPATRA includes activities in different phases studying both the preparation and functionalization of *n*HAP and its effects on the plants. More specifically, the extraction of *n*HAP from bones of various animals will be studied, together with their functionalization with P Solubilizing Bacteria (PSB) and urea. We will study various approaches for applying *n*HAP to plants, as well as how it is distributed within plants and its effectiveness in terms of NUE. Plant growth will be assessed with experiments carried out at growth chamber and mesocosm scale on maize (*Z. mays*) as a model plant. Along with this, focused results will be:

- design and synthesis of efficient *n*HAP-based nanofertilizers;
- molecular/physiological traits of N and P uptake and plant allocation dynamics;
- reduction of N and P leaching from soil;
- crop physiological responses;
- increased crop yield and quality and enhanced NUE.

CLEOPATRA outputs respond to EU expectations of reducing 2030 fertilizer use by at least 20%, nutrient losses by at least 50% while ensuring soil fertility and providing high-quality food.

O3 INULIN COATED ZnO NANOPARTICLES AS BIOSTIMULANTS FOR PROMOTING GROWTH OF VICIA FABA L. SEEDLINGS

Marilena Carbone (a), Silvia De Rossi (b), Domenica Donia (a), Gabriele Di Marco (b), Bianca Gustavino (b), Ludovica Roselli (a), Antonella Canini (b), Angelo Gismondi (b)
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The pursuit of "zero hunger" is challenging and requires a new approach to agricultural methods, where biotechnologies play a pivotal role. A way to sustain the requests of larger food production, for a constantly increasing world population, is ensuring a high ratio of crop yield/seeds. Recently, ZnO-NPs have gained interest in plant science as new fertilizers, and they are considered a solid solution to the problem of soil Zn deficiency. In the present contribution, we explored the employment of inulin, a fructan extracted from chicory root, as coating agent of ZnO Nanoparticles (ZnO@inu NPs). The goal is the achievement of an advanced material for potential implementation in food production systems in view of its biostimulating effects. Naked and inulin coated ZnO-NPs were synthesized according to purposely implemented green protocols and characterized with multiple techniques to determine their crystallographic phase, average particle size, and degree of coating. Fava beans were let grow in culture medium supplemented with NPs at two different concentrations: 50 and 100 mg/kg. Furthermore, besides the control, four different experimental conditions were considered for each concentration, i.e., using ZnO-NPs alone, inulin alone, a mixture of the two and ZnO@inu NPs. Germination and biometric analyses were performed, and multiple tests were carried out to determine the lipid peroxidation, the amount of Reactive Oxygen Species (ROS), the total flavonoid content and the Zn accumulation in roots and leaves, by X-Ray Fluorescence (XRF). The main mechanism of action of ZnO-NPs was hypothesized through quantitative PCR analysis of specific cellular enzymes, responsible for ROS production, hydrogen peroxide decomposition, flavonoid biosynthesis and progression of cell line. In addition, cytotoxicity and genotoxicity tests were carried out at the highest concentration of NPs. The combined studies indicate a potential biological activity of ZnO@inu NPs in promoting growth and development of *V. faba* L. seedlings, acting at a post-germinative phase, likely by stimulating the mitotic activity through a ROS/MDA-dependent molecular signaling. Inulin as a coating agent for the ZnO-NPs favoured the bioavailability of this nanomaterial and its adsorption into plant tissues, without altering their bioactivity but mitigating any side effects. In fact, cytotoxicity and genotoxicity tests proved the inulin coated nanoparticles to be harmless. As for the accumulation, Zn was mostly present in roots or in leaves when administered at low or at high concentrations, respectively.

Contributed oral presentations

Toxicology and risk assessment

Chairpersons

Alberto Mantovani, Marco Silano

O4 DO NANOPARTICLES USED FOR SUPPLEMENTATION IN FOOD AND FEED CAUSE EPIGENETIC MODIFICATIONS?

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Iron deficiency is a global public health problem caused not only by several diseases, but also common in elderly, adolescence and in pregnant or menstruating women. Unfortunately, therapeutic iron supplementation affects the gastrointestinal tract with significant side effects such as nausea, diarrhoea, and constipation. To overcome these limitations, it has recently been suggested the use of iron nanoparticles that are considered safe with limited side effects, highly bioavailable, and do not change taste and colour of the fortified foods. Moreover, the use of food additives, such as iron oxides and hydroxides (E172), has increased in recent years. Since these additives contain nanosized particles, the assessment of their toxicological risk becomes an important aspect to ensure safety for consumers. Despite the progresses in the field of nanotoxicology, however, a question that remains unanswered is the role of epigenetics in nanotoxicity. Epigenetics is a complex network of mechanisms that control gene expression in a potentially heritable way without changing the DNA sequence. Indeed, the cause of many human diseases can be found in the alteration of the genomic distribution of epigenetic marks that affects the transcriptome. Therefore, we have studied the changes that occur in the genome-wide distribution of H3K27ac, H3K4me1, H3K9me2, and H3K27me3 histone modifications and compared them with the transcriptome after exposing NIH-3T3 cells to iron-based magnetic nanoparticles (i.e., Fe_2O_3 and Fe_2O_3@Co NPs). This can be done thanks to the use of the 'omics' approaches to study the epigenome (e.g., chromatin immunoprecipitation coupled with massively parallel sequencing - ChIP-seq) and the transcriptome (e.g., RNA-sequencing - RNA-seq). Our results have shown that the variation in the genomic distribution of the H3K27ac marks has caused a change in the transcription pattern and that this has been primarily due to the modulation of the activity of the enhancers. Moreover, we have shown that there are specific epigenetic marks that are not due to iron release from the studied iron-oxide NPs, but to the NPs themselves. This analysis, although complete as far as it concerns the entire genome (i.e., genome wide), has been performed only following a single exposure. Therefore, we are currently examining the effect of different exposure times. Our results, taken together, suggest that an alteration of the epigenetic landscape is a key mechanism in defining the gene expression program changes resulting in nanotoxicity.

O5 AMORPHOUS SILICA NANOPARTICLES AFFECT LPS-DEPENDENT ACTIVATION AND IMMUNE METABOLIC REWIRING OF PRIMARY HUMAN MACROPHAGES

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Amorphous Silica Nanoparticles (ASNP) are used in a variety of commercial products and are considered endowed with low acute toxicity. However, the effects of chronic exposure to ASNP as components of the E551 additive on the gastro-intestinal barrier are not fully characterized. By using a variety of biological models, several studies have found pro-inflammatory effects of ASNP; however, the modulation of the response of innate immunity cells to natural activators (PAMPs or DAMPs) as well as their activation-associated metabolic reprogramming has not been thoroughly investigated. We have exposed human macrophages, derived from peripheral blood monocytes (monocyte-derived macrophages, MDM) to non-cytotoxic doses of ASNP (food grade pyrogenic NM-203 nanoparticles obtained from the JRC Repository) for 24 h. When challenged with LPS, these cells exhibit a markedly lower induction of pro-inflammatory cytokines (TNFα, IL-6). The above effect is associated with the suppression of NF-κB activation and the abnormal intracellular sequestration of the TLR4 receptor. Moreover, ASNP also prevent the late LPS-dependent induction of the anti-inflammatory cytokine IL-10 and of Glutamine Synthetase (GS), the enzyme responsible for the synthesis of Glutamine (Gln) which is involved in the late anti-inflammatory skewing of activated macrophages. Here we show that intracellular Gln undergoes biphasic changes during LPS treatment of MDM, with an early decrease followed by a delayed (24 h) restoration. The time course of the late increase corresponds to the induction of GLUL, the gene that encodes for GS. Along with GS increase, LPS treatment also causes the massive induction of SLC38A5, the gene coding for the bi-directional Gln transporter SNAT5. Both GLUL and SLC38A5 inductions are suppressed by the pre-exposure to ASNP. ASNP can perturb human macrophage activation by LPS. In particular, the secretion of both pro-inflammatory and anti-inflammatory cytokines is hindered, and the metabolic changes associated with the late anti-inflammatory metabolic shift are suppressed. Considering the intestinal environment, such perturbations of the innate immune cell response to microbial components may alter the equilibrium between intestinal mucosa and microbiota. The suppression of an efficient acute response and of late anti-inflammatory feedback mechanisms may favour chronic inflammatory bowel disease. The validation of this hypothesis will require advanced *in vitro* or adequate *in vivo* models.

O6 THE SURFACE CHARGE OF POLYSTYRENE NANOPARTICLES IMPACTS ON THEIR PENETRATION AND SAFETY

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A significant portion of industrial plastics commonly used for food packaging is degraded into Micro- and Nanoplastics (MNPs). Animals and plants interact with these bodies through inhalation, ingestion, skin, and cuticle absorption. MNPs enter inside the cells activating an endosomal process well known as the "trojan horse" mechanism. The interaction of these MNPs with different biological substrates depends on the constituent material, particle morphology, and z-potential. This study was aimed at evaluating how the modification of the surface charge of Polystyrene (PS) Nanoparticles (NPs) may affect the interaction with the host and the toxicity *in vitro* and *in vivo*, i.e. in the nematode *Caenorhabditis elegans*. First, we characterized fluorescent PS NPs (positively and negatively charged) through DLS and AFM to assess the dimension, surface charge, and morphology. Then, we treated HEK 293 cells (i.e., epithelial cells from human kidney embryos widely used in toxicology research) with the PS NPs and developed a platform combining fluorometric measures, imaging, and toxicity analyses. The internalization pathway was investigated using both pharmacological inhibitors of endocytosis (chlorpromazine and amiloride) and labeling intracellular vesicles. Finally, the potential *in vivo* toxicity of the PS NPs was evaluated by administering them to *C. elegans* and determining their effect on the main physiopathological features. Both positive and negative PS NPs had a spherical shape, ranged in diameter 100±30 nm, and were monodispersed; they only differed in z-potential (+50 mV and -9 mV). PS NPs entered into cells mainly by clathrin-mediated endocytosis and were rapidly entrapped by lysosomes. The amount of internalization and kinetics was markedly higher for positive NPs, probably due to their electrostatic attraction with the cell membrane. Positive NPs were rapidly internalized in HEK 293 cells and caused a dose-dependent reduction of cell growth and viability. Similarly to *in vitro* studies, worm exposure to positive but not negative NPs caused a dose-related toxicity resulting in defects in motility, pharyngeal function, reproduction, and development. The findings obtained underline the crucial role played by the surface charge of NPs in their interaction with biological membranes.

Poster Contributions

Agri-food applications and analytical determination

P01 DEVELOPMENT OF METHODS FOR THE DETERMINATION OF PARTICULATE MATERIALS IN FOOD IN THE ISS NANOMATERIAL CHARACTERIZATION FACILITY

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Adequate characterization of nanomaterials, encompassing chemical identity and physicochemical forms, is essential for safety assessment. The physicochemical parameters of a nanomaterial change in various environments and the characterization has to target a specific stage of the life of the material, its intended use and potential interaction with biological systems. In the case of food applications, the characterization strategy should be applicable to different scenarios/requirements: the nanomaterial as manufactured (in the pristine state), as delivered for use in food products, as present in the food matrix, as used in toxicity testing, or as present in biological fluids and tissues. In order to deal with the multifaceted challenges posed by the characterization of nanomaterials and their analytical determination in complex matrixes such as food and biological samples, a fit-for-purpose facility has been established at the Department Food Safety, Nutrition and Veterinary Public Health. The core of the facility is a unique trace element-free clean room laboratory, featuring state-of-the-art equipment and analytical instrumentation such as single particle-ICP-MS and AF4-UV-MALS-DLS-ICP-MS. This structure is the National Reference Laboratory (NRL) for nanomaterials in food (www.iss.it/en/lnr-nanoali) and is one of the six EU expert laboratories supporting the European Commission Joint Research Centre (Ispra, Italy) in the area of regulatory analytical method development and harmonisation via the Nanomaterials in Food Laboratory Group (NIF-LAG). As an example of current activities in analytical method development, a strategy for assessing the absence of titania particles in food products on the EU market following the E 171 ban as a food additive is presented herein. Food-grade titanium dioxide, known as E 171 in the EU, is a widely used food additive which, owing to the light-scattering effect of TiO_2 particles occurring in the particle size range of 200–300 nm, is used as a whitener. It is a polydisperse material composed by anatase (rarely rutile) particles, with a constituent size typically ranging 30-350 nm. Following the safety re-assessment of the European Food Safety Authority (EFSA) in 2021, E 171 has been banned as a food additive in the European Union (EU) in 2022. Testing compliance of food products on the EU market with the E 171 ban poses significant challenges to official control laboratories. Whereas TEM or SEM analysis coupled with elemental analysis (by, e.g., EDX) represents the confirmatory approach providing certainty of E 171 presence along with the capability to characterize the size of the constituent particles and their number-based size distribution, single-particle ICP-MS (spICP-MS) has emerged as an ideal technique for E 171 analysis with widespread availability in testing laboratories, higher sample throughput, and lower analytical costs. However, efficient screening methods are needed to identify samples to be tested by this particle- and elemental-specific method. Determination of the total titanium concentration by ICP-MS is a practicable option and was investigated in the present study. The foundation of such an approach are the relatively low and uniform Ti concentrations in food, unless manmade TiO_2 food additives are added. In addition to E 171, this encompasses pearlescent pigments consisting of mica platelets coated with TiO_2 nanoparticles, whose use is also illegal. Here we describe the analytical strategy we developed for assessing the absence of titania particles in food products on the EU market, founded on total Ti determination via ICP-MS as screening method, followed by spICP-MS analysis as confirmatory method. A tailored sample preparation, i.e. a microwave digestion method resulting in solubilization of TiO_2 particles for total Ti, and a chemical extraction procedure of TiO_2 particles for spICP-MS analysis, was set up.

P02 CELLULOSE-BASED NANOMATERIALS IN AGRICULTURE: SUSTAINABLE CROP PROTECTION AND SMART PACKAGING

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Since cellulose is one of the main component of agrofood-derived waste, it represents a renewable and affordable source for nanosized organic materials synthesis. Cellulose Nano Crystals (CNC) are rod-shaped nanomaterials derived from the crystalline portion of cellulose, with an average length ranging from 100 to 500 nm and an average diameter of 5-20 nm. CNC show interesting and unique properties like a high mechanical strength, a high elastic modulus and a low thermal expansion coefficient. Moreover, the abundant presence of hydroxyls groups makes these nanomaterials convenient to be used as nanocarriers for active compounds, making them suitable in materials engineering, electronics and pharmaceutics, while potential applications of CNC in agriculture and food industry have just begun to be explored. CNC have showed antimicrobial properties against phytopathogenic bacteria belonging to *Pseudomonas* and *Xanthomonas* genres, such as *P. syringae* pv. *tomato* (Pst), the causal agent of the bacteria speck of tomato, *P. savastanoi* pv. *savastanoi* (Psav), the causal agent of the olive knot disease, *X. arboricola* pv. *corylina* (Xac), the causal agent of hazelnut bacterial blight, *X. euvesicatoria* pv. *perforans* (Xp), the causal agent of the bacterial spot of tomato. Interestingly, CNC can decrease the motility of bacterial cells and their ability to stick to surfaces, leading to a reduction in biofilm production. As consequence, when applied to leaves, CNC can decrease the epiphytic survival of the inoculated bacteria. Furthermore, CNC have been functionalized with chitosan hydrochloride and gallic acid, using starch as excipient, showing the capability of inhibiting *P. syringae* pv. *actinidiae* (Psa) the causal agent of kiwifruit bacterial canker), Pst and Psav and of eliciting plant immune responses by an up-regulation of SAR related genes. Similarly, CNC have been positively tested for controlling *Fusarium graminearum* (Fg) and *Fusarium culmorum* (Fc), the causal agents of Fusarium head blight and Fusarium crown root, respectively, showing the concrete possibility of using cellulose-based nanopesticides to control even plant fungal pathogens. Given the properties showed by CNC, their use in post-harvest control has been investigated too. CNC have been successfully used in PVA and PLA-based films with carvacrol and lignin, showing antimicrobial properties against *Pectobcterium carotovorum* subsp. *odoriferum* (Pco), *X. vesicatoria* (Xv) and *X. arboricola* pv. *pruni* (Xap), the causal agents of bacterial soft rot, tomato bacterial spot and peach bacterial canker, respectively. The obtained evidences highlight the possibility of developing eco-friendly films for smart packaging that can enhance the shelf life of fruits and vegetables. To validate the aforementioned results, more detailed researches are in progress with the aim of investigating the biocompatibility and the safety of CNC.

P03 LIGNIN-BASED NANOCARRIERS FOR THE CONTROLLED RELEASE OF ACTIVE INGREDIENTS

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The traditional methods for crop protection require repeated applications of large volumes of active ingredients at high initial dosages. Moreover, the non-controlled delivery of pesticides and fertilisers causes a time-limited protection and their ubiquitous presence in the environment, inducing pesticide resistance and soil/water/food chain contamination. The controlled release of these pesticides and fertilisers represents thus a strategy to increase the efficiency of their delivery, reducing costs and pollution, and improving environmental sustainability. In this context, lignin-based micro- and nanocarriers loaded with these active ingredients are promising materials, due to their biocompatibility and stimuli-responsive behaviour. In this study, a nanocomposite based on lignin and silica mesoporous nanoparticles is proposed as a platform for the controlled release of copper ions for administration to plants. Silica nanoparticles were synthesized from rice husk and were exploited for the adsorption of lignin, thanks to their high specific surface area. On the other hand, softwood Kraft lignin was extracted in acetone and its acetone-soluble fraction was used for the preparation of the nanocomposite, acting as a chelator for copper ions (maximum adsorption capacity (0.154 g_{Cu2+}/g_{lignin}). Three different lignin:silica nanoparticles ratios were used (1:1, 2:1 and 1:2) to prepare the composites and were loaded with copper ions in acetone. The dimensional analysis performed using DLS revealed that the size of the composites loaded with copper ions was higher than the one of the sole lignin-silica nanoparticles composites (180-230 nm), which could be ascribed to the formation of agglomerates in which copper ions act as bridging species connecting primary particles. The 1:1 ratio composite was tested for the release of copper ions in aqueous solution at three different pH, namely 5.5, 7 and 8. Interestingly, the release kinetics at pH 8 resulted faster than the one at pH 7 (11% *vs* 8% copper ion release over a period of 200 hours, respectively), while at pH 5.5 the release rate was detected to be negligible over 400 hours. This behaviour could be correlated to the solubility of lignin in aqueous solution, which increases increasing the pH of the medium: it is therefore possible that the desorption of copper ions from the nanocomposites is favoured at higher pH due to higher solvation interactions with water. These preliminary results represent a promising starting point for the possible application of these fully biobased nanocomposites for the controlled release of active ingredients for agri-food applications.

P04 ADVANCED AND SUSTAINABLE NANOMATERIALS TO COUNTER THE SPREAD OF PATHOGENS IN THE FOOD INDUSTRY

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Foodborne diseases are an important cause of morbidity and mortality, worldwide. Most of these are caused by the consumption of foods or beverages contaminated by biological agents. Many food-borne pathogens, such as *L. monocytogenes, S. aureus, Salmonella spp.* and *E. coli*, are able to form biofilms and colonize the food-contact surfaces of large-scale food production systems, posing serious food safety problems and exposing the workers to biological risk. Furthermore the resistance of some pathogens to common disinfectants may cause their persistence in food-processing plants and other food-associated environments. These evidences therefore justify the growing interest in the identification of new prevention and mitigation measures for biological risk in the food sector, in addition to those already in use (e.g. surface disinfection). Novel size-related properties of nanomaterials are successfully applied into many fields, including the processing, packaging and safety in food industries. The combination of polymers with nanomaterials may enable the design of antimicrobial nanocomposites that could be used to counter the spread of pathogens on surfaces in food-processing plants, with the final aim to mitigate biological risk for humans, animals and the environment. Since nanoparticles show potential toxicity risk for human health, the development of new materials involving the manufacturing and use of nanomaterials should be conducted following the Safety and Sustainability by Design (SSbD) approach. As part of the project "PROTEZIONE" between INAIL and University of Padua, the *in vitro* bactericidal activity of two commercial Epoxy Resins (ER1 and ER2), yet unexplored as to their antimicrobial properties, has been assessed according to ISO22196:2011. Known concentrations of *E. coli* ATCC® 8739 and *S. aureus* ATCC® 6538P were deposited on ER samples. Bactericidal activity was evaluated calculating the average Reduction (R) of the Colony Forming Units (CFU) per cm^2 in ER samples compared to controls. The results show that the ER1 is effective (R=2.36 Log CFU/cm^2) against *E. coli* but not against *S. aureus*. As for the ER2, it has antibacterial activity both against *E. coli* (R=6.68 Log CFU/cm^2) and *S. aureus* (R=3.75 Log CFU/cm^2). These ER materials are the basis for developing new nanocomposites bearing suitably modified nanoclays. Therefore the manufacturing process of ERs with nanoclays are analyzed according to ISO and OECD guidelines, in order to evaluate potential risk and introduce safety-by-design measures to mitigate hazard and exposure at the early stage of the innovation process. The preliminary findings are promising in view to develop new antimicrobial nanocomposites taking into account SSbD principles.

P05 SAFE AND SUSTAINABLE NANO-ENABLED ANTIMICROBIALS TO REDUCE THE PRESENCE OF CONTAMINANTS OF EMERGING CONCERN IN THE AQUATIC ENVIRONMENTS

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Contaminants of Emerging Concern (CECs), such as antibiotics and Antimicrobial Resistant (AMR) bacteria associated to intensive fish and animal farming, represent a great threat to environmental and human health. Nanoparticles (NPs) and Nano-Enabled Products (NEPs) emerged as novel antimicrobial agents with proven efficacy against AMR bacteria, nevertheless, their safety and sustainability must be evaluated at an early (design) phase. Indeed, uncertainties persist about their safety and environmental sustainability, as evidenced by toxicological and Life Cycle Assessment (LCA) studies. Most importantly, the increase in Nanomaterials (NMs) manufacture and use may lead to inappropriate disposal into the aquatic environment, representing a potential risk to non-target species. In this context, the AMROCE project* aims at reducing the spread of AMR bacteria in the aquatic environments through a platform of novel nano-antimicrobial products. Specifically, the project will develop antimicrobial/antibiofilm fish cage nets and wastewater filtration membranes through polymer nano-engineered bulk- and surfaces with metal-oxide NPs (e.g., CuO). Marine-derived nano-formulated antimicrobial agents and antibiofilm enzymes are proposed as an alternative to antibiotics for fish and animals. To contribute to the development of safe and sustainable by design nano-biocidals, the project investigates their efficacy and assesses their associated risk in exploitation scenarios through the implementation and integration of both *in vivo* and *in silico* methodologies. Evaluation of the safety and sustainability of water-based copper oxide (wCuO) NPs and wCuO-based NMs, such as coated, co-extruded polymers and filtration membranes, along with nano-formulations of marine-derived NPs (i.e., oils from microalgae) and quorum quenching enzymes (i.e., acylase) are investigated. Both NPs suspensions and leachates obtained from the polymeric materials were characterized by means of TEM, DLS and ICP-OES. Nanosafety of the novel nanotechnology-embedded products was assessed by using zebrafish (*D. rerio*), a promising model organism for high-throughput developmental and behavioural screening. The aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity (FET) test (OECD n. 236) by exposing zebrafish embryos to increasing NPs concentrations or to polymers-derived leachates for 96 hours and screened every 24 hours for lethal and sub-lethal endpoints. Acute toxicity was analysed by calculating the Lethal Concentration 50 (LC_{50}) and the Effective Concentration 50 (EC_{50}). To further investigate the toxic potential at sub-lethal levels, morphometric analyses were performed following the FET test, as well as through behavioural evaluations on embryos at earlier development stages. Dedicated LCA studies are performed to assess the NMs environmental sustainability. Within the inventory phase, AMROCE partners provided primary data and information about the NPs and the NMs synthesis/production processes, which were further modelled. Secondary data were derived by the Ecoinvent 3.7 database. NPs and NMs associated impacts were assessed with the CML 2001 impact method by using the OpenLCA software. In line with the safe and sustainable by design approach, a comparative assessment was performed by increasing NPs concentrations in the NMs formulations. The suggested integration of novel nano-eco toxicology assessment and standard LCA studies represents a potentially effective methodology to provide a comprehensive and harmonised framework supporting decision-making at design stage for safe and sustainable nano-enabled antimicrobials within the field of fish and animal farming.

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P06 BOVINE MILK-DERIVED EXTRACELLULAR VESICLES AS NEW NANODRUG DELIVERY SYSTEM FOR BIOACTIVE COMPOUNDS

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Extracellular Vesicles (EVs), which can be isolated from almost any biological fluid, are gaining attention as a novel nanodelivery system for drugs. In particular, raw cow milk represents a low-cost source of extremely biocompatible nanovesicles for nanodrug delivery. Milk EVs (MEVs) can be loaded with bioactive molecules that have problems of solubility, e.g. like curcumin (Cur), and thus improve the bioavailability of the compound. In view of the serious obstacle to isolation efficiency given by the presence of casein micelles, which are similar in size to EVs, we first optimized the method of isolating EVs from raw cow's milk. The improved isolation procedure of MEVs, i.e. leading to higher purity, unaltered quality of EVs and homogeneous size, consisted in an enzymatic treatment for casein removal combined with size exclusion chromatography. The extensive characterization of isolated MEVs was performed with western blots, high-resolution microscopy, cryoTEM, dynamic light scattering together and thin-layer chromatography. The isolated MEVs were loaded with curcumin (CurMEVs) and, taking advantage of the intrinsic fluorescence and optical properties of curcumin, the passive loading efficiency, stability and solubility were determined. The morphology of CurMEVs, structural integrity and loading efficiency were also evaluated under different storage conditions and pH levels that mimicked those of the human stomach over time. The maximum loading efficiency of curcumin was found after 3 hours of incubation with MEVs, with higher stability and solubility than free curcumin. Indeed, CurMEVs maintain their integrity when stored at varying temperatures and at different pH levels for more than six months. Finally, by using an *in vitro* intestinal barrier model, we investigated the feasibility of using CurMEVs for oral administration, testing their toxicity and transepithelial crossing ability. The effects of our nanoformulations on the gastrointestinal system, an essential parameter for determining the safety and efficacy of a potential food supplement, were evaluated. This study lays the foundations for the development of a nontoxic and easy-to-manufacture food supplement with a positive environmental impact in view of the reuse of dairy production waste material.

P07 INTEGRATED ANALYTICAL PLATFORM BASED ON ELECTRON MICROSCOPY FOR ADVANCED CHARACTERIZATION OF METAL NANOPARTICLES IN WHEAT

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The relationship between human health and metal Nanoparticles (NPs) is one of the emerging topics in the last decades. As for metal contaminants in wheat-based products, including those present as NPs, they can be of natural origin, e.g. originating from the soil, or can be introduced during the various stages of the durum wheat chain, from cereals to processed products. To ensure safety for human health, quality control of raw materials is crucial, but the development of a quantitative analytical method to measure the presence of NPs is still challenging today. In fact, isolation from complex sample matrices, heterogeneity and polydispersity of natural NPs, very low concentration levels, and dynamic processes such as alteration of NP physicochemical properties are challenging issues affecting NP analysis in foods. The aim of this research was to develop a method based on Scanning Transmission Electron Microscopy (STEM)-with Energy-Dispersive X-ray Spectroscopy (EDX) for the characterization and quantitation of metal NPs in durum wheat samples of different geographical origin. Preliminary investigations were carried out by Environmental Scanning Electron Microscopy (ESEM)-EDX and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to obtain the information on the chemical nature, form and size of the studied particles. On the basis of these findings, careful development of the multi-step sample preparation method for STEM-EDX was performed, involving matrix removal, dispersion by probe sonication, and enrichment steps which resulted in the optimization of particle counting, particle representativeness on the grid and minimization of matrix effects. The resulting multifaceted characterization strategy was applied to the analysis of durum-wheat samples from Italy, Mexico, USA, and Australia. Al, Fe, Mg, Ca, Mn, Cs, and Ti at μg/g were determined by ICP-MS. Among these elements, only Fe and Ti were detected in nanoparticulate form by both ESEM-EDX and STEM-EDX; in addition, STEM confirmed that no particles in the smallest nanometer range (below 50 nm) were observed. The integrated approach based on the use of EM and ICP-MS techniques proved useful for advanced characterization and quantification of metal nanoparticles in wheat samples. In particular, promising results were obtained using the STEM-EDX method where samples at low NP concentrations could be deposited representatively on an EM grid and analysed at the per-particle level. The method can be used for quality control ensuring food safety, and for implementing regulations in a control setting.

Poster Contributions

Toxicology and risk assessment

P08 ASSESSING NANOCELLULOSE UPTAKE AND CROSSING OF THE HUMAN INTESTINAL EPITHELIUM USING THE CACO-2/HT-29 MTX/RAJI B CELLS TRICULTURE MODEL

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In the EFSA-funded project NANOCELLUP, the assessment of the uptake and potential crossing of the human intestinal epithelium by Nanocellulose (NC) was one of the main pillars of the considered IATA. However, for a carbonaceous material such as NC, such studies are complicated by the challenge of detecting 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. *In vitro* models based on a single cell line (e.g. Caco-2 cells) do not properly represent the complex gut environment and are poor in simulating the barrier properties of the human intestine. 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 different human-derived cell lines (Caco-2 cells, HT29-MTX cells, and Raji B lymphocytes) holds promise in this respect and is being investigated in an OECD project focusing on internalization/translocation of inorganic nanoparticles. In NANOCELLUP, improvements/adaptations to meet the complexity of carbon-based nanofibres as NC were addressed with the aim to develop a specific SOP applicable to all NC materials. The analytical challenge of identifying NC crystals and fibres was addressed by fluorescence detection. It was considered that fluorescent labelling is potentially prone to alter the properties of NC, affecting cellular uptake. Thus, fluorescent staining was used for the three selected Tier 2 NC materials. Two alternative methods - namely (i) Calcofluor White (CFW) or (ii) Protein with a Carbohydrate Binding Module and N-terminal Green Fluorescent Protein (GFP-CBM) - were used and compared in terms of selectivity and sensitivity. 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 in Tier 1 of the project, three NCs were selected to enter Tier 2, in which assessment of intestinal uptake and crossing was addressed. These were (i) one material composed of Cellulose Nanocrystals (CNC), (ii) one Nanofibrillated Cellulose (NFC), and one Bacterial NC (BNC). The CNC consisted in rod-shaped crystals with median length of ca. 170 nm, whereas NFC and BNC consisted in fibres with a length >1 μm. For all the three NC types, the diameter was very small, i.e. with a median value in the range 5-9 nm. The exposure concentration (30 μg/ml) was selected so as to be physiologically relevant and was markedly lower than in other existing studies. Another remarkable difference from previous studies was the demonstratedly effective dispersion of the test materials with a specifically developed SOP, which ensured maximal deagglomeration as well as stability in the testing media. Cell uptake was demonstrated for the three Tier 2 NC 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. Based on Tier 2 results, CNC was selected for Tier 3, in which repeated exposure conditions (i.e. three exposures lasting from the 14th to the 21st day of culture) were investigated with the triculture model. Repeated exposure led to an increase in CNC uptake as compared to single exposure and accumulation in lysosomes was also shown. Importantly, CNC intestinal barrier crossing was unmistakably demonstrated. Based on these results, it is concluded that for NC, as a non-digestible carbonaceous nanomaterial, a potential for 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.

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P09 AGGLOMERATION BEHAVIOUR AND FATE OF FOOD-GRADE NANOMATERIALS IN HUMAN GASTROINTESTINAL DIGESTION AND IN THE LYSOSOMAL ENVIRONMENT: THE CASE OF FOOD-GRADE TITANIUM DIOXIDE

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The fate of nanomaterials during human digestion is still poorly understood and available evidence indicates that the interactions with the Gastrointestinal (GI) environment may alter the physicochemical properties of ingested particulate materials and affect the intestinal uptake of the particles and their toxicological properties. New Approach Methodologies (NAMs) have enormous potential to improve mechanistic understanding of toxicokinetic and toxicodynamic processes at the nanoscale by focusing on human relevant models. For fate testing, the guidance of the European Food Safety Authority (EFSA) on the risk assessment of nanomaterials to ensure consumer protection prescribes, at lower tiers, the use of *in chemico* methods under GI and lysosomal conditions. For GI processes, the focus is on the small intestine (do particles dissolve or do they reach the small intestine as such, with potential uptake and crossing of the intestinal barrier?), and a quantitative approach is required (determination of the dissolution rate profile by measuring the mass fraction of the original material present as particles after 30 min of *in vitro* simulated intestinal digestion). A similar quantitative approach is proposed for testing the biopersistence of the particles that do not dissolve in the GI Tract (GIT) and may be taken up systemically (determination of the dissolution rate profile at 72 h in simulated lysosomal conditions to assess any potential for accumulation). Focus is on particles up to approximately 250 nm since available evidence suggests that specific cellular uptake mechanisms exist for particles within such size range. NANOPERSIST was a collaboration under the framework for access to the Joint Research Centre physical research infrastructures of the European Commission (agreement N° 35050/5) in which the use of the above mentioned acellular *in vitro* dissolution tests was applied to several food-grade particulate materials belonging to four main types - i.e. SAS (E 551), titanium dioxide (E 171), iron oxides/hydroxides (E 172), and zinc oxide as a nutrient source - with the objective of filling data gaps on fate and foster standardisation of these methods in view of subsequent validation. A state-of-the-art multi-technique approach was used for the physicochemical characterization of the particles in pristine conditions, after ingestion in fed and fasted conditions, and after lysosomal processing. The case study on E 171 is presented here. This is a widely used food additive which, following the recent (nanospecific) safety re-assessment of the European Food Safety Authority (EFSA), has been banned for such use in the European Union (EU). Other bodies worldwide have subsequently assessed the risk of titania as food additive with conventional (i.e. non-nanospecific approaches) coming to different conclusions, also on the basis of several simulated GI digestion studies showing increasing agglomeration along the GIT. Based on such evidence, these bodies questioned the relevance of the toxicity studies in which food-grade titania was accurately dispersed in water via sonication and administered via oral gavage or via drinking water. However, all of these studies used light scattering techniques to get information about the size of the TiO_2 particles in suspension, although these techniques are not suited for screening the presence of small particles or characterising Particle Size Distributions (PSDs) of polydisperse materials. As such, they are not accepted for regulatory safety assessment in the EU. With this background, in the present study we addressed the knowledge gaps and the uncertainties regarding the agglomeration behaviour and fate of E 171 in human GI digestion. A representative sample of E 171 was submitted to a thorough physicochemical characterization and its GI fate was studied by applying the *in vitro* GI digestion approach laid down in the EFSA guidance on risk assessment of nanomaterials, in both fasted and fed conditions. In addition, real food samples containing E 171 were also studied. Single particle ICP-MS was the key technique to characterize the agglomeration behaviour of E 171 and the obtained results showed that light scattering techniques used in earlier studies

delivered biased results. We demonstrated that, after simulated GID, in the small intestine E 171 at concentrations reflecting human exposure is well dispersed, with PSDs having ≥70% of the particles <250 nm. In addition, since TiO_2 particles were found to be resistant to GI dissolution, stability in lysosomal fluid was investigated. Biopersistence of the material in lysosomal fluid highlighted its potential for bioaccumulation.

P10 NANOPARTICLE BEHAVIOUR IN THE ORO-GASTROINTESTINAL TRACT - AN *IN VITRO* APPROACH

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Oral ingestion is highly relevant for hazard evaluation of engineered nanomaterials (ENMs) so European regulatory agencies and some international organizations emphasize the importance to evaluate the dissolution profile of ENMs in the digestive tract as well as their eventual internalization/translocation through the intestinal barrier. Currently, a new OECD Guidance Document (GD), led by Italy, is under preparation at the OECD-WNT (TGP 4.158). The GD is aimed to provide scientific basis to define conceptual framework and procedures for determining the intestinal fate of orally ingested ENMs using a two-step *in vitro* approach simulating the intestinal digestion of ENMs in the oro-gastrointestinal (OGI) tract and their interactions with the intestinal mucosa. Different protocols for *in vitro* digestion were developed, simulating physiological conditions in the human OGI tract after food consumption and many of them have been successfully applied to ENMs digestion. Usually, they cover the first steps of the digestion process, i.e., ENMs behaviour in mouth, stomach and intestine. In our approach digested samples were prepared according to a modified version of the Cascade *in vitro* digestion protocol. The dissolution assay was performed in sterile condition using realistic ENM concentrations starting from food daily intake values of three selected case study materials - SiO_2 [NM203], TiO_2 [NM104], and ZnO [NM110] - derived from JRC repository. Several research groups explored the use of advanced *in vitro* models of the intestinal barrier for studying ENMs internalization and translocation highlighting interesting application possibilities. In particular, the tri-culture model based on a co-culture of two human intestinal epithelial cell lines (Caco-2 and HT29-MTX) with the human hematopoietic cell line Raji-B is considered relevant for uptake/translocation of particulate matter. In the frame of the EU H2020 project NanoHarmony, Standard Operating Procedures (SOPs) for ENMs *in vitro* simulated digestion, uptake/translocation through *in vitro* intestinal barrier model, and analytical detection have been established and tested in an Inter Laboratory Comparison (ILC) exercise using the three case study ENMs, aiming to select the best conditions in terms of reproducibility and robustness of the tests. All the information gathered in NanoHarmony will feed the new OECD GD.

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P11 EXPLORING THE USE OF GUT-ON-A-CHIP MODELS FOR RISK ASSESSMENTS OF NANOFIBERS

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Nanocellulose (NC) is an emerging material in the food industry with possible future applications in food packaging, novel foods, and food additives. However, the potential hazard of ingested NC are not sufficiently characterised. Classical *in vitro* models based on a single cell line (i.e. Caco-2) are too far from mimicking the complexity of the intestinal environment *in vivo*. This limitation necessitates the development of more complex *in vitro* human intestinal models that recapitulate *in vivo*–like architecture and might therefore provide more physiologically relevant cellular responses, so that they can better predict the potential risk of NC for human health. The present exploratory project specifically pertained to the use and comparison of not yet validated novel complex 3D *in vitro* models to assess the potential toxicity, including immune reactivity, of NC in the gastrointestinal tract (GIT). Specifically, we used a static organotypic 3D system and a microfluidic-based organ-on-chip device, mimicking the peristaltic movements of a living intestine. Reliability and predictive value of these systems were further assessed by comparison with GI organoids. *In vitro* models were exposed to two types of NC, a Nanofibrillated Cellulose (NFC) and Cellulose Nanocrystals (CNC). To investigate potential effects of NC, specific functional and molecular endpoints were evaluated in the epithelial cell compartment, such as cell viability/cytotoxicity, (pro-) inflammatory responses, and integrity of the intestinal barrier. The results obtained indicated that NFC, but not CNC, at the highest concentration tested could modify the integrity of the intestinal barrier, without however affecting tissue viability. Compared with control inflammatory stimuli, exposure to both NCs did not result in a statistically significant increased production of inflammatory cytokines (IL-6, IL-8). These data might offer a starting point for future studies that allow evaluating the effect of NC following sub-chronic and/or repeated exposures, in the context of the advanced culture platforms developed in this work.

P12 LARGE POPULATION OF HOMOGENEOUS SPHEROIDS MODELS FOR TESTING TOXICITY AND DIFFUSION OF NANOMATERIALS

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The toxicity and the therapeutic effect of drugs need to be assessed on relevant cell models before they are tested on animal models or humans. Analogously, the safety of nanomaterials intended to be used in food should be tested for toxicity on relevant and reliable models. In recent years, the use of 3D cell models has been recognized as a step forward in complexity and relevance in biological cell assays. These models can mimic the complexity and the biological barriers of tissues better than conventional 2D systems. Nowadays, protocols are available to produce 3D cell models for many cell types. One of the common problems in 3D cell models is the variability in shape and size of the 3D structures, leading to a scarce reliability of the assays. We have developed and improved methods for the culturing of 3D spheroids in microwells arrays that could also mimic the mechanical properties of tissues. Large populations of up to several hundreds of homogeneous 3D tumor spheroids can be produced reliably and their growth and molecular properties can be characterized *in situ* during and after growth or treatment with drugs or nanostructures. It is possible to characterize the drug distribution in the spheroids in order to gain insight into their penetration properties and persistence. Recently, we obtained 3D heterotypic cell spheroids where 3D cell structures are made of different cell types so that more complex and reliable tissue models can be obtained. Additionally, it is possible to exploit the 3D bioprinting technology to obtain complex cell mixtures and achieve 3D heterotypic cell models with a range of compositions and treat them with drugs or nanostructures in the same environment. We trust that this technology will prove useful in reliably and quantitatively testing the toxicity and other biological effects of food-relevant nanomaterials as well as other nanostructures.

P13 METHODOLOGICAL APPROACH FOR THE EVALUATION OF POTENTIAL TOXIC EFFECTS OF MICRO- AND NANO-PLASTICS

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Due to their extraordinary characteristics, plastics are used worldwide in thousands of everyday products. Micro and Nanoplastics (MNPs) are solid polymers made of particles with an average size of less than 5 mm and 100 nm, respectively, arising from the degradation of larger plastic pieces released into the environment. The widespread presence of MNPs has raised public concern for their potential hazard to human health. The MNPs used in this pilot study are Polystyrene Latex Beads 100 nm in size (PS-NPs), which constitute an excellent model for the standardization of the methodological approaches to investigate toxic effects of greater production and wider use MNP as well as MNP derived from bio-plastics. Since ingestion represents the main route of human exposure to MNPs, the human colon carcinoma cell line Caco-2 was adopted as a suitable cell model to study the *in vitro* toxicity of MNPs. Semi-confluent Caco-2 cells were exposed for 4, 24 and 48 hours to increasing concentrations (25-100 μg/ml) of PS-NPs and the effect on DNA integrity and cell redox was then evaluated by alkaline comet and glutathione assay, respectively. Furthermore, *in vivo* studies using the nematode *Caenorhabditis elegans* aimed at evaluating the effects of PS-NPs on nervous and reproductive systems, and on the production of Reactive Oxygen Species (ROS), were carried out. Preliminary results showed a significant increase of DNA damage and a reduction in intracellular glutathione concentration at the highest dose (100 μg/ml) after 24 hours of treatment. *In vivo* assays showed a clear effect of PS-NP exposure on the locomotion behavior of young adults, a reduction of the deposition rate that worsened in the second and third generation of exposed worms, and a strong increase of ROS. The present study intends to contribute to the development of innovative and reliable methodological approaches for evaluating potential toxic effects due to human exposure to MNPs.

P14 *SACCHAROMYCES CEREVISIAE* FUNCTIONAL DELETOMICS AS A MODEL FOR MECHANISTIC STUDY OF NANOMATERIALS WITH APPLICATION IN THE MEDICAL AND FOOD SECTORS

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Quantum Dots (QDs) are promising nanomaterials with antimicrobial properties, which are tested for being incorporated into biopolymeric systems for food packaging purposes. These type of nanomaterials are attracting interest for a wide variety of applications. As an example, Zinc Sulphide Quantum Dots (ZnS QDs) are considered interesting candidates for gene delivery and editing in eukaryotes model systems. The increasing interest in this type of nanomaterials rises questions on the risks related to their utilization in condition of chronic exposure. To go beyond toxicological studies, unravelling the mechanistic impact of QDs on humans and the ecosystem requires the use of genome-wide approaches. We used functional deletomics, a robust method which is based on the screening of almost complete, knock-out mutants in haploid configuration of *S. cerevisiae*. Phenotype of deletion mutants found to be hypersensitive to ZnS QDs was further validated. From a Gene Ontology perspective, it was found that deleted genes in the identified mutants were involved in mitochondrial functions, with a crucial role in mitochondrial DNA stability. Furthermore, the attention was focused on the comparison between the wild-type strain BY4742 and the ZnS QDs hypersensitive mutant *pos5Δ*, whose deletion is related to a mitochondrial NADH kinase. RNA-seq analysis show how mitochondria, cell wall, endosomes and vacuolar compartments can be considered as cellular hotspots involved in the QDs response. The approaches that utilize *S. cerevisiae* as a functional tool could be suitable to identify potential specific biomarkers of exposure and effect, even at very early exposure stages and low concentration. From an operational point of view, yeast deletome can be considered as an alternative platform to animals and animal cells in cellular studies.

P15 EPIGENETIC DETERMINISM IN CELLULAR RESPONSES TO NEW NANOMATERIALS IN THE HEALTH AND FOOD SECTORS

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New nanomaterials such as Quantum Dots (QDs) are currently included in gene therapy protocols and are also materials with potential applications in food packaging. Understanding the environmental and health risk of their use and dispersion in the environment can benefit from the application of genome wide-approaches. When such approaches are used, in addition to structural genomic evidences (DNA level), also the regulatory aspects of the cellular responses are of relevance. Regulation of gene expression in human cells models, i.e. hepatocytes and macrophages, after exposure to QDs was studied at two levels. First, miRNAs regulation was considered using an almost complete set of primers and probes for 754 human miRNAs. This approach showed that the epigenomic regulation at this level can determine the fate of the exposed cells with reagard to two different pathways, i.e. apoptosis and/or autophagy. The second approach focused on the level of global mRNA sequencing performed on cells treated in the same conditions (as for miRNA). This approach partly confirmed the *in silico* evidence but also showed new variations, particularly at the level of mitochondrial function. The epigenomic involvement in response to QDs exposure had a role in the model response with the activation of nuclear function like RAS signaling and the increase in intracellular calcium.

P16 EU POTENTIAL PROJECT: A PLATFORM TO ASSESS NANOMATERIAL SAFETY FOR RAPID COMMERCIALIZATION

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The development of Advanced Nanomaterials (AdNMs) is taking an ever-increasing role within global production, including the food and beverage industry. A few peculiar physico-chemical features may make AdNMs extremely competitive in many sectors and in different fields, such as electronics, energy, biomedical, and food. On the other hand, their ability to interact with many substrates has opened questions about their possible impact on human and animal health and the environment. In the last decade, many studies have been carried out to determine AdNMs effects. Despite the many publications, the data are still somehow fragmentary and controversial. In order to provide new tools that make it possible to move from perception to assessment of the risk associated with nanomaterials before the transition from research & development to large-scale production, the Mario Negri Institute for Pharmacological Research, jointly with 11 other groups, has devised and created an integrated methodological platform. This project was funded by the European Union, within a call on digital sustainability (Horizon CL4 2022 Digital Emerging 01-35). Briefly, POTENTIAL looks at the entire pathway of physico-chemical characterization and ecotoxicity assessment of AdNMs, with the intent of developing standard protocols for 1) Imaging of nanomaterials in complex matrices and biological tissues; 2) Detection of changes in nanomaterial properties throughout their lifecycle; 3) Assessment of ecotoxicology and health hazard and development of C. elegans standardised methods for the evaluation of acute, sub-acute and long-term toxicity; 4) Grouping (i.e., organise categories) and Read-across (i.e., model behaviour across AdNM types). The research activities are guided by crosstalk with international regulatory bodies: the Organisation for Economic Cooperation and Development (OECD), the International Standards Association (ISO), and the European Committee for Standardisation (CEN). The partnership includes 7 research centres and 5 SMEs.

The project has received funding from the European Union's Horizon Europe Research and Innovation Actions programme under Grant Agreement 101092901 (website: www.potential-eu.eu). It started on January 1st 2023 and will last for 4 years, until January 2027.

P17 FAIRIFICATION OF NANOMATERIALS GENOTOXICITY DATA TO IMPROVE THEIR REUSABILITY IN RISK ASSESSMENT

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A great amount of data on the safety of nanomaterials have been produced over the last decades, mainly driven by regulatory pressure towards their safe use. Although nanosafety data are essential to support risk assessment, for the development of predictive models and in general for advancing knowledge on mode and mechanisms of toxic action, their effective reuse is hampered by several obstacles. A great stimulus for improving the management of scientific data comes from the FAIR principles (i.e., Findable, Accessible, Interoperable, and Reusable), which summarize the key characteristics that data and metadata must have to optimize reusability. During the H2020 Gov4nano project, the challenge of advancing nanosafety data FAIRness was addressed, largely supported by the Nanosafety Data Interface-NDI (https://search.data.enanomapper.net/). The work presented here illustrates a case study performed within the project on the reusability of *in vitro* Comet test data from several EU projects (i.e., NANoREG, NanoGenoTox, NanoReg2), available in the NDI. The Comet test results of different TiO_2, SiO_2, and ZnO nanoforms were retrieved from the NDI, and issues related to quality assessment, curation and translation were analyzed and addressed together with FAIRification needs. In this process, the recent EFSA assessment of TiO_2 as a food additive (E 171) has been taken as a reference for data reuse in the regulatory field. Results of the case study supported the creation of a Template Wizard for *in vitro* Comet data. This tool was implemented in the NDI to facilitate the data import workflow through a user-friendly interface, incorporating domain-experts' needs and automating several aspects of FAIR. Assessment elements that the EFSA panel deemed necessary to evaluate the relevance of each study's results are available in the Template Wizard. This ensures the streamlining of collection and evaluation of data to be used in food-safety risk assessment and other regulatory frameworks. The results of the Gov4nano project may inform FAIRification needs of the large amount of data expected to be produced in the near future, as the result of the flourishing research on the application of New Approach Methodologies in the risk assessment of nanomaterials. Moreover, a GO FAIR Implementation Network called AdvancedNano was launched to collect experience gained so far and smooth the process of data FAIRification for all stakeholders (www.go-fair.org/implementation-networks/overview/advancednano). This community brings together experts from different fields with the common intent of maximising the value of scientific data generated within nanosafety research, fostering the implementation of FAIR principles.

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