ISTISAN CONGRESSI 16|C2

ISSN: 0393-5620 (cartaceo) • 2384-857X (online)

Second National Conference

Nanotechnologies and nanomaterials in the food sector and their safety assessment

Istituto Superiore di Sanità
Rome, April 29, 2016

ABSTRACT BOOK

Edited by
F. Cubadda, F. Aureli, A. Raggi,
M.C. Barea Toscan and A. Mantovani

ISTITUTO SUPERIORE DI SANITÀ

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ISSN 0393-5620
ISTISAN Congressi
16/C2

Istituto Superiore di Sanità
Second National Conference. Nanotechnologies and nanomaterials in the food sector and their safety assessment. **Istituto Superiore di Sanità. Rome, April 29, 2016. Abstract book.**
Edited by Francesco Cubadda, Federica Aureli, Andrea Raggi, Maria Cristina Barea Toscan and Alberto Mantovani
2016, viii, 39 p. ISTISAN Congressi 16/C2

This volume gathers the abstracts of the contributions presented at the "Second National Conference on nanotechnologies and nanomaterials in the food sector and their safety assessment", organized by the Department of Food Safety 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 products.
Key words: Nanomaterials, Nanoparticles, Food safety, Risk assessment.

Istituto Superiore di Sanità
Secondo Convegno Nazionale. Nanotecnologie e nanomateriali nel settore alimentare e loro valutazione di sicurezza. Istituto Superiore di Sanità. Roma, 29 aprile 2016. Riassunti.
A cura di Francesco Cubadda, Federica Aureli, Andrea Raggi, Maria Cristina Barea Toscan e Alberto Mantovani
2016, viii, 39 p. ISTISAN Congressi 16/C2 (In inglese)

Questo volume raccoglie i riassunti dei contributi presentati durante il "Secondo Convegno Nazionale nanotecnologie e nanomateriali nel settore alimentare e loro valutazione di sicurezza", organizzato dal Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare dell'Istituto Superiore di Sanità. Il volume offre una panoramica sulle applicazioni, la normativa, la determinazione analitica, la tossicologia e la valutazione del rischio dei nanomateriali nei prodotti alimentari.
Key words: Nanomateriali, Nanoparticelle, Sicurezza alimentare, Valutazione del rischio.

Conference Chair: Francesco Cubadda

Scientific Secretariat: Federica Aureli

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The skilled assistance of Maria Cristina Barea Toscan for the organization of the event is gratefully acknowledged.

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Il Rapporto è disponibile online sul sito di questo Istituto: www.iss.it

Citare questo documento come segue:

Cubadda F, Aureli F, Raggi A, Barea Toscan MC, Mantovani A (Ed.). *Second National Conference. Nanotechnologies and nanomaterials in the food sector and their safety assessment. Istituto Superiore di Sanità. Rome, April 29, 2016. Abstract book.* Roma: Istituto Superiore di Sanità; 2016 (ISTISAN Congressi 16/C2).

Legale rappresentante dell'Istituto Superiore di Sanità: *Gualtiero Ricciardi*
Registro della Stampa - Tribunale di Roma n. 119 del 16/5/2014 (cartaceo) e n. 120 del 16/5/2014 (online)
Direttore Responsabile della serie: *Paola De Castro*
Redazione*: Paola De Castro, Egiziana Colletta* e *Patrizia Mochi*
La responsabilità dei dati scientifici e tecnici è dei singoli autori, che dichiarano di non avere conflitti di interesse.

Viale Regina Elena, 299 – 00161 Roma

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Director, Directorate General for Food Hygiene and Safety and Nutrition, Ministero della Salute

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Director, Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità

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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 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.

PREFACE

Nanotechnologies deal with the application of scientific knowledge to manipulate and control matter in the nanoscale – i.e. approximately 1 to 100 nm – in order to make use of size- and structure-dependent properties and phenomena distinct from those associated with larger sizes of the same material. Nanotechnologies are introducing dramatic changes in virtually all industry sectors by enabling management of characteristics such as material size, shape, morphology, chemical composition and molecular configuration for the improvement or development of new process and product properties.

In the food sector, three main categories of products/applications of nanotechnologies and nanomaterials can be identified, namely agricultural production (e.g. nano-formulated agrochemicals and animal feeds), food processing (nano-sized ingredients, additives, nutritional supplements and functional foods), and food contact materials.

Although making materials smaller can generate novel and useful properties, concerns have been raised on potential risks related to the interactions of nano-sized materials at the molecular or cellular levels, which may ultimately harm human health and the environment. There is insufficient knowledge on how altered physicochemical properties and potentially increased systemic bioavailability of engineered nanomaterials may influence their toxicological properties. Moreover, approaching the safety assessment of products of nanotechnology is a challenge, since new concepts and tools for safety assessment of nanomaterials are needed. Current toxicity testing approaches used for conventional substances are still suitable starting points for risk assessment of engineered nanomaterials but they may need methodological modifications since they appear to be inadequate to detect all aspects of potential toxicity of nano-sized materials.

For hazard characterization, the relationship of any toxicity detected to the various dose metrics that may be used, such as size and other physicochemical parameters of engineered nanomaterials, has to be explored since mass concentration alone (as used for conventional substances) is clearly insufficient. In particular, decrease of particle size in the nanoscale has been identified as a main determinant for the increased toxicity of different materials and, along with shape and morphology (aspect ratio), plays a pivotal role.

Applications of nanotechnology to the food sector pose the issue of the real consumer exposure to nanoparticles through consumption of food and beverages. If particles are absorbed in the gut, there is a potential for internal systemic exposure. Once in the body nanoparticles may cross biological barriers, including placenta, and the issue of potential toxicity arises especially for materials which may bioaccumulate in tissues.

Last but not least, a science-based risk characterization should deal with the novel challenges posed by the specific aspects of nanomaterials, from both standpoints of hazards and exposure.

This volume gathers the abstracts of the contributions presented at the *'Second national conference on nanotechnologies and nanomaterials in the food sector and their safety assessment'* organized by the Department of Food Safety and Veterinary Public Health on 29 April 2016. Since several years now the Department is committed to the assessment of the potential impact of the use of engineered nanomaterials on food safety and consumers' health. Following the first conference organized on the same topic in 2013, the meeting was

an occasion for the experts of the Department, along with experts from the European Commission (DG Joint Research Centre, Ispra), the European Food Safety Authority (EFSA), and other research centres, to offer an up-to-date overview of this rapidly growing area from the standpoint of food safety.

The conference was held under the patronage of the Ministry of Health, which itself contributed by presenting the legislative framework on 'nano food ingredients', such as vitamins, minerals or other substances that contain or consist of engineered nanomaterials, under the new Novel food Regulation. The meeting stimulated interest from researchers, stakeholders and the public and promoted discussion on cutting-edge topics for science and risk assessment in the agri-food system.

Francesco Cubadda
Conference chairman
National scientific expert in the EFSA Network for Risk Assessment of Nanotechnologies in Food and Feed

Alberto Mantovani
Director of the Food and Veterinary Toxicology Unit

Oral session 1

Chairpersons

Giuseppe Ruocco, Umberto Agrimi

NANOTECHNOLOGIES IN THE FOOD SECTOR: PROBLEMS AND PERSPECTIVES FOR SAFETY ASSESSMENT

Francesco Cubadda
Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Existing or potential applications of manufactured nanomaterials in the agri-food sector include agricultural production (*e.g*. pesticides, feed additives), direct use in food as ingredients or additives, and food contact materials. As a result, according to the different use scenarios, nanomaterials - or the chemicals resulting from their degradation/dissolution (if any) - might be found in food as residues, as intentionally added substances or due to migration from packaging.

Apart from intentional use of engineered nanomaterials for their specific (*i.e.* nano-related) properties, nano-sized materials may be present in food additives and food contact materials because the bulk material contains a nano-fraction as a consequence of the production processes. Both situations, however, require risk assessment of the nano-fraction, no matter how small it is in percentage of the bulk material (either as fraction of particles in the number size distribution or as mass fraction).

The development and harmonization of analytical techniques and tools in support of nanomaterial risk assessment is a challenging task. Size is the decisive parameter (along with chemical identity) for regulatory purposes (see the EC definition of nanomaterial); in the meanwhile, in the context of safety assessment a comprehensive physicochemical characterization is required, where a number of additional parameters - *e.g*. morphology, surface properties, dissolution - may be equally important.

Physico-chemical properties are critical to point out the toxicological hazards of specific nanomaterials. As an example, for nanomaterials with a certain degree of solubility, dissolution and thus loss of the particulate nature is often invoked to imply lower risk. Few materials, however, are readily soluble and while dissolving, smaller - potentially more toxic – particles are generated. Silver nanoparticles, proposed as antimicrobial agents in food-related applications, depending on the coating/stabilizing agent may partially dissolve in the GI tract, which however does not rule out the possibility of local effects, including alteration of the mucosa-associated microbiota and modulation of the gut-associated immune response. If particles are absorbed through the intestinal wall, their bioavailability to cells is inversely related to their size; once inside the cells, toxicity appears to be mediated by the intracellular release of silver ions. At the other extreme, titanium dioxide nanoparticles are extremely insoluble and biopersistent, and their long retention in target organs of deposition is of concern for potential long-term adverse effect.

One main challenge in the risk assessment of nanotechnologies is the fact that nanomaterials with apparently slightly differences in physicochemical properties may pose significantly different hazards and risks. At present, the knowledge on the relationships

between physicochemical properties and nanomaterials effects is limited. Targeted research will clarify to what extent grouping and read-across approaches may help in making the best use of available information and guide risk assessment, thus minimising the need for extensive animal testing.

THE NEW NOVEL FOOD REGULATION AND THE USE OF NANOMATERIALS IN FOOD

Bruno Scarpa, Valeria Di Giorgi
Directorate General for Food Hygiene and Safety and Nutrition, Ministero della Salute, Rome, Italy

In order to guarantee the public safety, the market of food that have not been consumed to a significant degree in the Union before May 1997 has to undergo a premarketing authorization procedure to assess the safety for human consumption according to the Regulation 258/97/EC on novel food.

Food or food ingredient that can be classified as engineered nanomaterials (ENMs), different from additives, flavourings or enzymes, are considered novel in the meaning of category f) of Regulation 258/97 as "foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances". Therefore an authorization procedure must be followed prior to their marketing.

The current EU rules on novel food needed to be updated because of the evolution of technological developments and scientific advice. Because of that and after conducting an impact assessment of the cited regulation, the Commission presented the first proposal to amend it in 2008, but agreement could not been reached.

In 2013, the Commission presented again a new proposal of amendment of Regulation 258/97, the agreement was reached in 2015 and the new novel food Regulation was adopted (namely Regulation EU 2015/2283).

The more important change of the new regulation is that novel food would be subject to a simpler, clearer and more efficient authorization procedure fully centralized at EU level, which should enable safe and innovative food to be placed on the EU market faster without compromising a high level of public health.

The European Food Safety Authority (EFSA) will assess the safety of the novel food, for which an authorization has been required, while the Commission will manage the application and will propose a decision for authorization of a novel food that is found to be safe.

Moreover, in the new regulation the categories have been defined in details, specifying, among others, that ENMs, as defined in the new legislation, require a novel food authorization before being used in food. The applicants must also prove that the latest methods of analysis have been used for testing the ENMs for which they are requiring an authorization.

For this reason, the definition of ENM, currently stated in Regulation EU 1169/2011, will be included under this new regulation. Moreover, the cited regulation provides that the Commission should, by means of delegated acts, adjust and adapt the definition of ENM set out to technical and scientific progress or to definitions agreed at international level.

The new rules will apply since the 1st of January 2018.

Oral session 2

Chairpersons

Alberto Mantovani, Bruno Scarpa

SOFT NANOMATERIALS IN THE AGRI-FOOD SECTOR

Catia Contado
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Products based on nanotechnology or containing nanoparticles (NPs) are found in the entire food chain, from cultivation (agriculture), to the industrial processing and packaging of foods. Nanoscale materials can be naturally occurring, may be intentionally added or may be the result of unintentional contamination.

Intentionally added NPs are frequently used to improve taste, flavour, colour, texture, and consistency of foodstuffs, to increase absorption and bioavailability of nutraceuticals and health supplements, to develop food antimicrobials. Engineered nanomaterials result very useful also in food processing, food packaging, and storage include monitoring of food quality, safety, and biosecurity (for example, nanosensors for traceability and monitoring the condition of food during transport and storage).

By focusing the attention on the intentionally added NPs, their functionalities (*e.g.* release of food additives) depend on the physicochemical properties of NPs (size and size distribution, surface area, shape, solubility and dissolution, reactivity, coagulation or aggregation state, chemical composition, etc.) and on the biological matrices (compounds that are present in the matrix and thermodynamic conditions).

In this presentation, some examples of NPs used in the food chain are given, by distinguishing them between soft and hard nano-entities. Since the agricultural and food samples are heterogeneous systems, which may contain a mixture of natural and engineering NPs of different composition, their detection and characterization are usually very difficult and complex. In particular, nano-emulsions, micelles, nano-liposomes, solid lipid nanoparticles or nanostructured lipid carriers, biopolymers can be well characterised during their formulation by using many of the conventional analytical techniques (imaging, separation and spectroscopic techniques), but the sample pre-treatment necessary to reduce, for example the food matrix complexity, might introduce important alterations which make their *in situ* analysis sometimes almost impossible.

HARD NANOMATERIALS IN THE AGRI-FOOD SECTOR

Federica Aureli
Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

The so-called "hard nanomaterials" are presently the ones that are most widely used in food applications, especially in food processing and food packaging. Apart from that, they are in the spotlight because some of these nanomaterials - particularly some insoluble and possibly biopersistent inorganic materials - have been associated to potential safety issues.

A number of authorised food additives have been shown in recent years to contain a nano-sized fraction, such as E171 (TiO_2) and E551 (SiO_2). The safety of these food additives is now being assessed in the light of up-to-date scientific evidence in the EFSA re-evaluation programme; TiO_2 is also a feed additive and is being evaluated jointly for both uses. The recently completed re-evaluation of the safety of elemental gold (E175) pointed out the need to include in the specifications of this food additive the mean particle size and particle size distribution, as well as the percentage (in number) of particles in the nanoscale. For elemental silver (E174) the EFSA concluded that the information available was insufficient to assess the safety of silver as food additive, pointing out the lack of data on toxicity studies on elemental silver or E174 and the unknown particle size distribution of E174. Contrary to TiO_2, SiO_2, or Au, elemental silver has an appreciable solubility and this is especially important in the case of nano-sized silver since the release of silver ions is of concern.

Several hard nanomaterials have been authorized for use as additives in plastic materials and articles intended to come into contact with food, *i.e.* carbon black, silicon dioxide, titanium nitride, kaolin, and the copolymer "methacrylic acid, ethyl acrylate, n-butyl acrylate, methyl methacrylate and butadiene". In most cases, the risk assessments were based on zero exposure scenarios as there was no appreciable migration into the food.

Characterization of hard nanomaterials is not trivial at their pristine state, and it is by far more challenging if they are incorporated in complex matrices such as food. In the latter case, valid protocols for sample preparation (extraction of particles) are prerequisite for accurate analytical measurements. If the metric selected in the EC's definition of nanomaterial as the only defining property of the material, *i.e.* size (1-100 nm), is considered, still a suite of analytical techniques is needed since at the moment there is no single technique alone that is able to cover the whole necessary size range and all the different nanomaterials types. Caution should be exercised in comparing results, however, because different particle size analysis methods measure fundamentally different parameters (*e.g.* gyration radii, hydrodynamic diameters, etc.). In addition, non-spherical particle are characterized by multiple 'sizes'.

An overview of advanced ICP-MS-based methods for the simultaneous determination of particle size and mass concentration of the most important class of hard nanomaterials in food, *i.e.* the inorganic ones, will be given.

THE EUROPEAN COMMISSION'S DEFINITION OF NANOMATERIAL: IMPLEMENTATION AND TECHNICAL CHALLENGES

Hubert Rauscher
Joint Research Center-Institute for Health and Consumer Protection, European Commission, Ispra, Varese, Italy

In the European Union (EU) Nanomaterials (NMs) are explicitly covered in several regulatory sectors, such as food (Novel Food Regulation), cosmetics (Cosmetic Products Regulation) or biocides (biocidal products Regulation), with certain consequences for products containing such materials. Moreover, EU legislation covering other consumer products and horizontal legislation, *e.g.* on chemicals (REACH), in principle applies to nanomaterials even if it does not specifically address them. Legislative provisions that explicitly address NMs need to refer to a definition to identify and distinguish them from other materials. The European Commission (EC) published a "Recommendation on the definition of nanomaterial" to promote consistency in the interpretation of the term "nanomaterial" for legislative and policy purposes in the EU. This definition is not legally binding but serves as a reference that is broadly applicable across different regulatory sectors and can be adapted to specific product legislation. The EC's definition of nanomaterial uses size (*i.e.*, size range 1-100 nm) as the only defining property of the material.

The implementation of any definition of nanomaterial which is based on quantitative criteria requires validated and harmonised measurement methods to correctly classify a material. The use of a multi-method approach is recommended for that purpose, since at the moment no individual method alone is able to cover the whole necessary size range and all the different types of NMs. An important issue to implement a definition is the specification at which stage(s) of its life cycle a nanomaterial should be identified, because the NM can change at each of these steps, *e.g.* in terms of size, agglomeration/aggregation state or surface properties. There are challenges in the choice of equipment (technique), protocols for sample preparation and measurement, and possibly protocols for conversions of the test result into a parameter of the definition. For these reasons, existing methods need to be further developed and improved, especially if they are to be applied in complex matrices such as food.

There is an ongoing large-scale effort by regulators, industry and research institutes, that addresses these challenges in an integrated approach. The strategic objective of that project (NanoDefine) is to provide the affected industries and regulatory agencies with the tools that support the implementation of the EC definition of nanomaterial in all relevant regulatory contexts. The project is working on real world test materials (industrial raw materials, products) and includes a comparative evaluation of techniques following uniform criteria. It provides guidance to manufacturers and regulators and aims at producing an easy-to use e-tool that guides the user through the entire analysis and decision process to classify a material as nano or non-nano according to the EC definition of nanomaterial.

Oral session 3

Chairpersons

Alberto Mantovani, Francesco Cubadda

NANOMATERIALS IN AGRI/FOOD/FEED: UPDATE FROM THE EUROPEAN FOOD SAFETY AUTHORITY

Reinhilde Schoonjans
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Nanomaterials are currently present in agri/food/feed products and their potential use is on the rise (see EFSA nano inventory: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/621e.pdf). Before being placed on the market, such agri/food/feed products are subject to scientific risk assessment carried out by the European Food Safety Authority, and a following decision for authorisation by the European Commission and the EU Member States.

To carry out such risk assessment, data are needed as well as appropriate test methods. In the EFSA guidance for risk assessment published in 2011, approaches are outlined on how to address human health risks (see EFSA Scientific Committee; Scientific Opinion on Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain). Until today, however, not many full safety assessments for nanomaterials in agri/food/feed are completed worldwide and challenges remain to exist both with respect to data availability and test methods. EFSA has identified four main areas that will be updated in the guidance: new legal requirements (see EC Recommendation of 18 October 2011 on the definition of nanomaterial http://eur-lex.europa.eu/), advancements in physico-chemical characterisation of nanomaterial, best practice for ADME studies and lessons learned from toxicological studies. Moreover, EFSA also plans to develop Environmental Risk Assessment Guidance, covering the environmental fate and impact of nanomaterials in agri/food/feed products, particularly nanopesticides and nanoformulations. The purpose is to help stakeholders and risk assessors in the Member States to prepare authorisation dossiers and ultimately to help protect the consumers' safety.

These activities will be carried out building on the knowledge generated by *e.g.* European Research projects (*e.g.* NanoReg, NanoDefine, NanoFate), the OECD working group for nanomaterials, and national research as reported by the EFSA Nano Network (see latest annual report of the EFSA network with Member States experts: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/939e.pdf).

OECD ACTIVITIES AND PRIORITIES FOR NANOMATERIALS SAFETY

Isabella De Angelis
Department of Environmental and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy

The exponential development and commercial use of Manufactured Nanomaterials (MNs) raise to the scientific community and national and international institutions relevant questions regarding their potential unintended hazards to humans and environment. In particular, a debate is taking place on whether MNs need special regulations to deal with those potential risks.

As response, OECD (Organisation for Economic Cooperation and Development) launched in 2006 a strategic programme, named Working Party of Manufactured Nanomaterials (WPMN), focused on the safety assessment of MNs in order to assist countries in the implementation of national policies to ensure their responsible development and safe use.

Priority objective of WPMN is to promote international cooperation on human health and environmental safety aspects of MNs both for regulatory purposes and for ensuring that approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonized standard.

One of the main objectives of the activity of WPMN has been to establish whether the methods for safety testing and assessment of chemicals are suitable for NMs. In this regard, and after seven years of work, WPMN published an important and shared recommendation in which the main conclusion is: "*The approaches for testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials"*.

Moreover, WPMN completed the Sponsorship Programme in which many delegations worked together to undertake the safety testing of 11 commercially important MNs. To date, it is one of the largest studies of its type and the results have recently been declassified and published on a dedicated public web site.

In 2013 the second phase of WPMN activities started. It is characterized by a shift to science from regulatory validity and by horizontal transfer of information between priority areas of intervention (inhalation toxicity, ecotoxicity and environmental fate, physical-chemical properties, genotoxicity, toxicokinetics, categorisation/ grouping and read-across).

For the near future, WPMN has identified four main areas of action:

- testing and assessment, focused on methods and strategies to address MNs safety;
- exposure measurement and mitigation guidance development for occupational settings, human exposure from consumer products, and environmental exposure;
- life cycle considerations, *i.e.* the broader environmental issues associated with MNs and relative tools;
- risk assessment and regulatory programmes specifically targeted to MNs safety, an issue on which information exchange and comparative analysis amongst countries will be extremely important.

Poster Contributions

P01 REPEATED ORAL ADMINISTRATION OF LOW DOSES OF SILVER IN MICE: EFFECTS ON CENTRAL NERVOUS SYSTEM

Marcella De Maglie (a,b), Claudia Cella (c,d), Simona Argentiere (c,d), Fabio Fiordaliso (e), Alessandro Corbelli (e), Marilena D'Amato (f), Federica Aureli (f), Andrea Raggi (f), Francesco Cubadda (f), Cristina Lenardi (c,d), Eugenio Scanziani (a,b), Camilla Recordati (b)
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The toxicity of silver nanoparticles (AgNPs) is being widely investigated because they are extensively proposed in a broad range of applications. Concerns arise because AgNPs may cause adverse effects in target organs for deposition (*e.g.* liver) but also in other sites such as the Central Nervous System (CNS). *In vivo* studies demonstrated that AgNPs can damage the blood-brain barrier (BBB) and neurons, affect neurotransmitters concentration and upregulate oxidative stress-related genes in rats. The aim of this study was to investigate the biodistribution and potential adverse effects on the CNS induced by repeated oral administration of low doses of AgNPs or silver ions.

Fully characterized 10 nm citrate-coated AgNPs at two doses, namely 0.25 and 1 mg/kg body weight (bw) and the equivalent dose of silver acetate (AgAc) were administered to male CD-1(ICR) mice by oral gavage once a day for 28 days. Mice were sacrificed at the end of treatment (28d) and after a recovery period of additional 28 days (56d). Mice underwent necropsy and organs (intestine, liver, spleen, kidney, brain) were collected for histology and silver quantification by ICP-QQQ-MS analysis. Quantification of astrocytes (GFAP) and microglial cells (Iba1) was performed in the hippocampus by immunohistochemistry and digital image analysis. In order to assess BBB damage, hippocampus was additionally examined by transmission electron microscopy (TEM).

At 28d, the highest silver concentration was found in the brain, followed by liver and spleen. Tissue levels were slightly higher after exposure to AgAc than AgNPs, and dose-dependent. After the recovery period (56d) silver was still detected in the brain in all treated groups, although in a lower amount, confirming its persistence in this organ. No histopathological changes were found in the examined organs. An increase of GFAP and Iba1 immunoreactivity was detected in treated mice after 28d, regardless of the source of silver. TEM analysis of the hippocampus demonstrated splitting of basement membrane of the capillaries, and swelling of astrocytic perivascular end-feet in both AgNPs- and AgAc-treated mice.

In conclusion our study highlighted accumulation and long retention of silver in the brain after oral administration at low exposure levels. Immunohistochemical and ultrastructural investigations of hippocampus revealed morphological alterations involving the BBB and microglial cells indicating potential neurotoxic effects.

P02 TiO_2 NANOPARTICLES *IN VIVO* TOXICITY IS GREATLY INFLUENCED BY THEIR PHYSICOCHEMICAL FEATURES

Federica Morelli (a), Francesco G. Viscotti (a)), Paolo Bigini (a), Fabio Fiordaliso (a), Luca Iannarelli (b,c), Andrea M. Giovannozzi (b), Valter Maurino (c), Giuseppe Spoto (c), Gianmario Martra (c), Vasile-Dan Hodoroaba (d), Erik Ortel (d), Andrea M. Rossi (b), Luisa Diomede (a)

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TiO_2-based products are widely used in several applications, including as colorant additive in food. It has been recently showed that about 36% of food-grade TiO_2 is in nanosized form representing a potential source of nanoparticles (NPs) for humans and raising questions about its safety. Limited data exist on how the physicochemical features of such NPs, influence their ability to localize, accumulate and trigger toxic effects in living organisms. 2D chemical imaging analysis based on Confocal Raman Spectroscopy was here applied to study the detailed spatial distribution of TiO_2 NPs with well defined shapes and dimensions, in living organisms. C. *elegans* has been utilized in this study as an alternative tool to match the organ-specific bio-accumulation of NPs with their biological effects. This nematode shares the 65% of genes with humans and a large percentage of stress pathways are conserved making the C. *elegans* as a reliable and versatile system for easily recapitulating the key molecular mechanisms underlying complex toxicological features.

In the present study rod and bypiramidal shaped engineered TiO_2 NPs with a primary particle size of 100 nm and 50 nm, respectively, and different agglomeration states, were synthesized and widely characterized. In addition, commercial and food-grade with spheroid shape TiO_2 NPs (E171) were used. All kind of TiO_2 NPs were put in the medium to feed the nematode. A non-invasive and label free methodology (the Raman microscopy) was exploited to monitor the bioaccumulation of TiO_2 NPs in the worm. The high spatial resolution chemical imaging and the specificity of Raman technique in the localization of TiO_2 NPs allowed to assist tissue-specific toxicological studies. All the considered TiO_2 NPs, regardless of their size and shape, accumulated in the pharynx, pass through the biological barriers and reached the reproductive apparatus. In particular, rod shape NPs resulted the most toxic ones, causing a great impairment of the pharyngeal function, reproduction activity and larval growth, indicating that rod-shape, more than bipyramidal and spherical shape, allow NPs to interact with biological systems.

This Raman-nematode combined approach, being rapid and low expensive can be applied for a first screening of the ability of NPs to accumulate and exert toxicological properties *in vivo*. According to the 3Rs guiding principles on the use of animals in

scientific research, this approach offers also the advantage that the ethical issues involving the use of vertebrates can be addressed since it can guide the design of tissue-specific toxicological evaluations, contributing to reduce the number of animals.

P03 AN INTEGRATED PLATFORM TO EVALUTE THE INTERACTION BETWEEN E171 AND THE GASTRO-INTESTINAL TRACT

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Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in many industrial fields, in particular as food additive (E171) in whitening of gums, candies and sweets. E171 generally show a wide size-distribtuion, with an appreciable

percentage of particles in the nano-range. The exposure to commercially available E171 (food grade TiO_2) has been estimated to be up to 3 mg/kg bw per day on average, but might be higher in special cases (including children) depending on food consumption patterns.

In recent years, a number of studies highlighted potential adverse effects of TiO_2 NPs ingestion in *in vivo* models, but the actual impact of these NPs upon chronic oral exposure is still far from being elucidated. Ingestion of TiO_2 NPs makes the gastro-intestinal tract one of the main potential target organs. However, there is a lack of systematic investigations focusing on the GI tract and involving analysis ranging from the single cell to the whole body level.

In this study we used an integrated platform to evaluate the interaction between TiO_2 NPs and biological matter at increasing levels of complexity. First, the behavior of the E171 sample was investigated in water and culture medium and a thorough characterization carried out by TEM and Nanosight. Afterwards, the potential for local effects (at the GI level) was investigated using the human Caco-2 cell line. Then we resorted to the nematode C. *elegans* as *in vivo* model, to assess the accumulation and identify functional effects. Finally, effects of a 3-week administrations of E171 to healthy immunocompetent mice via gavage, at 5 mg/kg bw per day were investigated.

A slight increase of apoptotic markers expression and an over-expression of inflammatory cytokines was observed in cells incubated for 24 hours at high doses (100 µg/ml TiO_2 NPs), without a significant reduction of cell survival. E171 (2 µg/ml, 2 hours) selectively accumulated in the pharynx of the C. *elegans* with an associated impairment of

the functional pharynx activity. No significant reduction of the life-span was observed. An organ dependent response was found in mice treated orally with E171. The stomach homogenates showed an overexpression of inflammatory genes, whereas an anti-inflammatory response was observed in the intestine of treated mice compared to controls. The assessment of TiO_2 biodistribution and histopathological analyses are in progress.

The authors acknowledge the EU FP7 Quality Nano Transnational Access Program for financial support.

P04 90-DAY ORAL TOXICITY STUDY ON SAS IN RATS: USE OF STATE-OF-THE ART APPROACHES FOR CHARACTERIZATION OF NANOMATERIAL DISPERSION AND TISSUE DEPOSITION

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Synthetic Amorphous Silica (SAS) is a nanomaterial used as food additive (E551). In order to investigate potential adverse effects following dietary exposure, a sub-chronic oral toxicity study (OECD TG 408) including the assessment of gross and histopathology, biomarkers of liver/renal function, thyroid and steroid hormone levels, genotoxicity, immunotoxicity, and reproductive toxicity, was carried out. Male and female Sprague Dawley rats were exposed daily by gavage to 0, 2, 5, 10, 20, or 50 mg/kg bw of a pyrogenic SAS used in food applications - NM-203, provided by the EC-JRC - for 90 days.

NM-203 batch dispersions were prepared on a daily basis in Milli-Q water (0.1 µm-filtered) at 5 mg/ml and were thoroughly characterized. A suite of characterization techniques was used, namely Asymmetric Flow Field Flow Fractionation (AF4) coupled on-line with UV and Multi-Angle Light Scattering (MALS), Analytical Centrifugation (LUMISizer), and Dynamic Light Scattering, comparing two instruments employing different algorithms to obtain particle size distributions. A reference material (ERM FD-100) was employed as quality control in each analytical batch. The adopted dispersion protocol was compared to the protocol previously developed in the NANOGENOTOX project (www.nanogenotox.eu), used as a reference. Dispersion quality was similar, notwithstanding the almost double mass concentration of NM-203 used in the current study. Noticeably, the proportion of particles in the nano-range was higher with the new protocol.

For the tissue deposition study, the analytical work has been carried out in clean room conditions using state-of-the-art ICP-MS/MS technology to overcome spectral interferences in the determination of silicon. This allowed to obtain a limit of detection for total silicon in tissues (0.2 mg Si/kg) that was 175 times lower to that reported in the other available 90-day oral study on SAS, thus enabling accurate detection of the minute amounts of deposited silica resulting from administration of relevant low doses of the test material. A bovine liver QC test sample was used for quality control of the analytical determinations and the found value was in excellent agreement with the target value. Silicon tissue levels were found to increase in a dose-related manner in spleen, whereas a high inter-individual variability was detected for the small intestine, liver and brain; nevertheless, generally higher levels were detected at the highest dose level.

In order to gain an insight into the presence of silica particles in tissues, livers from females treated at the highest dose are being analyzed by single particle-ICP-MS/MS.

The outcome of the sp-ICP-MS/MS study will be compared with results obtained using a variety of electron microscopy techniques.

This study is partially supported by the EU Framework 7 Programme, contract no 310584, NANoREG Project.

P05 FROM FOOD SAFETY TO STUDIES ON FORMS AND FATE OF NANOPARTICLES IN BIOLOGICAL SYSTEMS: A FIT-FOR-PURPOSE NANOMATERIAL CHARACTERIZATION FACILITY

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Adequate characterization of nanomaterials, encompassing chemical identity and physico-chemical forms, is essential for safety assessment. The physico-chemical 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, *e.g.*, food/feed 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/feed products, as present in the food/feed 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 our department. The core of the facility is a trace element-free clean room laboratory. The sample preparation room has the highest cleanliness (ISO 6 class), achieved through a positive pressure gradient obtained through HEPA-filtered air; inside the area a laminar flow work bench is located for ultra-clean treatment of analytical samples.

The facility features a complementary analytical platform based on state-of-the-art ICP-MS(/MS) instruments for analysis of inorganic nanoparticles along with a variety of other techniques, either present in the facility or made available by partner laboratories, including single particle-ICP-MS, Asymmetric Flow Field Flow Fractionation (AF4)-UV-MALS-ICP-MS, HDC-ICP-MS, HPLC-ICP-MS, DLS, ELS (Electrophoretic Light Scattering), APC (Analytical Photo-Centrifugation), BET, and SEM/TEM/ESEM-(EDX).

As regards to nanoparticle detection and characterization in liquid suspension and complex matrices, activities carried out in the facility mainly focus on: (i) characterization of nanomaterials in terms of size, size distribution, mass/number concentration, chemical composition; (ii) determination of minor inorganic components and impurities which might have toxicological significance; (iii) development of dispersion protocols.

The other main area of work consists in advanced sample preparation methods for undertaking studies on the fate of nanomaterials in the food chains, namely: (i) forms and fate of nanoparticles in biological systems; (ii) their stability and dissolution in synthetic and biologically relevant media; (iii) *in vitro* simulated gastrointestinal digestion of nanoparticles and nanoparticle-containing food matrices; (iv) discrimination of soluble and particulate forms in biological tissues and fluids after *in vivo* nanoparticle administration; (v) chemical speciation of the soluble fraction originating from dissolution of inorganic nanoparticles; (vi) biodistribution/ADME studies.

Examples are given concerning the detection of unlabelled oxide nanomaterials (SiO_2, TiO_2) in food and biological samples; this problematic issue complicates the safety assessment of these widely used nanomaterials. Novel approaches using state-of-the-art analytical platforms are important for generating new data to support risk assessment of nanomaterials in food.

P06 ADVANCED ICP-MS-BASED METHODS FOR THE ANALYTICAL DETERMINATION OF INORGANIC NANOMATERIALS IN FOOD

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Inorganic nanomaterials (metals, their compounds, and oxides) - *e.g.* Ag, SiO_2, TiO_2, ZnO, TiN, Fe oxides/hydroxides, nanoclays - are a prominent class of materials with current or projected applications in the food sector ranging from food additives, to antibacterial agents, to food packaging. It is thus essential to have analytical methods available to detect and characterize them as such, in food or - for food contact materials - food simulants (migration tests).

State-of-the-art mass spectrometric techniques for the analytical determination of inorganic nanoparticles in dispersion and (after proper sample extraction) in complex matrices have recently become available. Being based on atomic mass spectrometric, they are element-specific (*i.e.* provide information on the chemical identity) and have the potential to measure size, size distribution, number and mass concentration of particles. Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) is based on time resolved analysis of diluted nanoparticle dispersions using short dwell times (≤10 ms). Each particle gives rise to a signal clearly distinguishable from random background noise and, by means of appropriate algorithms, signal frequency distributions are converted into size frequency distributions. In principle, the signal arising from ionic (*i.e.* soluble) forms of the element constituting the particles, if any, can be distinguished from that due to the presence of the particles themselves. Therefore, it is a particle-specific technique with sizing capability, presently having limitations mainly in the size detection limits (from ~10 to several tens nm, depending on the element). Another powerful technique is asymmetric flow field flow fractionation (AF4), which provides separation of particles according to their size, combined on-line with optical detectors for size determination (MALS, DLS, UV) and elemental detection/quantification by ICP-MS. With AF4-(UV-MALS)-ICP-MS particles having diameters down to 1 nm can be determined with the additional advantage of multi-detector capability.

Our laboratory was one of the few - on a worldwide level - that participated to the three interlaboratory studies promoted by the European Commission on the application of these techniques to food analysis, namely two interlaboratory studies on sp-ICP-MS determination of Ag nanoparticles in aqueous or ethanol dispersions and in chicken meat, respectively, and the first interlaboratory study on AF4-ICP-MS determination of Ag nanoparticles. However, far more challenging is the application of these techniques to the characterization of oxides such as SiO_2 and TiO_2 owing to a number of issues, the main being spectral interferences in the detection of Si and Ti. We developed a multi-method approach for the simultaneous determination of particle size and mass concentration of

synthetic amorphous silica nanoparticles over the size range of 20-200 nm by AF4 coupled with online MALS and ICP-MS/MS detection. Ongoing work deals with sample preparation methods for nanosilica characterization in E551-containing food samples.

P07 OPTIMIZATION OF DATA ACQUISITION PARAMETERS FOR SINGLE PARTICLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (SP-ICP-MS)

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Advances in nanotechnology are forecast to have a major impact on a wide segment of industries, including the food sector. In particular, silver nanoparticles have a number of applications that are of specific interest for the production of food or food contact materials.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can be used to measure individual Nanoparticles (NPs), using a technique called single particle ICP-MS (sp-ICP-MS). This allows simultaneous determination of particle number, concentration, size and size distribution.

Current ICP-MS systems are capable to use shorter dwell times (below 1 ms) and acquire data continuously, with no settling time between measurements. This give the opportunity to make several measurement with one particle event, allowing integration of the signal from a single NP and significantly reducing the chance of overlapping signals from multiple particles. This also give the possibility to use a wider range of sample dilution factors.

We have developed a sp-ICP-MS dedicated software module that we have used in this work, where optimized measurement conditions using gold and silver NP standard materials are discussed. With a robust plasma and a 0.1-0.2 ms dwell time the method demonstrated excellent performance for the analysis of silver e gold NPs with a good accuracy, within the expected size distribution.

The method described here is of clear interest for applications in the fields of the detection of silver NPs in food products, or released from food contact materials, as well as for the safety assessment of such silver nanoparticles-containing products.

P08 DEVELOPMENT AND USE OF NUCLEAR AND RADIOANALYTICAL TECHNIQUES IN FOOD NANOTOXICOLOGY RESEARCH

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Ingested Nanoparticles (NPs) present in foods or materials that become in intimate contact with foods may pose health risks. Consumers can ingest NPs as nanosized components of inorganic food additives (colorants, UV filters, anti-caking and antimicrobial agents), and as products potentially released from NPs-containing food contact materials (*i.e.* packaging nanocomposites containing nanosilver and nanoclay as diffusion barriers and antimicrobial agents). Nanosized fractions (<100 nm) have been identified in common food-grade additives (titanium and silicon dioxides, E171 and E551) suggesting that other bulk additives may contain nanosized components (calcium carbonate (E170), vegetable carbon (E153), iron oxides/ hydroxides (E172), metallic gold (E175) and silver (E174)). In addition, TiO_2, now available as TiO_2NPs, is intentionally added to food as nano-additive. Furthermore, an experimental study showed how AgNPs, applied on pears in a similar manner to that of pesticides, were trapped in the peel and also could reach the pulp. So, before NPs are used in foods and food-related materials, relevant safety information should be available within the public domain.

In this context, we have started a program on the application of nuclear and radiochemical techniques in food nanotoxicology research. Here, based on the use of the nuclear reactor Triga Mark II of the University of Pavia we report examples of activities concerning: (i) the development of instrumental neutron activation analysis-INAA for the determination of the elements constituent of metal-based NPs of potential interest in the food applications. The availability of the rabbit channel (thermal neutron flux of 7.4×10^{12} neutrons cm^{-2} s^{-1}) allows the rapid and sensitive detection of short lived radionuclides induced by irradiation of the samples for seconds/ minutes followed by a prompt radioactive counting by computer-based gamma ray spectrometry; (ii) a study of the penetration of the radiolabeled ^{198}AuNPs ($T_{1/2}$=2.7 d) into quail eggs during their cooking; (iii) the analysis of Ag in plastic food containers containing nanosilver (irradiation of solid disks taken from the containers and measurement of the induced ^{108}Ag ($T_{1/2}$=2.4 min)); (iv) an investigation of the degree of dispersion of nano-clay in polymeric films for food contact applications (determination of main element constituents (Al, Mg, Ti, Si) of the nanoclay (cloisite-Na^+) via the simultaneous detection of the neutron induced ^{28}Al ($T_{1/2}$=2.4 min), ^{27}Mg ($T_{1/2}$=9.5 min), ^{51}Ti ($T_{1/2}$=5.7 min) and ^{29}Al ($T_{1/2}$=6.7min)).

P09 DISSOLUTION OF SILVER NANOPARTICLES BY AN *IN VITRO* HUMAN DIGESTIVE ASSAY

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Nanotechnology is one of the major scientific revolutions that the food industry has increasingly experienced over the last years. In particular, the agro-food market has exploited the well-known biocidal activity of silver nanoparticles (AgNPs), employing them in packaging material as additives or to extend the shelf life of consumer products. Since the human ingestion is possible, the need to get information about the possible adverse effects raised by these NPs on health is therefore urgent. Also, the knowledge of AgNPs fate, as well as, the identity of the molecular species arising from biotransformation processes in human biological fluids (*e.g.* the simulating gastrointestinal juices) is fundamental. These studies are important for the creation of novel and more proper models of risk assessment, which may take into account the possible dangerousness of AgNPs when used in the productive food chain. Ultimately, they may also be useful to improve the risk perception of stakeholders for such innovation.

Within this context, our work aimed at studying the dissolution behaviour and fate of AgNPs when in contact with synthetic human digestive juices by using a range of complementary analytical techniques. We implemented an *in vitro* model to simulate the oral ingestion and the passage along the gastrointestinal tract, with salts and proteins composition, pH differences and transit times alike the in vivo digestion. Results showed that AgNPs progressively aggregated during the passage along the digestive compartments and partially dissolved in ions. The ions were present in two different states, namely free or chelated to the digestive matrices and the bioavailability was higher in the stomach and intestine than in the mouth.

Overall, these results indicate that the molecular species bioavailable for duodenal absorption are variegated being represented by a polydisperse matrix containing low amount of AgNPs in their primary state or agglomerates/aggregates, free and chelated ions.

P10 EFFICACY AND SAFETY OF NANOMATERIALS USED IN NUTRACEUTICALS

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The use of nanomaterials and nanotechnology in nutraceuticals is becoming increasingly important for the innovation of existing products. Nanotechnology is used to increase efficacy of active ingredients, formulation stability, and dispersibility of products. However, nanofoods raise some concerns regarding their safety. These concerns were recently taken into account in the revision of the European Novel Food Regulation, which states that every food not significantly consumed in the European Union before May 1997 for human consumption has to be considered a Novel Food. Nanomaterials are explicitly considered among novel foods, which will need to undergo an approval procedure before being placed on the market. This provision makes all nanofoods virtually subjected to EU authorization from January 2018. Authorization is based on a scientific evaluation by the European Food Safety Authority (EFSA), which requires the adoption of state-of-the-art approaches and methods, both to demonstrate efficacy and safety.

The development of nanotechnology-based novel foods needs to be inherently safe in all the innovation chain steps. This approach, called Safe Innovation, is under development and demonstration within the H2020 project NANOREG2, after being initiated in the FP7 project NANoREG. Safe Innovation represents the framework in which to structure support to Companies wanting to innovate products and processes, providing technical, pre-regulatory and regulatory support in single parts or along the whole innovation chain.

In this work it is presented an integrated approach to address efficacy and safety of nanomaterials in nutraceuticals, supporting Companies in the production of nano-based formulations taking into account regulatory requirements and standardized and state-of-the-art methodologies. The procedure starts with the support in the identification of the best product and nutraceuticals formulation, taking into account different factors, including the overall compatibility with the potential nano-carriers and nano-formulations. Testing of the selected alternatives is carried out with an *in vitro* metabolic model, simulating the digestive process along the GI in different conditions. This method is following the currently available EFSA guidelines approach establishing the tiered exposure assessment for nanomaterials. By applying this model, newly developed formulations are evaluated for their bio-accessibility and bio-availability, as well as for their potential toxic effects. The whole system is supported by state-of-the-art characterization instrumentation and methodologies, and by early-on regulatory preparedness assessment, followed by support for authorization to market. In this poster, ECSIN approach is well-described and case studies reported.

P11 USE OF SILVER NANOPARTICLES IN POULTRY PRODUCTION: FOOD SAFETY IMPLICATIONS

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Increasing amounts of engineered Nanoparticles (NPs) have already found their way into different consumer products also in the agri-food sector, but the potential risks for humans following the exposure to Nanomaterials (NMs) is still a matter of study. In this context, the development of reliable analytical methods for detection and quantification of NPs in complex matrices is crucial and plays a key role both for exposure assessment in the framework of risk assessment/management as well as for the reinforcement of existing regulation.

Among the different NPs used, silver nanoparticles (AgNPs) have aroused a lot of interest thanks to their antimicrobial activity and currently find different applications in the food industry *e.g.* in food contact materials. AgNPs are also seen as potential candidates to replace antibiotics in animal husbandry and few studies have recently focused on this new application.

These studies mainly consider animal behaviour and metabolic effect but lack of any consideration about NPs residual accumulation in animal edible tissues and thus, on the consequent impact on consumers.

Given the above premises, a 3-week long *in vivo* experimentation was carried out in order to verify the possible AgNPs accumulation in animal tissues (meat, liver, kidney) and products (eggs) of hens treated repeatedly with AgNPs.

For this purpose the recently emerged single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) analytical approach was used to determine the presence of AgNPs in the different matrices and to test its potential to discriminate between ionic Ag and AgNPs. Moreover, the sp-ICP-MS results were compared with those obtained with classical analytical techniques like Atomic Absorption Spectroscopy (AAS) for quantitative residual total Ag determination and Electron Microscopy (EM).

This study shows that the use of AgNPs in animal farming results in the presence of such materials in food products and hints at the potential risk of the use of AgNPs in animal husbandry for direct exposure of consumers to NMs. From an analytical perspective results confirm the potential of sp-ICP-MS to detect AgNPs in complex matrices and its applicability in routine analysis.

P12 PROTEOMIC RESPONSE TO CADMIUM SULFIDE QUANTUM DOTS IN MODEL PLANTS

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A fuller understanding of the interaction between plants and engineered nanomaterials (ENM) is of topical relevance since the latter find applications also in agriculture and in the food industry. The aim of this work was to develop a toxicogenomic approach to assess the risk posed by ENMs to food crop using Arabidopsis thaliana as model plant.

In a previous study, the recognition of two independent A. thaliana insertional mutants displaying a greater level of tolerance than the wild type plant to exposure to Cadmium Sulfide Quantum Dots (CdS QDs) offered the opportunity to characterize the tolerance response at physiological and transcriptomic levels.

The proteomic analysis has been performed on crude protein extracts, obtained from whole seedlings of the two mutants and wild type, grown on agarized MS, treated with 80 mg/L CdS QDs and non-treated. The plants whole proteome has been separated by 2D gel electrophoresis, and an analysis using PDQuest software has been carried out on qualitative/quantitative differentially abundant proteins between wild-type and mutants. Proteins whose abundance was statistically (Student's t test) different in response to the experimental conditions were identified by MALDI-TOF/MS and database search to infer their possible role in the plant response, and in particular in the resistance to CdS QDs. In addition, some physiological analyses were performed in order to evaluate oxidative stress levels in treated plants, in particular, the content of hydrogen peroxide and the levels of lipid peroxidation.

An integrated study using a global proteomic approach to understand the response to nanoparticles in plants, together with physiological and transcriptomic analysis, may lead to a better understanding of some of the main genetic, molecular and physiological mechanisms at the basis of plant response to stress induced by ENM.

P13 NANOTECHNOLOGY IN FOOD: A PATH MODEL ANALYSIS FOR CONSUMER WILLINGNESS TO BUY

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Nanotechnology represents a new frontier in food science with a great potential in many food sectors: to improve sensory characteristics, health and safety. Since nanotechnology is an emerging field, few studies have analysed the interaction between the new technology and human population or environment, and the potential hazards of certain nanotechnology applications. A number of studies have examined the public's benefits and risks perceptions of nanotechnology as well as public attitudes toward nanotechnology in the US and in Europe. However, there has been limited research conducted in Italy that evaluates the factors influencing the public's attitudes toward nanotechnology. Therefore the aim of the study is to evaluate the influence of risk perception on the acceptance of nanotechnology food. The theoretical framework we adopted is a Path Model in which we assume that perceived benefits and perceived risks influence willingness to buy "nanotechnology food". The model included variables such as trust in science, trust in food industry and retail, attitude toward technology, preference for healthy food, preference for organic products and preference for food supplements.

Data was collected by means of an online survey administered on May and June 2015 to a sample of 500 respondents representing food purchaser in Italy. To achieve our goal a structured questionnaire was submitted to consumers and Principal Component Analysis, followed by Structural Equation Model, was performed. To estimate the parameters the STATA 10 program was used.

Results show that trust in science and institutions has a significant effect on perceived benefits and perceived risks; preference for healthy food is correlated with perceived risks but not with perceived benefits. The model shows that perceived benefits explain a large part of variance in willingness to buy.

P14 MICROWAVE-ASSISTED GREEN SYNTHESIS OF STABLE SILVER-CONJUGATED FLAVONOID NANOPARTICLES

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In the past few years, a growing number of studies on bio-inspired nanomaterials was published. The biological synthesis of Nanoparticles (NPs) generally involves a "bottom-up" approach wherein biomolecules mediate reductive processes and stabilize nanostructures. Several compounds from the primary and secondary metabolism of higher plants (*i.e.* carbohydrates, organic acids, polyphenols) have been demonstrated to exert antioxidant and reducing properties in *in vitro* experiments.

Catechins are an important class of dietary Flavonoids (FLs) with promising use as therapeutic agents due to their powerful antioxidant activity and numerous biological properties. Grape pomace is a valuable source of these compounds and at the same time represents a waste for the winery industry, which is currently used mainly for animal feed, organic fertilizers, ethanol production, or directly disposed as a waste.

In this study, we investigated the possibility of a bio route for the green synthesis of silver nanoparticles using catechin, epicatechin gallate and a mixture of these compounds as both reducing and capping agents.

Flavonoid-Loaded silver nanoparticles (FL-AgNPs) were synthesized with the assistance of Microwave (MW) irradiation and the reaction was carried out for 40 s at 700 W.

Obtained FL-AgNPs were characterized by means of different spectral, electrochemical and morphological analysis and their stability over time assessed. Finally, FL-AgNPs properties were compared to those of certified commercial AgNPs.

The synthesized FL-AgNPs showed UV-vis profiles similar to that of commercial AgNPs, with a sharp Surface Plasmon Resonance (SPR) peak centered at 426 nm, and were highly stable over time. The same results were obtained with Cyclic Voltammetry (CV) measurements using screen-printed electrodes. FL-AgNPs showed a behavior similar to that of commercial AgNPs, characterized by a reversible electrochemical behavior with an oxidation potential of 109 mV and a reduction, less pronounced, around - 144 mV. FL-AgNPs were nearly spherical, with an average size of 25-35 nm. Moreover, results showed that the nature of FLs employed for the synthesis strongly affected the intensity of SPR peak, pointing out the role of the structure-activity relationship of FLs in the biosynthesis of AgNPs.

This study highlighted the potential of flavonoids from grape pomace as green agent for bio-inspired nanomaterial synthesis. This innovative synthetic method of FL-AgNPs is simple and convenient to handle, without using hazardous chemicals.

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Stampato da Tipografia Facciotti srl
Vicolo Pian Due Torri 74, 00146 Roma

Roma, aprile-giugno 2016 (n.2)