Nanotechnologies in the Food Sector: problems and perspectives

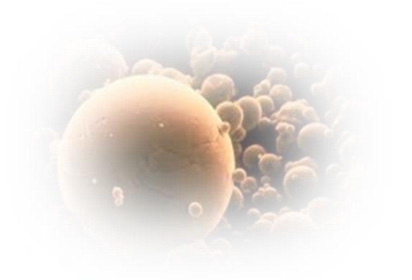
Francesco Cubadda

Dept. Food Safety, Nutrition and Veterinary Public Health

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

Rome

francesco.cubadda@iss.it



National Scientific Expert in the EFSA Network for
Risk Assessment of Nanotechnologies in Food and Feed

NANOINNOVATION 2016

Roma, 21 settembre 2016

❑ ISO definition of nanomaterial (2015)

International Organisation for Standardization
(ISO/TS 80004:2015)

‘nano-object’: discrete piece of material with one, two or three external dimensions in the nanoscale

‘nanoscale’: length range approximately from 1 nm to 100 nm

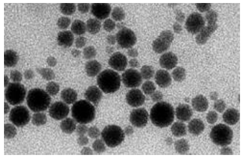
‘nanoparticle’: nano-object with all external dimensions in the nanoscale *where the lengths of the longest and the shortest axes of the nano-object do not differ significantly*

nanom

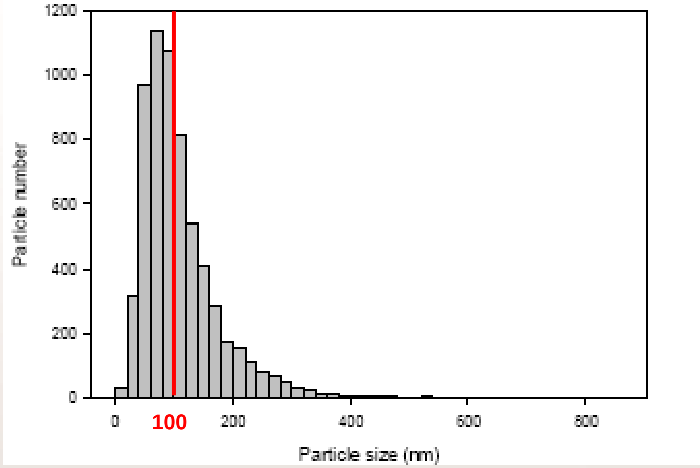
nano-object
any external dimension in the nanoscale

Nano-object
(1, 2 or 3 external dimensions in the nanoscale)

Nanoparticle
(3 ext. dimensions In the nanoscale)



Particle size distribution



European Commission’s definition of nanomaterial for legislative and policy purposes in the EU

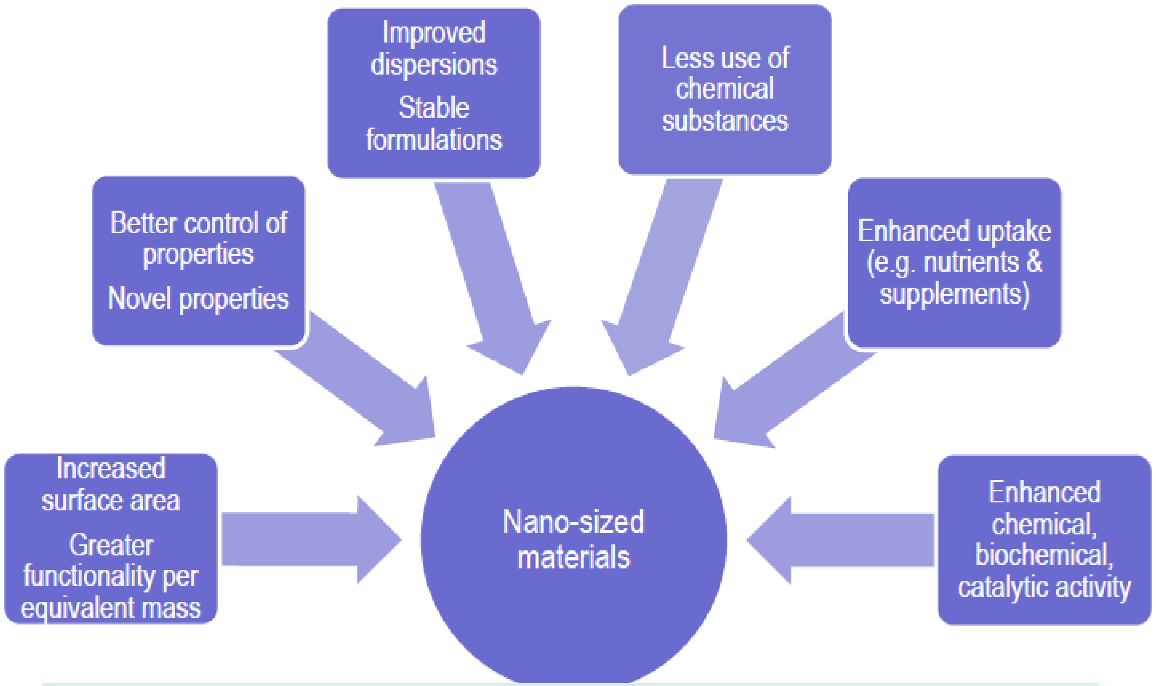
Based on the percentage of particles with one or more external dimensions in the size range 1-100 nm

Characterization of size and size distribution is essential

Nanotechnologies: nano-enabled functions/performances

- **Nanotechnology**: Application of scientific knowledge to manipulate and control matter in the nanoscale in order to make use of size- and structure-dependent properties and phenomena distinct from those associated with larger sizes of the same material

- A number of **nanotechnology applications in all industrial sectors** are emerging since management of characteristics such as material size, shape, morphology, enable the improvement or development of new process and product properties



Functions/performances of interest in the food sector

Nanotechnology applications in the food sector

Agricultural production

Food processing

Packaging & storage

e.g. **nanocomposites, nano-coatings and other food contact materials**

e.g. **nanopesticides, other nanosized agrochemicals**

e.g. **nanosized additives, nanoencapsulates, nutritional supplments**

Situation today in the EU

- Authorisation required for whatever application in the food sector: EFSA evaluates possible health risks
- The new Novel food Regulation gives the rules for the use nano-ingredients in food (since 2018)
- Some nanosized additives authorised for use in plastic food contact materials
- No nanosized additives authorised for use in food, but some «older» additives have been found to be partially in nanoform e.g. TiO_2 (E171) and especially SiO_2 (E551) (both under EFSA re-evaluation)

Nanotechnology applications in the food sector

According to the different use scenarios, nanomaterials - or the chemicals resulting from their degradation/dissolution (if any) - might be found in food…

Agricultural production

	Function
	Binding and coloring micro-organisms, Detection of contaminants, mycotoxins and …

as residues

Food processing

Application	Function
Nanoemulsions, micelles	Use of less fat and emulsifiers, stable … products
… textured food products	
Nano-sized additives	
Nutritional and nutrac…	
Nanofilters	

as intentionally added substances

Packaging & storage

Nanotechnology	Application	
Nanocomposites	Incorporating nanoparticles into a p… composite	Improving strength of … properties, biodegradation
Na…	Incorporating nanomaterials onto the packaging surface	Improving barrier properties
	…ating active …ticles with intentional …into- and …ent effect on the packaged food	Oxygen scavenging, prevention of growth of pathogens
"Intelligent" packaging materials	Incorporating nanosensors for fo…	Detection of food deterioration, … processing, handling and preparation

due to migration from packaging

Nanopesticides

Other exposure routes (*e.g.* for operators using nano-agrochemicals, incl. feeds)

There is potential for consumers' dietary exposure

Is there any risk? → **What are the (potential) hazards of ENMs?**

Fate in human body and potential hazards of NMs absorbed in the GI tract

Ingestion

Dissolution

Elimination /excretion

Translocation across gut epithelium

Absorption

Body Fluids (lymph, blood)

Distribution

Nanoparticles may cross **biological barriers**: BBB, Blood–testis barrier, Placenta

Potential Interaction(s)
Phagocytes
Cellular Response
Target Organs

SOLUBILITY

Dissolution

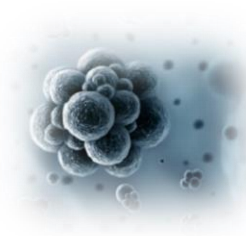
Slow, or No Dissolution

Retention

Metabolism

Excretion

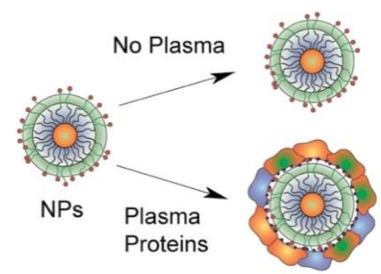
- Some NMs have a certain degree of solubility
- Dissolution (=loss of the particulate nature) is often invoked to imply lower risk
- Few materials, however, are readily soluble and while dissolving, smaller (potentially more toxic) particles are generated



Smaller particles generally absorbed more readily and faster than larger ones (greater uptake of NPs compared to respective microparticles)

SIZE

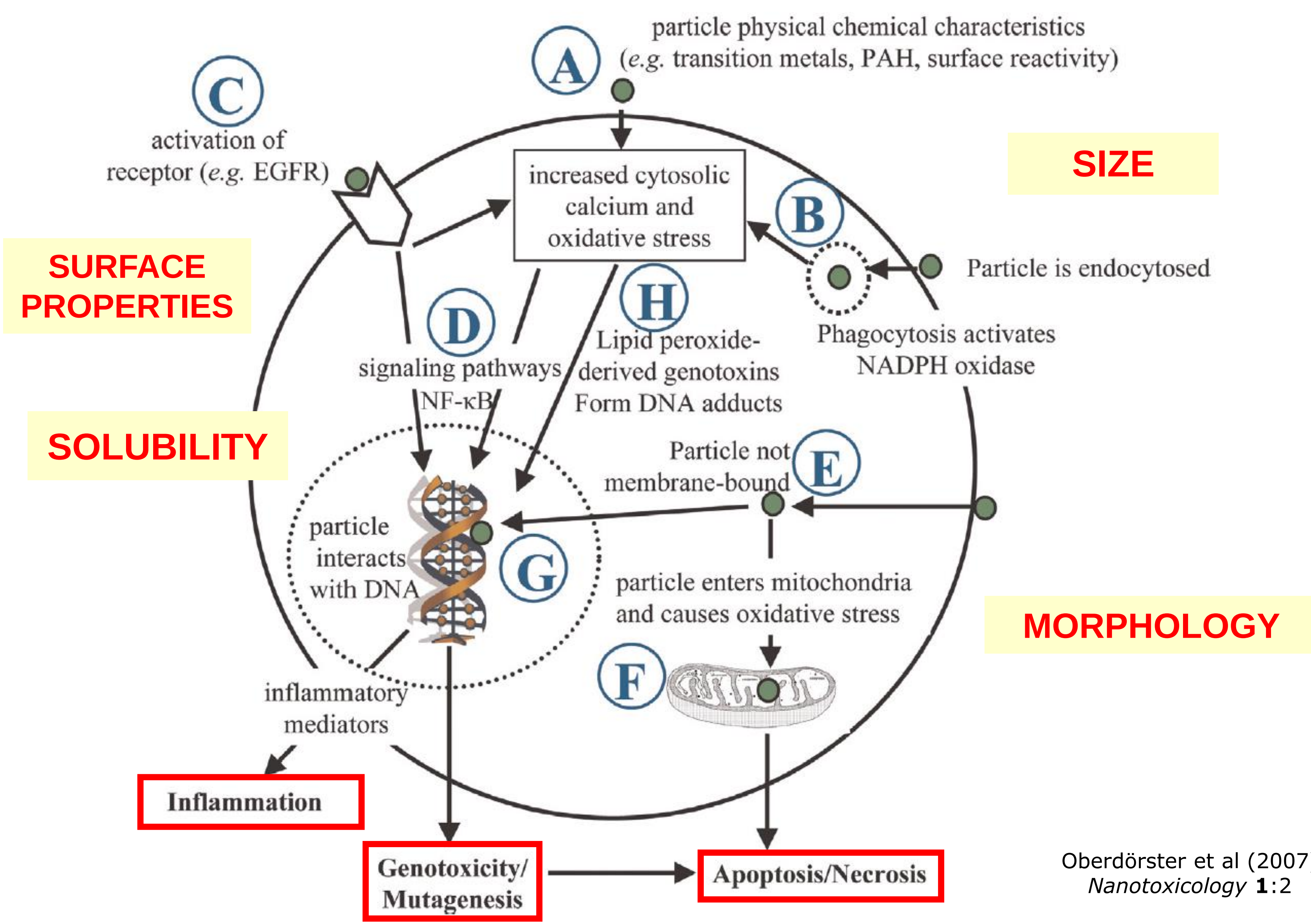
Surface properties and the existence of any surface **coating** critically affect uptake and fate in the body after absoprtion



Protein corona

SURFACE PROPERTIES

Fate in human body and potential hazards of NMs absorbed in the GI tract



Oberdörster et al (2007)
Nanotoxicology **1**:2

ARTICLES
PUBLISHED ONLINE: 12 FEBRUARY 2012 | DOI: 10.1038/NNANO.2012.3
nature nanotechnology

Oral exposure to polystyrene nanoparticles affects iron absorption

Gretchen J. Mahler[1], Mandy B. E
Raymond P. Glahn[3] and Michael

The use of engineered nanoparticles i
exposure to nanoparticles on human
polystyrene nanoparticles can influenc
an *in vivo* chicken intestinal loop mode
iron transport due to nanoparticle dis
(50 nm in diameter) had a lower iron
remodelling of the intestinal villi, whic
the *in vitro* and *in vivo* results su
toxicology studies.

Conclusions

The intestinal epithelial layer represents the initial gate that ingested nanoparticles must pass to reach the body. The polystyrene particles used in these experiments are generally considered non-toxic, but their interaction with a normal physiological process suggests a potential mechanism for a chronic, harmful, but subtle response. Similar disruptions in nutrient absorption could be possible in relation to other inorganic elements such as calcium, copper and zinc, which require passive or active transport systems for them to be absorbed through the intestinal epithelium. Fat-soluble vitamins such as vitamins A, D, E and K are absorbed only after micellization by pancreatic lipase[45]. Hydrophobic, charged nanoparticles could disrupt the formation of micelles, micelle interactions with the epithelial layer, and/or nutrient diffusion through the phospholipid membrane.

- Development and harmonization of **analytical methods and tools in support of nanomaterial risk assessment** is a challenging task

Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed *(April 2011)*

- **Comprehensive physicochemical characterization** (size, size distribution, morphology, surface properties, dissolution, etc.) is required for 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**
  - **as present in biological fluids and tissues**

Challenges of NM characterization in support of risk assessment

- ❑ Development and harmonization of **analytical methods and tools in support of nanomaterial risk assessment** is a challenging task

- ❑ **Comprehensive physicochemical characterization** (size, size distribution, morphology, surface properties, dissolution, etc.) is required for 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**
  - **as present in biological fluids and tissues**

Food Nanosafety Team @ ISS

Department of Food Safety and Veterinary Public Health

A major area of research:

- ❑ **Analytical determination of ENMs** (pristine, in food, in biological tissues)

State-of-the-art techniques

NM characterization facility

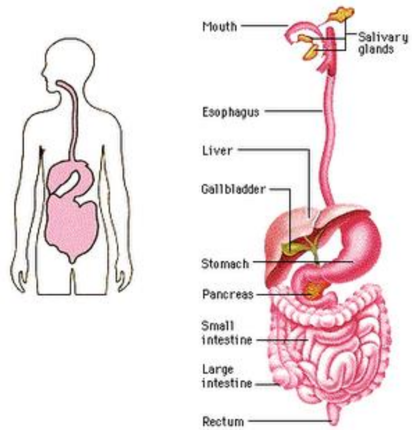
Italian National Health Institute: what we do

Food Nanosafety Team @ ISS

Department of Food Safety and Veterinary Public Health

Other areas of research:

- ***In vitro* studies**, incl. assessment of ENM modification/ degradation/dissolution after ingestion (simulated GI digestion)



- ***In vivo* oral toxicity studies**
(ADME/biodistribution, repeated dose toxicity, focus on effects on endocrine/development/reproductive system)

NANOGENOTOX

NANoREG

Generation of new data to support risk assessment of ENMs in food by the National Authority and the EFSA*

* *EFSA Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed*

efsa European Food Safety Authority

FONDAZIONE FILARETE

SAPIENZA UNIVERSITÀ DI ROMA

rivm National Institute for Public Health and the Environment Ministry of Health, Welfare and Sport

anses

Vrije Universiteit Brussel

Nanotoxicology, 2013; Early Online, 1–9
© 2013 Informa UK, Ltd.
ISSN: 1743-5390 print / 1743-5404 online
DOI: 10.3109/17435390.2013.822114
informa healthcare

Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat: focus on reproductive and endocrine systems and spleen

Roberta Tassinari[1], Francesco Cubadda[1], Gabriele Moracci[1], Federica Aureli[1], Marilena D'Amato[1],

http://informahealthcare.com/nan
ISSN: 1743-5390 (print), 1743-5404 (electronic)
Nanotoxicology
Nanotoxicology, Early Online: 1–8
© 2014 Informa UK Ltd. DOI: 10.3109/17435390.2014.969791
informa healthcare

ORIGINAL ARTICLE

Amorphous silica nanoparticles alter microtubule dynamics and cell migration

Laetitia Gonzalez[1], Marco De Santis Puzzonia[2*], Raffaele Ricci[2], Federica Aureli[3], Giulia Guarguaglini[2], Francesco Cubadda[3], Luc Leyns[1], Enrico Cundari[2], and Micheline Kirsch-Volders[1]

http://informahealthcare.com/nan
ISSN: 1743-5390 (print), 1743-5404 (electronic)
Nanotoxicology
Nanotoxicology, Early Online: 1–10
© 2014 Informa UK Ltd. DOI: 10.3109/17435390.2014.940408
informa healthcare

ORIGINAL ARTICLE

Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food

Petra C. E. van Kesteren[1], Francesco Cubadda[2], Hans Bouwmeester[3], Jan C. H. van Eijkeren[1], Susan Dekkers[1], Wim H. de Jong[1], and Agnes G. Oomen[1]

[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, [2]Istituto Superiore di Sanità (ISS) – National Health Institute, Rome, Italy, and [3]RIKILT – Wageningen University & Research Centre, Wageningen, The Netherlands

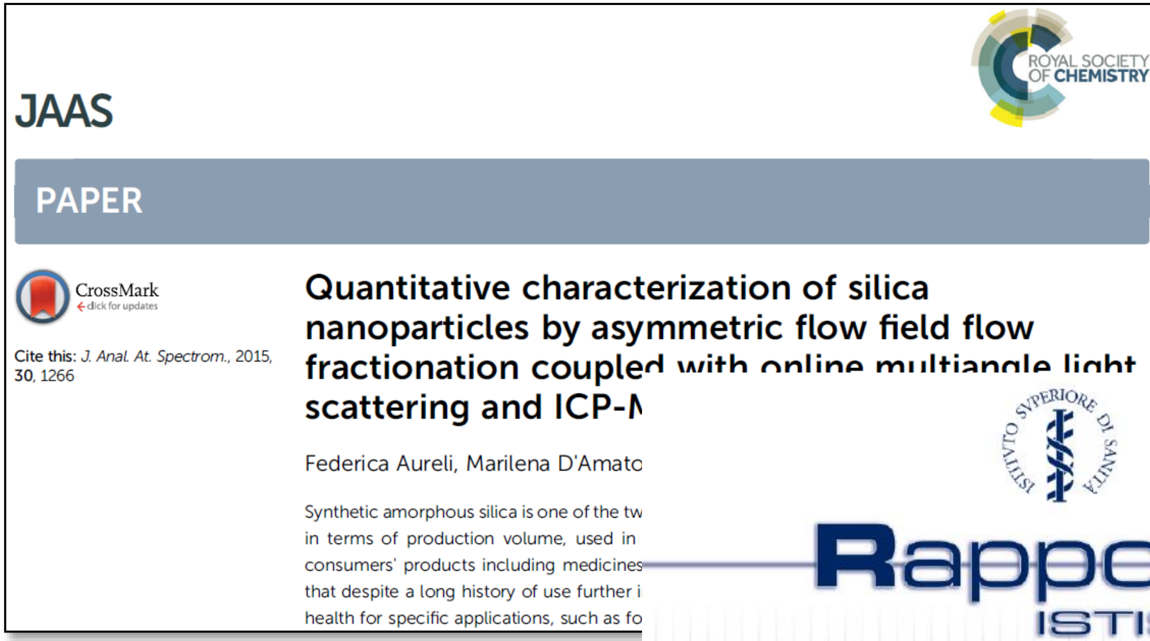
ELSEVIER
Trends in Food Science & Technology xx (2014) 1–22
TRENDS IN FOOD SCIENCE & TECHNOLOGY

Scientific basis of nanotechnology, implications for the food sector and future trends

M. Rossi[a,b,*], F. Cubadda[c], L. Dini[d], M.L. Terranova[e], F. Aureli[c], A. Sorbo[f] and D. Passeri[a]

[a]Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Via A. Scarpa 16, 00161 Rome, Italy
[b]Centro di Ricerca per le Nanotecnologie Applicate all'Ingegneria della Sapienza (CNIS), Sapienza University of Rome, Piazzale A. Moro 5, 00185 Rome, Italy (Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Via A. Scarpa 16, 00161 Rome, Italy. Tel.: +39 06 49766341; fax: +39 06 44240183; e-mails: marcorossi@uniroma1.it; marco.rossi@nanoshare.com)
[c]Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità – National Health Institute, Viale Regina Elena 299, 00161 Rome, Italy
[d]Department of Biological and Environmental Science and Technology, University of Salento, Prov.le Lecce-Monteroni, 73100 Lecce, Italy
[e]Department of Chemical Sciences and Technologies – MinimaLab, University of Rome Tor Vergata, Via Della Ricerca Scientifica, 00133 Rome, Italy
[f]European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO) – Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità - National Health Institute, Viale Regina Elena 299, 00161 Rome, Italy

Novel methods for NM characterization



JAAS

ROYAL SOCIETY OF CHEMISTRY

PAPER

CrossMark

Cite this: *J. Anal. At. Spectrom.*, 2015, **30**, 1266

Quantitative characterization of silica nanoparticles by asymmetric flow field flow fractionation coupled with online multiangle light scattering and ICP-M

Federica Aureli, Marilena D'Amato

Synthetic amorphous silica is one of the tw
in terms of production volume, used in
consumers' products including medicines
that despite a long history of use further i
health for specific applications, such as fo

National conferences

“Nanotechnologies and nanomaterials in the food sector and their safety assessment”

- ✓ **1st**: September 2013
- ✓ **2nd**: April 2016

Under the patronage of the Ministry of Health

With the participation of representatives of EFSA and the European Commission (JRC, Ispra)

Nano-silver (Ag)

- Silver nanoparticles, proposed as antimicrobial agents in food-related applications, depending on the coating/stabilizing agent may **partially dissolve in the GI tract**

- However **local effects**, including alteration of the mucosa-associated microbiota and modulation of the gut-associated immune response, are of concern

- 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**

- Increasing oral silver exposure (no matter if NPs or ions) appears problematic because of potential **neurotoxicity** even at very low exposure levels

- Re-evaluation of **silver as food additive** (E174) @ EFSA: it may be partially nanosized, insufficient data for risk assessment (data on size distribution & Ag ions release lacking)

The major issues included chemical identification an d characterisation of silver E 174 (e.g. quantity of nanoparticles and release of ionic silver) and similar information on the material used in the available toxicity studies. Therefore, the Panel concluded that the relevance of the available toxicological studies to the safety evaluation of silver as a food additive E 174 could not be established.

Toxicity of nano-Ag *vs.* ionic Ag *in vivo*

Recordati *et al. Particle and Fibre Toxicology* (2016) 13:12
DOI 10.1186/s12989-016-0124-x

Particle and Fibre Toxicology

RESEARCH Open Access

Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and size-dependent effects

Camilla Recordati[1*†], Marcella De Maglie[1,2†], Silvia Bianchessi[1], Simona Argentiere[1], Claudia Cella[1,3], Silvana Mattiello[2], Francesco Cubadda[4], Federica Aureli[4], Marilena D'Amato[4], Andrea Raggi[4], Cristina Lenardi[1,3,5], Paolo Milani[1,3,5] and Eugenio Scanziani[1,2]

Abstract

Background: Silver nanoparticles (AgNPs) are an important class of nanomaterials used as antimicrobial agents for a wide range of medical and industrial applications. However toxicity of AgNPs and impact of their physicochemical characteristics in *in vivo* models still need to be comprehensively characterized. The aim of this study was to investigate the effect of size and coating on tissue distribution and toxicity of AgNPs after intravenous administration in mice, and compare the results with those obtained after silver acetate administration.

Methods: Male CD-1(ICR) mice were intravenously injected with AgNPs of different sizes (10 nm, 40 nm, 100 nm), citrate-or polyvinylpyrrolidone-coated, at a single dose of 10 mg/kg bw. An equivalent dose of silver ions was administered as silver acetate. Mice were euthanized 24 h after the treatment, and silver quantification by ICP-MS and histopathology were performed on spleen, liver, lungs, kidneys, brain, and blood.

Results: For all particle sizes, regardless of their coating, the highest silver concentrations were found in the spleen and liver, followed by lung, kidney, and brain. Silver concentrations were significantly higher in the spleen, lung, kidney, brain, and blood of mice treated with 10 nm AgNPs than those treated with larger particles. Relevant toxic effects (midzonal hepatocellular necrosis, gall bladder hemorrhage) were found in mice treated with 10 nm AgNPs, while in mice treated with 40 nm and 100 nm AgNPs lesions were milder or negligible, respectively. In mice treated with silver acetate, silver concentrations were significantly lower in the spleen and lung, and higher in the kidney than in mice treated with 10 nm AgNPs, and a different target organ of toxicity was identified (kidney).

Conclusions: Administration of the smallest (10 nm) nanoparticles resulted in enhanced silver tissue distribution and overt hepatobiliary toxicity compared to larger ones (40 and 100 nm), while coating had no relevant impact. Distinct patterns of tissue distribution and toxicity were observed after silver acetate administration. It is concluded that if AgNPs become systemically available, they behave differently from ionic silver, exerting distinct and size-dependent effects, strictly related to the nanoparticulate form.

Keywords: Silver nanoparticles, Silver acetate, Dissolution, *In vivo* study, Mouse, Intravenous route, Tissue distribution, Toxicity, Hepatocellular necrosis, Hemorrhage

Summary of tox issues: NMs compared to 'traditional' chemicals

- Different and generally **higher bioaccessibility** in terms of
  - Gastrointestinal absorption
  - Ability to cross biological barriers (blood–brain barrier, blood–testis barrier, placenta)

- **Different biodistribution** in the body (*e.g.* different toxicokinetic parameters and different target organs)

- **Uncertainties on the forms and half-life** in the different tissues (What form? Particles? Their assemblies? Dissolving particles and all the intermediate species between nanoparticulate forms and soluble counterparts?)

- Different and presumptively **greater interaction with cellular components** (organelles, molecules) up to – possibly – the DNA

Conclusions

- Approaching the safety assessment of products of nanotechnology is a challenge, since **new concepts and tools for safety assessment of nanomaterials are needed**
- Improvement/adaptation of **analytical methods and toxicity testing approaches** is needed. **Novel risk assessment approaches** (*e.g.* internal dose) should be considered
- **Physico-chemical properties** are critical to point out the toxicological hazards of specific nanomaterials
- 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

Thank you for your attention