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Conference & Exhibition

4° Workshop AgriNanoTechniques
Nanomaterials for products and application in agriculture

From scientific knowledge to regulatory application: The nanomaterial intestinal fate case study

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Environment and Health Department

Multidisciplinary Unit on Nanomaterials and Nanotechnologies

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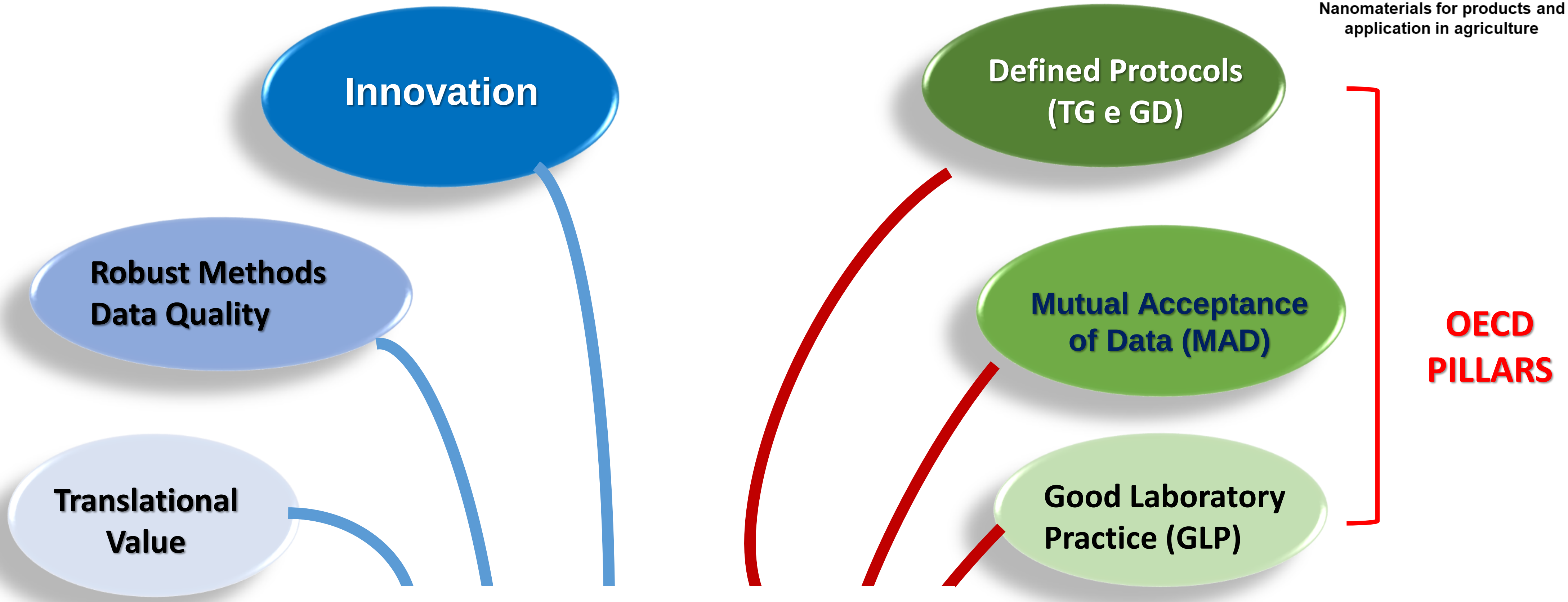


REGULATORY SCIENCE

Regulatory science plays a vital role in protecting and promoting global public health by providing the scientific basis for ensuring that food, consumer, and medical products are safe, properly labeled, and effective

ORGANISATION OF ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

Intergovernmental organisation

- 37 Member States representatives and European Commission
- Industry representation (Business and Industry Advisory Committee, BIAC)
- Animal Welfare organisations (ICAPO)
- Green NGOs
- Other Partners (i.e China, Malaysia, Thailand, South Africa)
- International Organisations (i.e. WHO, UNITAR, ISO TC229, UNEP)

Test Guidelines (TG) are harmonised test methods for the safety assessment of chemicals, including nanomaterials, addressing regulatory needs. Fixed test protocols with validity criteria. **Covered by MAD**

Guidance Documents (GD) can be a test method or they can provide technical guidance for the use of TGs. Scientific validation could be limited and based on published literature. **Not covered by MAD**

Both are discussed and agreed by OECD member states

OECD Guidance Document

Integrated in vitro approach for intestinal fate of orally ingested nanomaterials

Develop of a new Guidance Document (GD) reporting a testing strategy to determine Nanomaterials behavior in a simulated *in vitro* intestinal environment

Lead Country Italy
Contribute JRC, Luxembourg, Spain

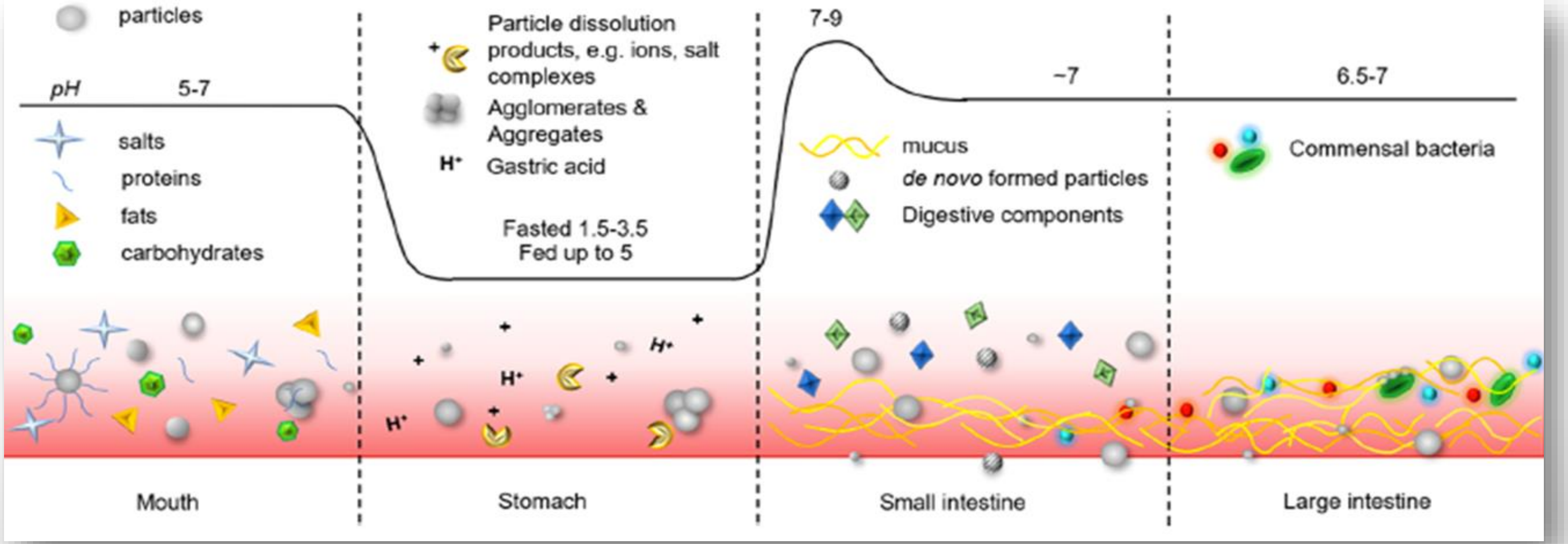
H2020 NanoHarmony EU project

Develop a set of reliable methods and guidelines, based on the translation of existing scientific knowledge into a form having regulatory relevance, able to support regulatory oriented research

Ministero della Salute

Preparatory study for the development of an OECD GD for the purposes of REACH obligations

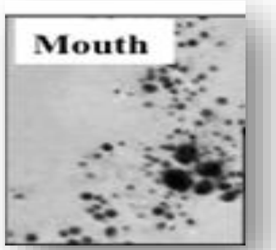
Parameters affecting the physicochemical properties and availability of particles throughout the oro-gastrointestinal passage



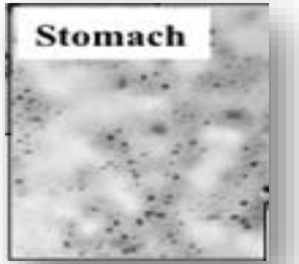
Kampf A., Chem. Res. Toxicology, 2020

IN VITRO ACELLULAR MODEL

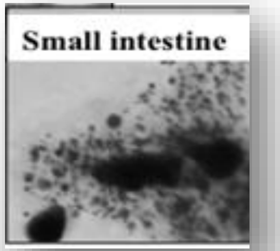
Mouth
Salivary juice



Stomach
Gastric juice



Intestine
Duodenal juice

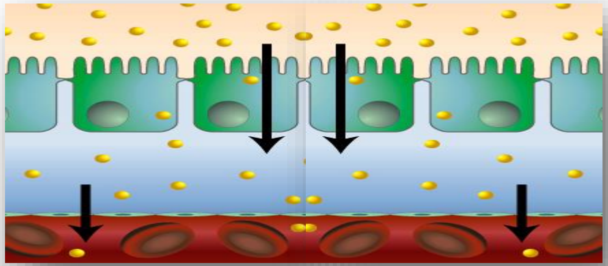


- ✓ **Does the material quickly degrade into non-nanomaterials in *in vitro* digestive tract conditions?**
- ✓ **Is there a potential for the material to be biopersistent and/or hazardous?**

EFSA Guidanceon risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health, 2018

IN VITRO CELLULAR MODEL

Intestinal Absorption



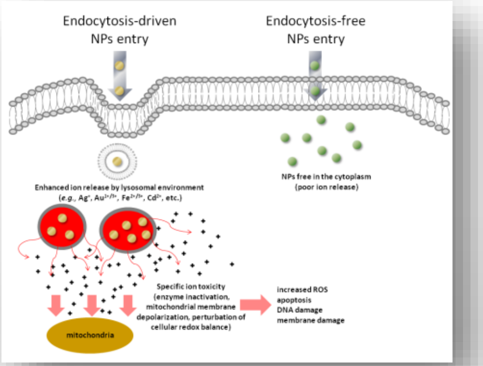
DISSOLUTION OF NANOMATERIALS

Dissolution is defined as the process of obtaining a solution containing the analyte of interest

Dissolution of NMs is the act of dissolving and the resulting species may be molecular or ionic.

Dispersion "refers" to the microscopic multi-phase system in which discontinuities of any state (solid, liquid or gas: discontinuous phase) are dispersed in a continuous phase of a different composition or state".

According to definition, **dissolution of nanomaterials will entail release of ions to the surrounding solvent where the rate of dissolution will be dependent on size, chemistry, solvent composition, and surface coating or functionalization of nanomaterials**. **ISO/TR 19057:2017(E)**



Sabella et al., Nanoscale (2014)

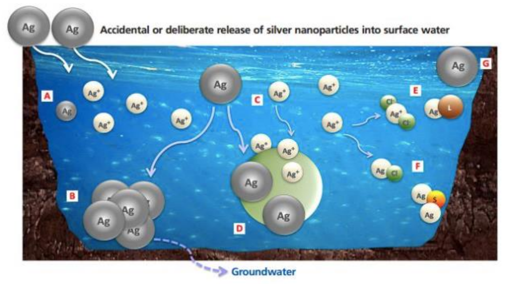
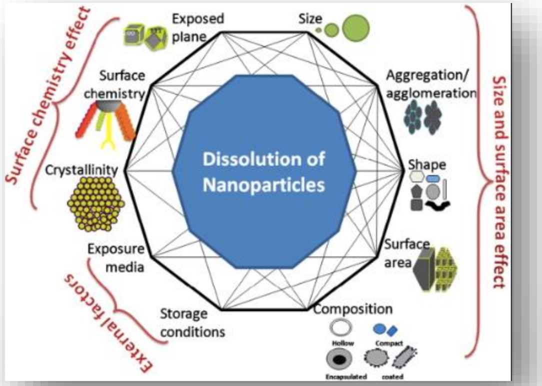


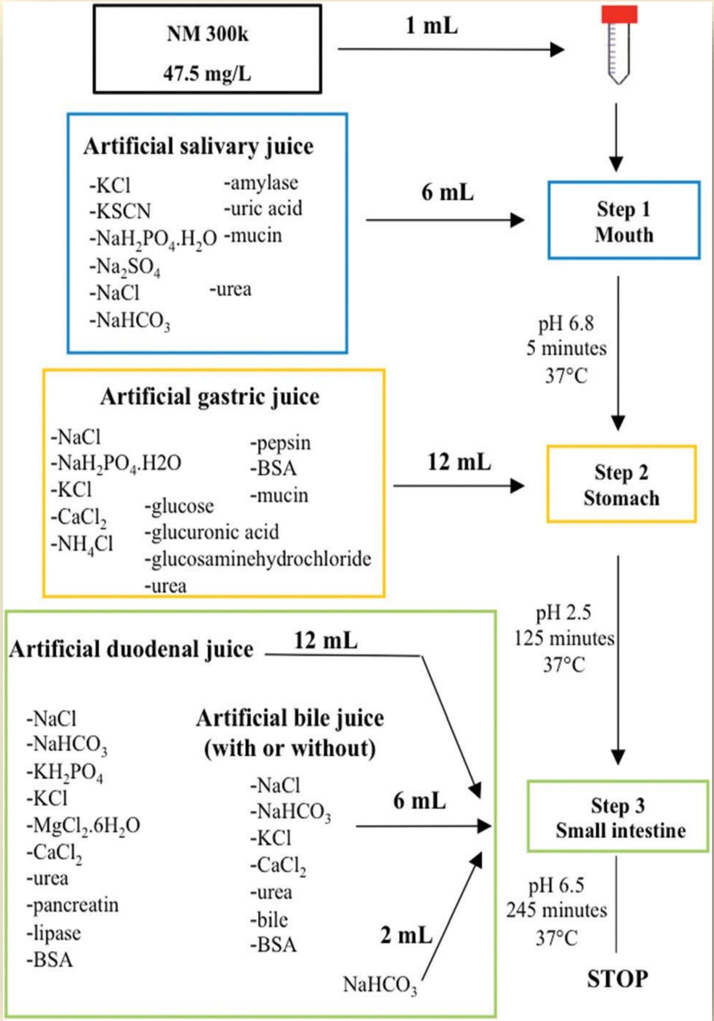
Figure 1. (A) Dissolution process leading to free ions release and smaller particles; (B) Aggregation into larger particles; (C, D) Adsorption of released Ag+ and nAg, respectively; (E) Formation of soluble complexes; (F) Reaction with other components in the water, which may result in precipitation; (G) nAg remaining stable.

Courtesy from AZONANO

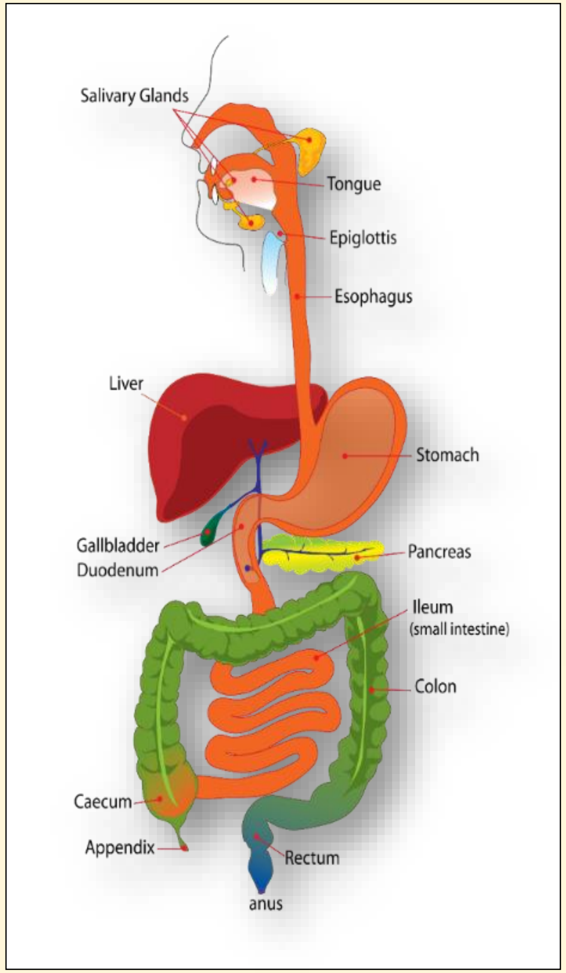


Misra et al., Science of The Total Environment Volume 438, 2012 , 225-232 (2012)

SIMULATED PHYSIOLOGICAL MEDIA



Bove P. et. al. Nanoscale, 2017



CONSECUTIVE DIGESTION *IN VITRO* ASSAY USING ARTIFICIAL DIGESTION FLUIDS

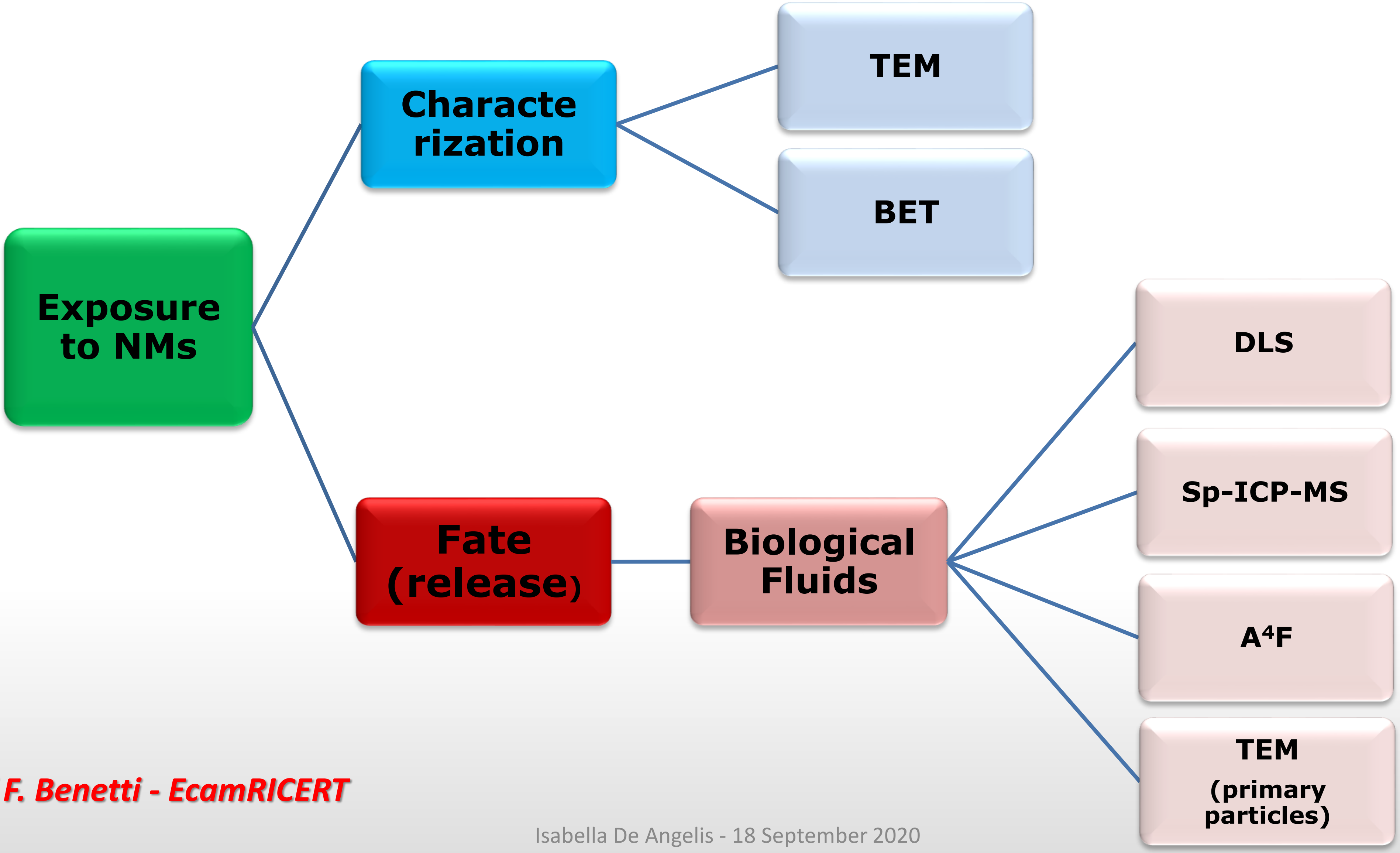
They simulate the different human digestion enviroments in the GIT through modulation of biochemical composition, pH differences and transit times, alike human *in vivo* digestion.

A pre-standardized procedure for this test was developed within the EU FP7 NANoREG project.
The assay was further validated by inter-laboratory (IL) comparison studies among different research groups within EU projects NANoREG and Gracious (H2020).

By literature research and IL comparison, effects on dissolution (k) rate of different juice compositions, ranging from simple solutions (only pH and salts, no enzymes) to more complex juices, was evaluated.

Courtesy of S. Sabella - IIT

NANOPARTICLES FATE FOLLOWING DIGESTION



Courtesy of F. Benetti - EcamRICERT

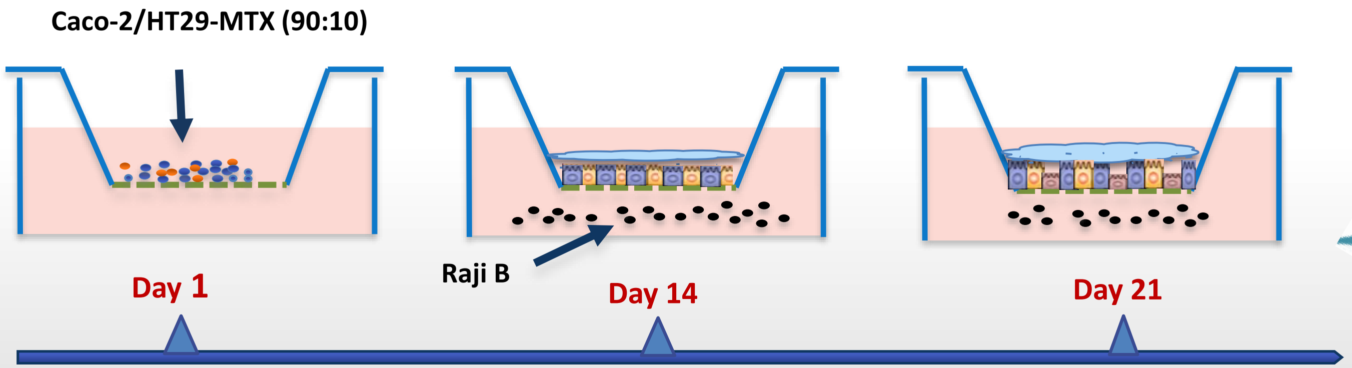
TOWARDS ADVANCED *IN VITRO* MODEL

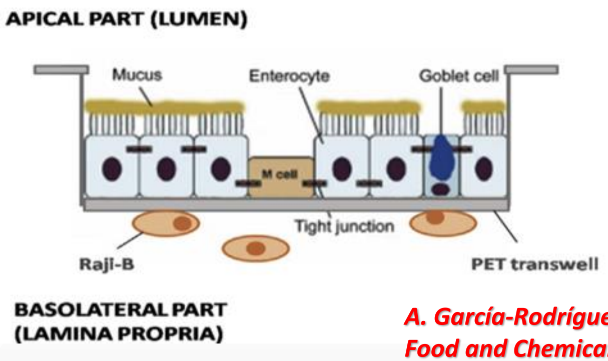
In vitro model based on a single cell line does not properly represent the complex gut environment. Complex intestinal *in vitro* barrier models were proposed to accomplish a more realistic *in vitro* system.

In particular, **mucus** has important defensive properties and strongly impacts nanoparticle mobility while **specialized M cells** are involved in particulate uptake.

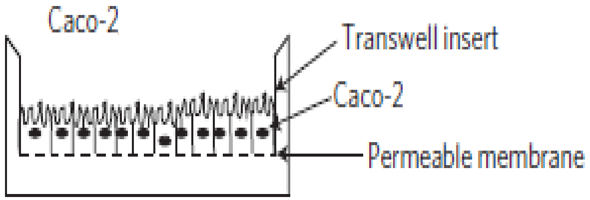
Incorporation of microfold (M) cells and mucus secreting cells into Caco-2 cell culture can enhance the physiological relevance of the intestinal *in vitro* models.

Increasing interest has been recently directed towards the triple co-culture model formed by Caco-2/HT29/Raji-B cells to quantitatively assess cell uptake rates and transport kinetics of NMs

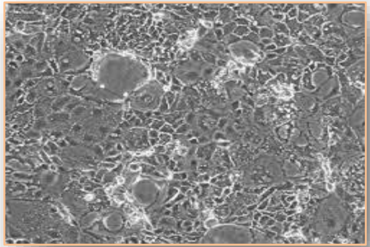


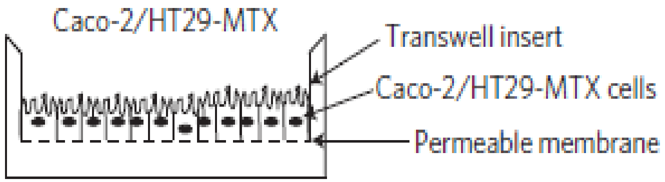


A. García-Rodríguez et al., Food and Chemical Toxicology 113 (2018) 162–170

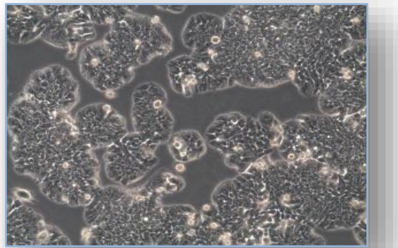


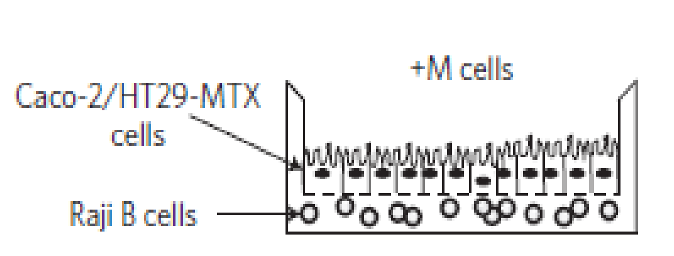
Differentiated Caco-2 cells form a polarized monolayer of enterocytes expressing an organized brush border with a dense network of tight junctions



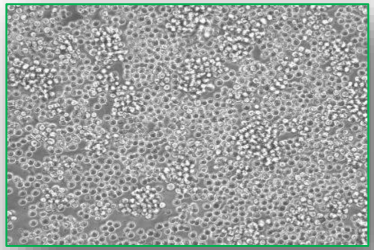


HT29-MTX, mucus-secreting goblet cells, are able to reproduce the mucus-secretion of the human intestine *in vivo*





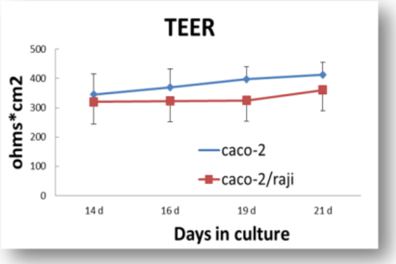
Raji B lymphocyte cell line induces Caco-2 differentiation into cells with an M cell-like morphology, thus recreating the human small-intestinal epithelial membrane

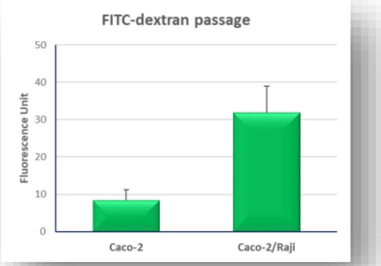


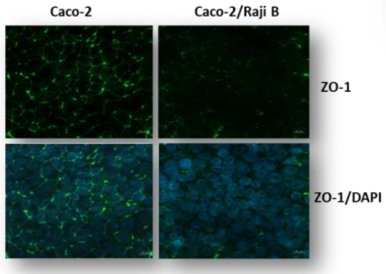
ENDPOINTS

BARRIER INTEGRITY MARKERS

- TEER
- LY or FITC-dextran passage
- ZO-1 expression

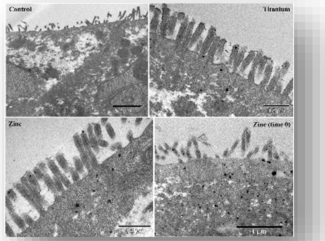


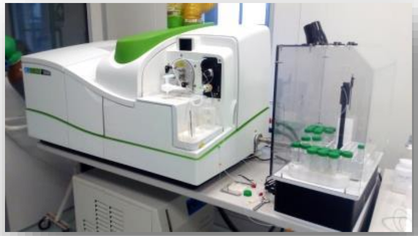




CELLULAR UPTAKE AND TRANSLOCATION INDICATORS

- TEM analysis
- Determination of NM concentration in AP/ BL compartments and in cell lysate

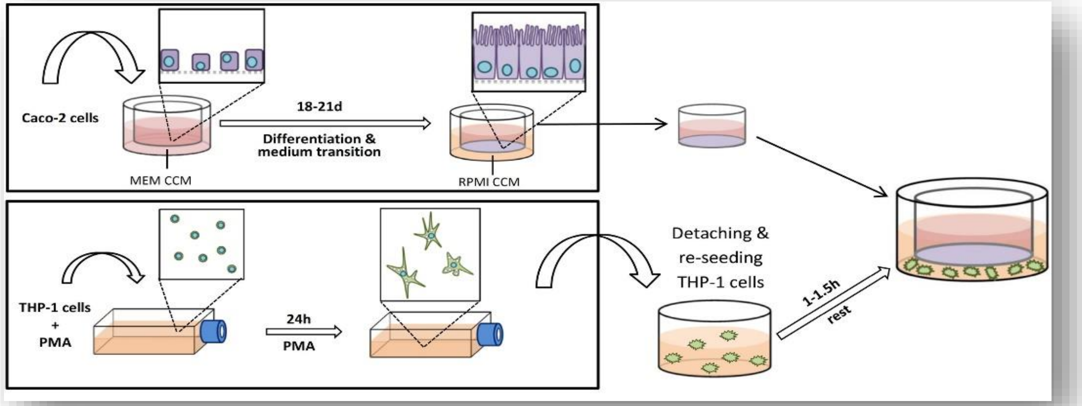




ICP-MS

INFLAMMATION INTESTINAL MODEL

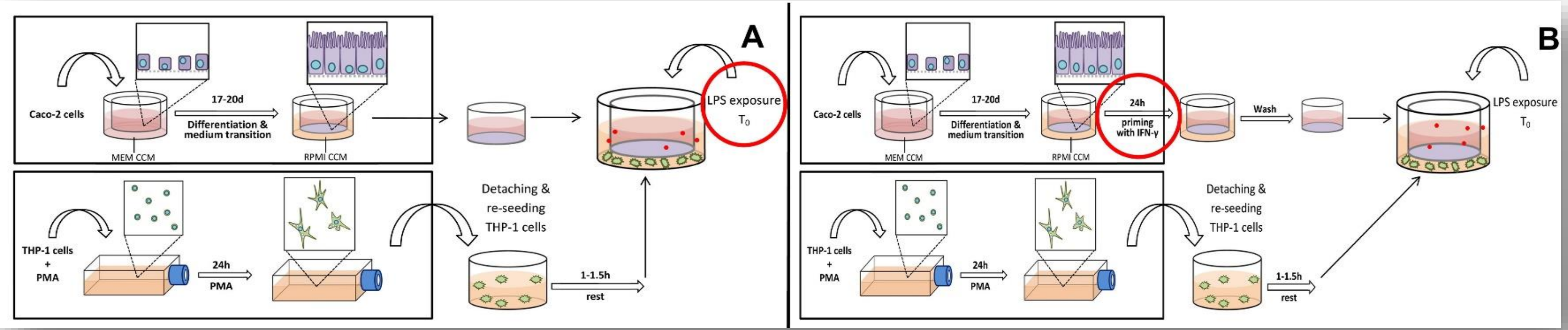
Stable physiological conditions



ENDPOINTS

- **Cytokine production**
- **NO production**
- **Inflammation effects on barrier integrity**

Controlled inflammation conditions




Toxicology in Vitro

Development of an *in vitro* co-culture model to mimic the human intestine in healthy and diseased state

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THANK YOU FOR YOUR ATTENTION

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