*International Workshop on Gap Analysis and Data Requirements to support Test Guideline and Guidance Document Development*

NanoHarmony

# DATA GAPS IDENTIFICATION RELATED TO INTESTINAL FATE OF INGESTED NANOMATERIALS

*Closing Plenary, November 5, 2020*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

# Data gaps identification related to intestinal fate of ingested nanomaterials

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## Tuesday 3rd November 15:00 - 17:00 (CET)

Chair: Isabella De Angelis (Italian National Institute of Health, Italy)
Rapporteur: Tommaso Serchi, (LIST-Luxemburg)

**Agenda**

15:00 Welcome and Introduction to Sessions (Isabella De Angelis)

15:10 Gap analysis approach and preliminary results: acellular model (Luisana Di Cristo)
15:20 Gap analysis approach and preliminary results: cellular model (Isabella De Angelis)
15:30 Gap analysis approach and preliminary results: analytical techniques (Federico Benetti)
15:40 Gap analysis approach and preliminary results: *in vitro/in vivo* correlation (Luisana Di Cristo)

15:50 Discussion on data gap analysis (see questions below)
16:45 Summary notes (Tommaso Serchi)
17:00 End of the session

**33 EXPERTS JOINED THE SESSION**



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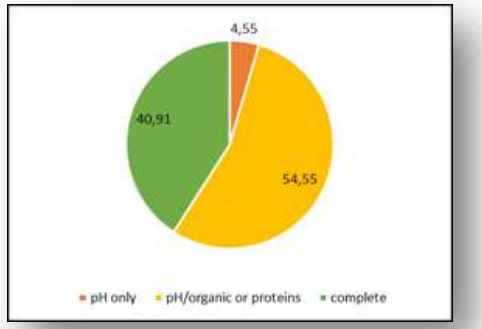
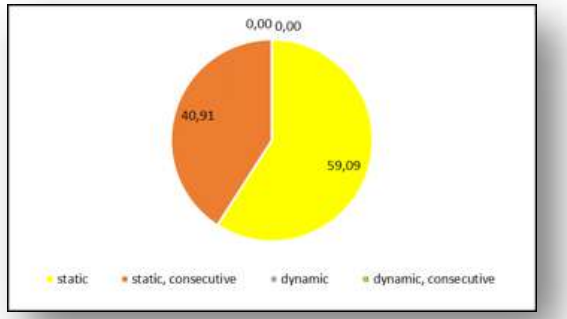
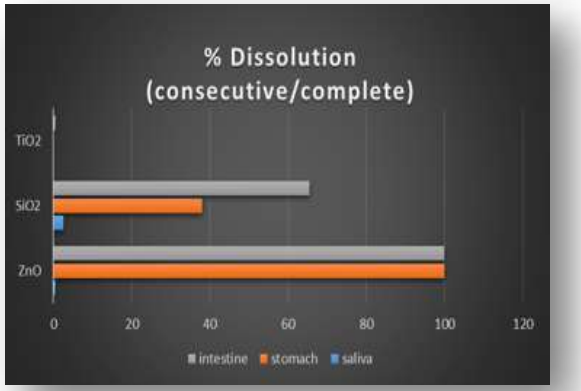
## 1. ACELLULAR MODEL CRITICAL GAP ANALYSIS OF STATE-OF-THE-ART: MAIN RESULTS

### Simulant juice composition




### Assay type




**ZnO quick dissolving (ca. 100% in stomach/intestine cond.) = high dissolution rate**

**Amorphous $SiO_2$ moderate dissolving (60-100% in stomach/intestine cond.) = medium dissolution rate**

**$TiO_2$ slow dissolving (0,4-1%, biodurable material) = low dissolution rate**

## MAIN GAPS EVIDENCED

- **Static/Consecutive set up with complete, simplified juices seems the gold standard method to have a clear depict of dissolution of ENMs, however more data are needed to substantiate;**
- **Few data on dissolution using simulant juice including food;**
- **Standard methods (for the preparation of GIT simulating juices) are widely applied, however no standardized/validated data on instrument set-up and relative methodologies.**



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## 2. CELLULAR MODEL CRITICAL GAP ANALYSIS OF STATE-OF-THE-ART: MAIN RESULTS

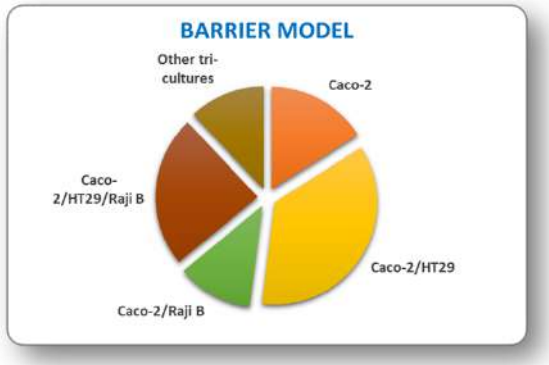
**Differentiated Caco-2 cells with different level of complexity (mono, bi or tri co-culture) were considered**

### Model characterization:

**Barrier integrity: TEER, LY/FITC passage, TJ (ZO-1)**

**Mucus production: Alcian Blue staining**

**M-cells identification: WGA, TEM**




### ANALYSIS FOCUSED ON 4 ENMs

### Zn, $SiO_2$, $TiO_2$, Ag

| ENMs | BARRIER INTEGRITY | UPTAKE | TRANS LOCATION |
|---|---|---|---|
| TiO2 | No effects | Low (adherent to apical membrane | Low |
| SiO2 | No effects | Low (associated brush border) | Very Low |
| ZnO | No effects | Not determined | Not determined |
| Ag | Only at 100 µg/mL after 96 h | Yes (time dependent) | Limited (similar to ions) |

### MAIN GAPS EVIDENCED

- Limited characterization and standardization of the co-culture model, particularly for M cells identification
- Lack of comparison between mono, di and tri-culture model in respect to ENM effects
- No studies conducted applying doses within the daily intake concentration range



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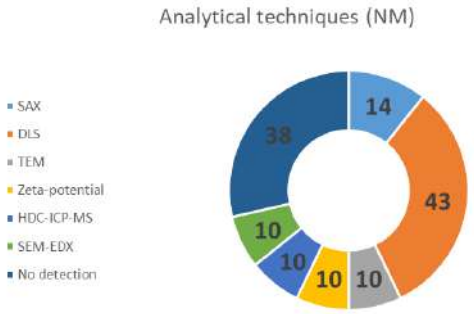
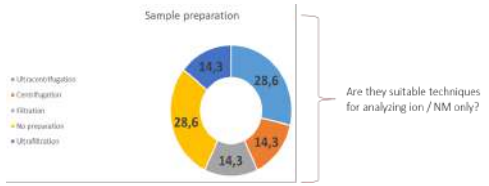
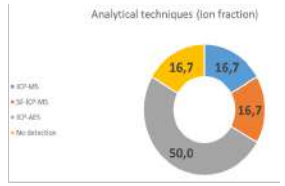
## 3. ANALYTICAL TECHNIQUES STATE-OF-THE-ART ANALYSIS: MAIN RESULTS

### ENMs – acellular model

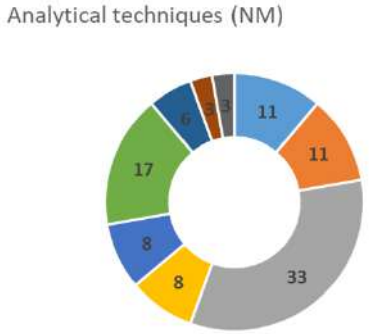


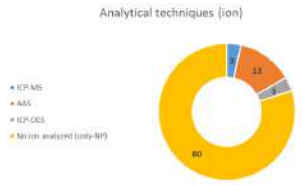

#### Ion fraction

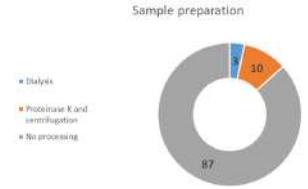



### ENMs – cellular model


## MAIN GAPS EVIDENCED

- Analytical approaches do not provide both number- and mass-based results;
- Sample preparations (e.g. (ultra)centrifugation, dialysis, ultrafiltration) do not separate precisely ENMs from ions;
- Analytical techniques are not suitable to distinguish between ions and ENMs, so under- or over-estimation can occur



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## 4. IN VIVO STATE-OF-THE-ART ANALYSIS AND *IN VIVO-IN VITRO* CORRELATION

**Focus on accumulation and clearance of the selected ENMs (Zn, $SiO_2$, $TiO_2$)**
**Biopersistance of ENMs is relevant for their biological effects and may impact on their long-term toxicity**
**The dissolution and biodegradation of ENMs are considered biodurability indicators**

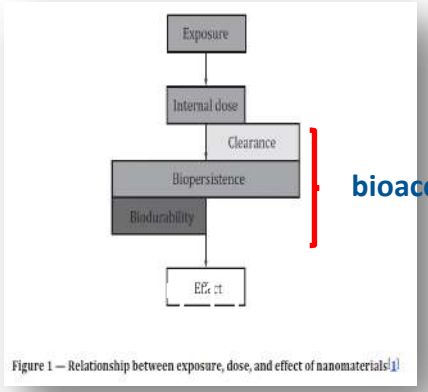
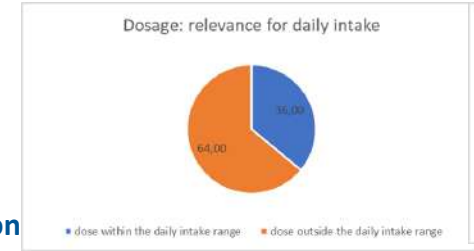
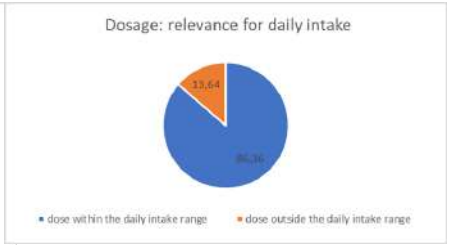


Figure 1 — Relationship between exposure, dose, and effect of nanomaterials[1]

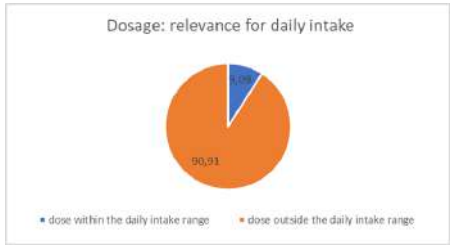
**In vivo model**




**Acellular model**




**Cellular model**




**Dosages of *in vivo*, acellular and cellular experiments are not comparable and sometimes not relevant for daily intake of selected ENMs**

### MAIN GAPS EVIDENCED

- **Feeding somministration (gavage instead of feeding exposure)**
- **Lack of relevance with the daily intake**
- **Few *in vivo-in vitro* correlation studies**



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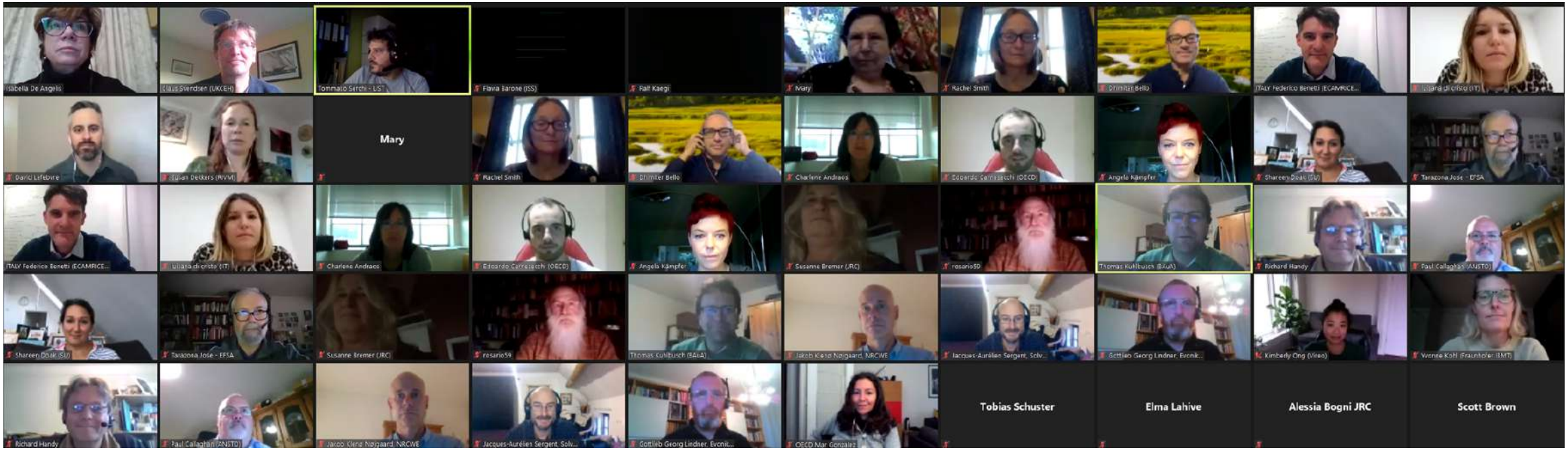


**ADDITIONAL SUGGESTIONS OR COMMENTS ARE WELCOME**

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**End of November**



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