Data requirements in Test Guideline and Guidance Document development for nanomaterials

NanoHarmony

DATA GAPS IDENTIFICATION RELATED TO INTESTINAL FATE OF INGESTED NANOMATERIALS

Webinar 16 December, 2020

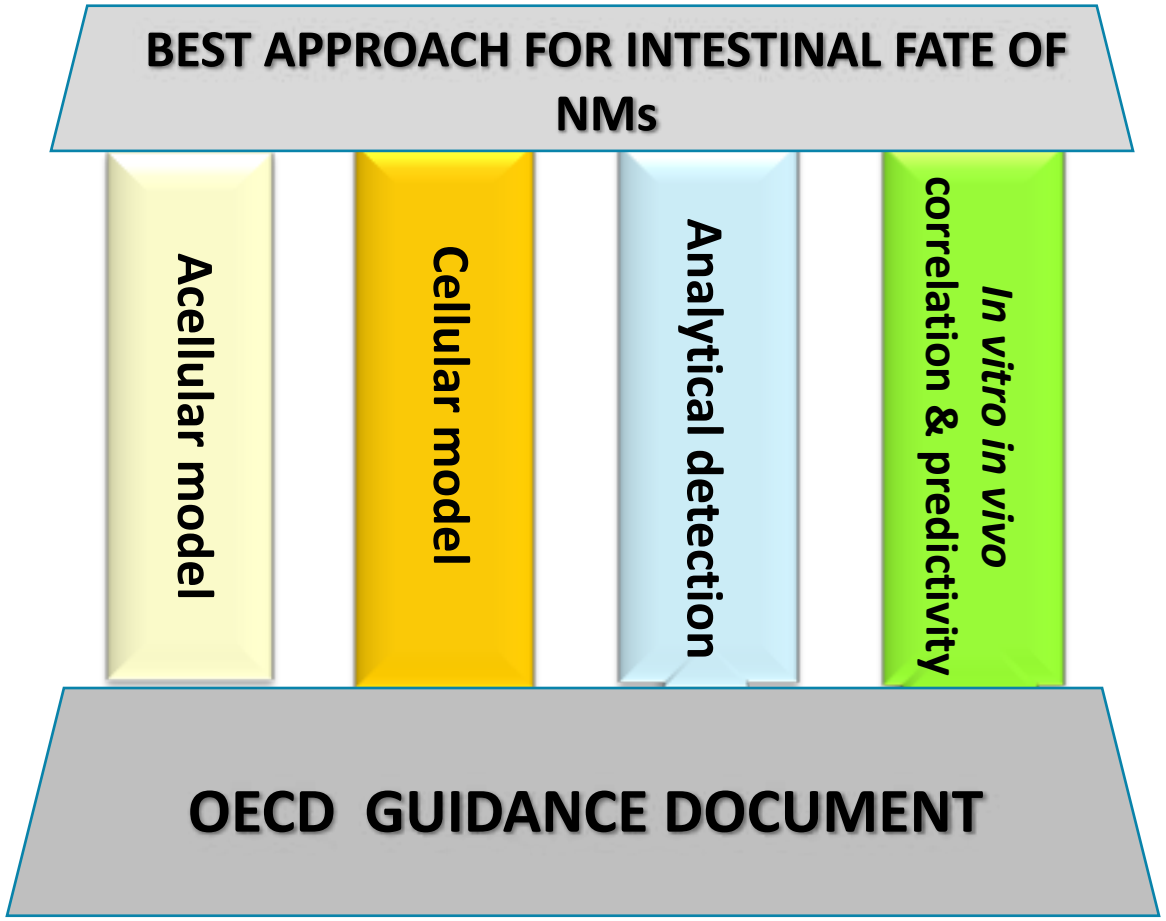
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Data gaps identification related to intestinal fate of ingested nanomaterials

NanoHarmony

WEBINAR AIMS

- ✓ Introduce the most relevant aspects discussed and the main feedbacks collected in the first Nanoharmony Expert Workshop
- ✓ Discuss additional feedback from the audience in order to obtain supplementary suggestions



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

NanoHarmony

CRITICAL ANALYSIS OF THE STATE-OF-THE-ART – METHODOLOGICAL APPROACH

SEARCH ANALYSIS via PubMed/google scholar

KEYWORDS oral ingestion, oral dissolution, Caco-2 and NPs, advanced *in vitro* barrier models and NPs, zinc oxide NPs, titanium dioxide NPs, silicon oxide NPs

YEAR RANGE Acellular model (2012-2020) Cellular model (2005 – 2020)

ANALYZED PAPERS (n ~ 25)

KEY PARAMETERS IDENTIFIED

ENM Physical Chemical properties (provider, size, shape, coating, material physical state, dispersant)

Acellular model (juice molecular compositions, instrument set-up: static, static consecutive, dynamic, dynamic consecutive)

Cellular model (cell line utilized, (co)-culture type, (co)-culture details & characterization, exposure time, endpoints)

ENM Dosage (acellular model: µg/mL to mg/mL or even higher) or **dose** (cellular model: µg/mL or µg/cm^2)

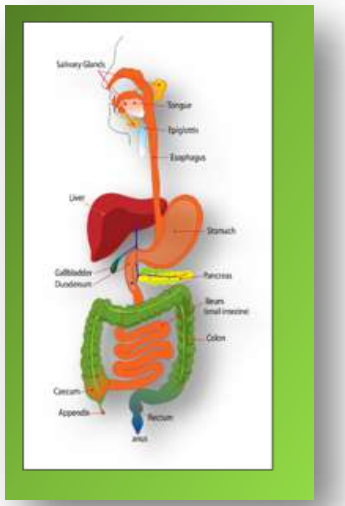
IN THE EXPERT MEETING METHODOLOGICAL APPROACH WAS DISCUSSED WITH THE EXPERTS AND AGREED WITH THEM

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

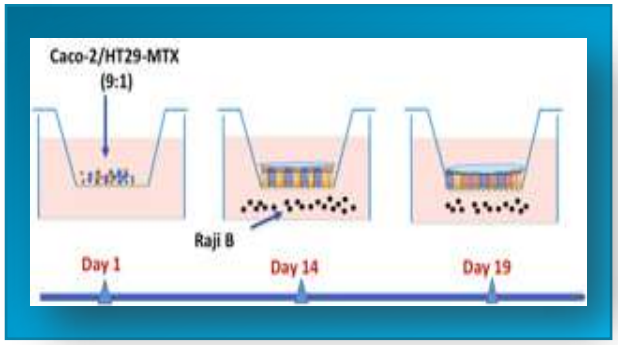
Data gaps identification related to intestinal fate of ingested nanomaterials

NanoHarmony

MAIN GAPS EVIDENCED - 1



- 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 needed to substantiate.
- Few data on dissolution using simulant juice including food.
- Standard methods (for preparation of GIT simulating juices) are widely applied, however no standardized/validated data on instrument set-up and relative methodologies are reported.



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

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

NanoHarmony

MAIN GAPS EVIDENCED - 2

- A large panel of techniques to quantify and characterize both qualitatively (presence/absence) and quantitatively (exposure) digested ENMs were identified.
- 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.

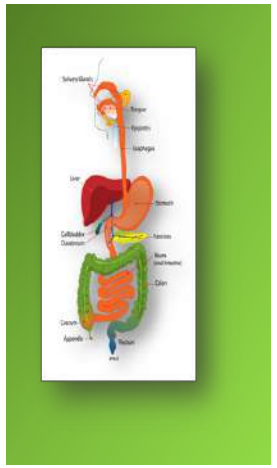
- Feeding somministration (gavage instead of feeding exposure)
- Lack of relevance with the daily intake.
- Very limited studies are found in literature for *in vitro/in vivo* correlation.
- Lack of diseased animal models.

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

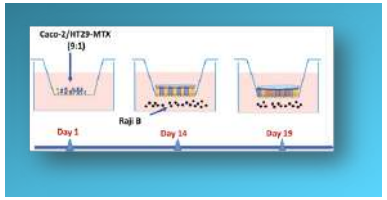
NanoHarmony

FEEDBACK FROM THE EXPERTS - 1



- Consecutive Protocol for *in vitro* simulated digestion is considered robust enough and could be considered as the gold standard. However, additional data for its standardization are still required.
- No studies using dynamic assay were found. Reasons of these data lack need to be further investigated
- Dissolution rate is a cross-cutting endpoint in NanoHarmony project. Clear definition and terminology harmonization of slowly, moderate quick, and quickly dissolving ENMs is need.

- Differentiated Caco-2 model can be considered as starting point since it has been extensively used & characterized and it is quite reproducible. However, Caco-2 cells need to be used under very well-defined conditions and some bias are inherent in the model (i.e. tumoral origin of the cells). This point have to be clearly reported in the GD.
- Bi-culture model of Caco-2/HT29 cells, due to the presence of mucus, could be a good model for EMNs uptake/translocation. To date, ENMs uptake/translocation data on this model showed a good reproducibility.
- Moreover, the choice of the model depends on specific purposes: some important information may be obtained only with the addition of lymphocityc/macrophagic cells (inflamed intestinal model).



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

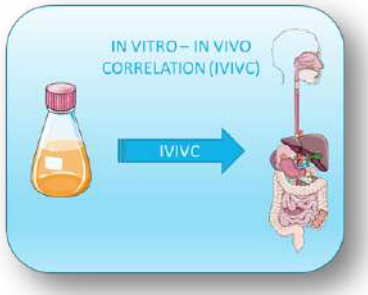
Data gaps identification related to intestinal fate of ingested nanomaterials

NanoHarmony

FEEDBACK FROM THE EXPERTS - 2

- Analytical techniques most frequently used for ENMs detection are DSL and ICP-MS, followed by SAX-HPLC
- The majority of the analyzed studies used only one analytical approach; multi-technical approach should be recommended.
- Importance of analytical data comparability and repeatability over time (some techniques may become obsolete). Again, the use of more than one analytical technique may be partially overcame this aspect.

- For *in vivo-in vitro* correlation link between experimental doses and daily intake is crucial. Generally, doses used in experiments with cellular models are not comparable
- Human GIT conditions are very different from experimental animals. For ENMs we have limited information on the capacity of animal models to predict the human situation. This aspect must be considered in the GD.
- In the GD the use of standard reference ENMs with a good *in vitro-in vivo* correlation should be suggested to take into account for lab-to-lab variability and changes in analytical sensitivity over time.



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

NanoHarmony

Thanks to all the experts who worked together

Please, use the chat for additional questions, clarifications, suggestions

isabella.deangelis@iss.it

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