

UNRAVELLING THE FATE OF EMERGING FOOD CONTAMINANTS IN THE GASTROINTESTINAL TRACT: A MULTIMODAL APPROACH FROM PFAS TO NANOPLASTICS

Marie Schuppe

PhD Student

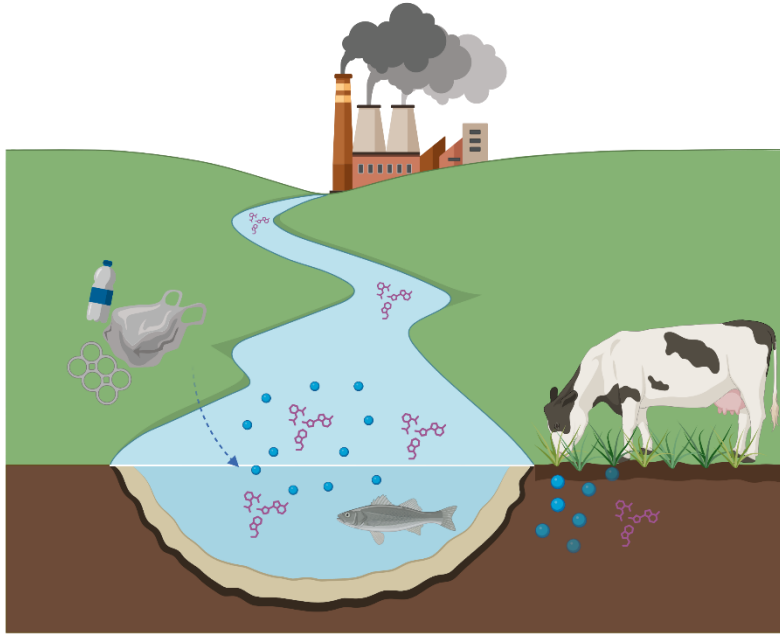
Advanced Instrumentation for Nano-Analytics (AINA)

Luxembourg Institute of Science and Technology (LIST)

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28/09/2026

CONTAMINANTS IN EVERYDAY LIFE



Animals



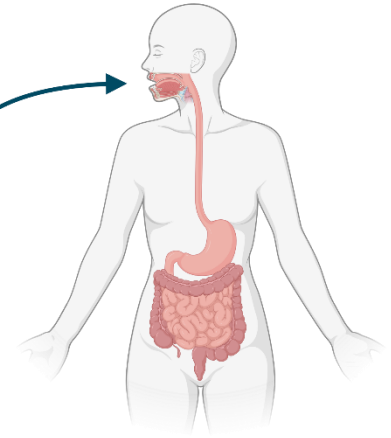
Seafood



Plants



Drinking water



- Physical particles: micro- and nanoparticles (e.g. TiO_2 , microplastics)
- Chemicals and substances (e.g. PFAS)
- Biological agents (bacteria, parasites, etc.)

WORKFLOW FOR THE INVESTIGATION THE IMPACT OF EVERYDAY CONTAMINANTS

The scientific expertise of the Luxembourg Institute of Science and Technology

- **Definition of an exposure case:** inhalation, ingestion, dermal contact, ...
 - Selection of the contaminants of interest
 - Selection of the appropriate *in vitro cell* model: pulmonary, intestinal, dermal, etc.
- **Toxicological assessment:** evaluation of cytotoxicity, metabolic functions, ...
- **Visualisation and localization at subcellular scale** of the contaminants in the biological system
- **Correlation of localization with toxicological impact**

THE CASE OF THE FOOD ADDITIVE E171

An example from study to European regulation



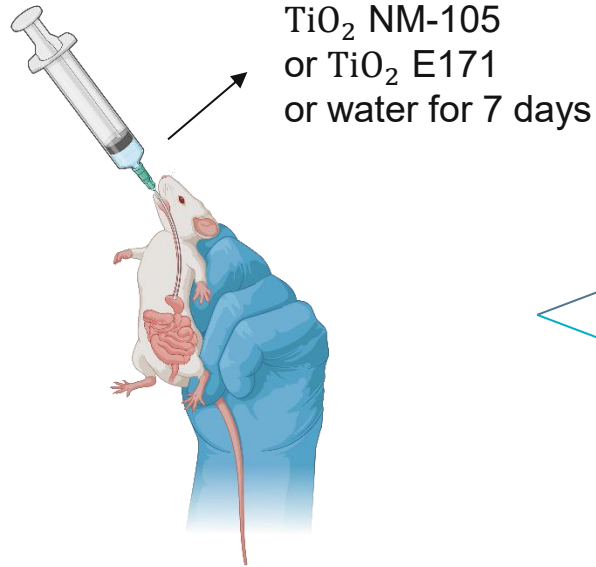
E171:

- former food-grade approved white pigment, made of titanium dioxide (TiO_2) nanoparticles
- [20 – 500 nm]

NM-105:

- TiO_2 nanoparticles, representative nanomaterial for the OECD tests on manufactured nanomaterials
 - [20 – 30 nm]
-
- Comparison of the impact of food-grade VS non food-grade TiO_2 nanoparticles
 - Use of *in vivo* models in collaboration with European partners: INRAE (FR), ANSES (FR), LIST (LU), EFSA (EU) ...

TOXICOLOGICAL ASSESSMENT AND LOCALIZATION OF THE CONTAMINANT



Toxicological assessment:

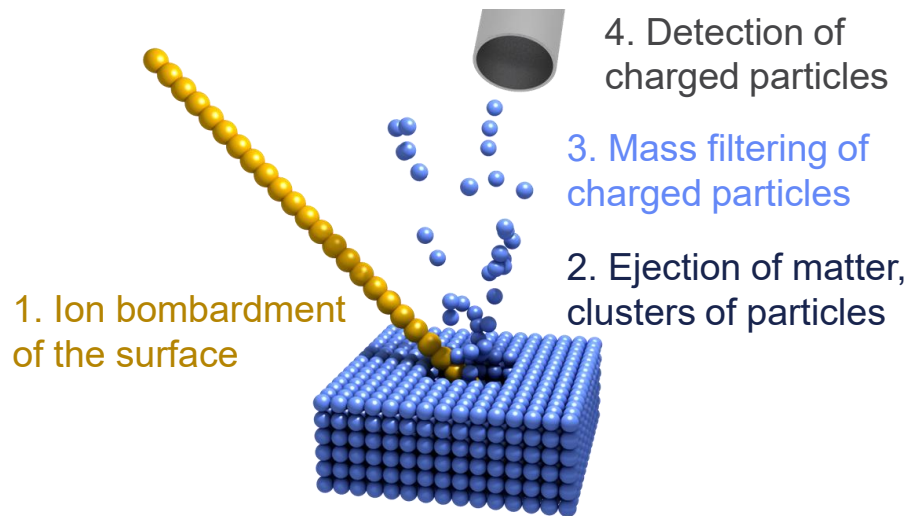
- Evaluation of cytotoxicity
- Immune response
- Inflammatory response
- Carcinogenesis

Morphological studies:

- Tissue imaging
- Inflammation
- Gut permeability
- Visualisation and localization of the contaminant

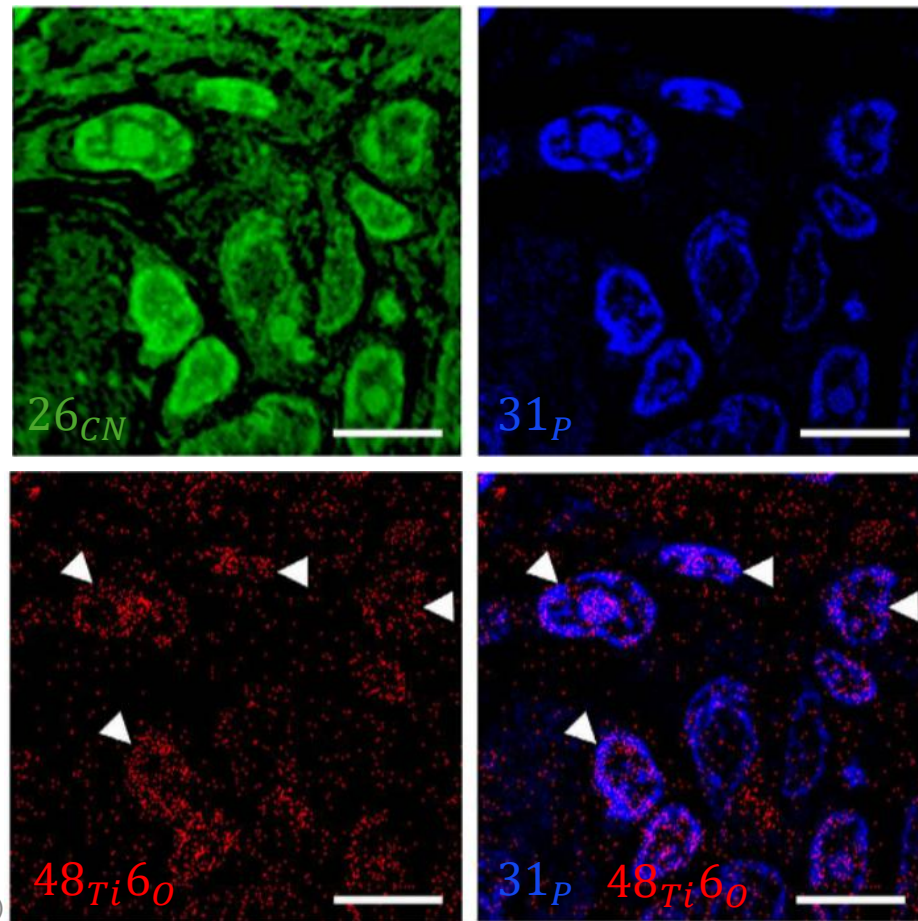
LIST CONTRIBUTION: VISUALISATION AND LOCALIZATION OF THE CONTAMINANT

Secondary Ion Mass Spectrometry (SIMS)



PhD course 2020, courtesy of Jean-Nicolas Audinot and Tom Wirtz

➤ Ion microscopy under high vacuum



NM-105 VS E171 NANOPARTICLES

Localization in tissue

- Similar accumulative behaviour for NM-105 and E171
- Localization of nanoscale contaminants in cells

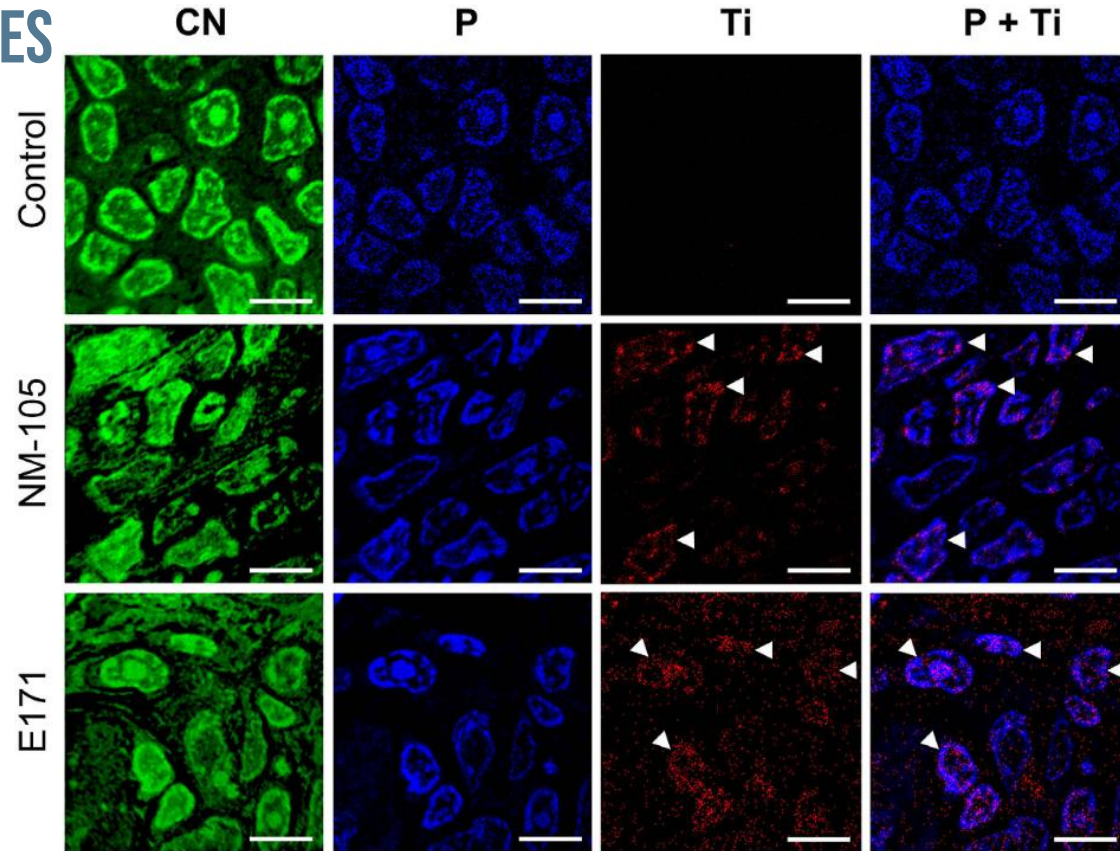


Figure 2. NanoSIMS analyses of subcellular Ti distribution in PP after 7 days of oral exposure to NM-105 or E171. The raster size was set to $20 \times 20 \mu\text{m}^2$. NanoSIMS images for the elemental distributions of carbon-nitrogen $^{12}\text{C}^{14}\text{N}$ (green), phosphorus ^{31}P (blue), and titanium oxide $^{48}\text{Ti}^{16}\text{O}$ (red) and the merged image of ^{31}P and $^{48}\text{Ti}^{16}\text{O}$ (blue/red) on ultra-thin sections of PP (scale bars, $5 \mu\text{m}$). The image overlay (P + Ti) shows Ti-rich zones in the nuclei of cells in PP (arrowheads).

Bettini, S. et al. Food-grade TiO_2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon.

Sci Rep 7, 40373 (2017). <https://doi.org/10.1038/srep40373>

THE CASE OF THE FOOD ADDITIVE E171

An example from study to European regulation

Toxicological assessment

- Immunity response (uptake and cellular type evolution)
- Inflammatory response
- Long-terms effects

Increase the risks for autoimmune diseases and colorectal cancer

Morphological study

- Similar accumulative behaviour for NM-105 and E171
- Localization of nanoscale contaminants in cells

European ban of the food additive E171 since August 2022



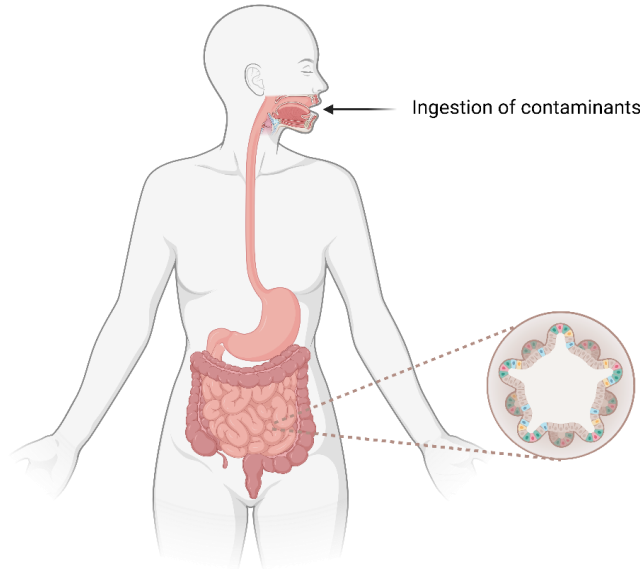
WORKFLOW FOR THE INVESTIGATION THE IMPACT OF EVERYDAY CONTAMINANTS

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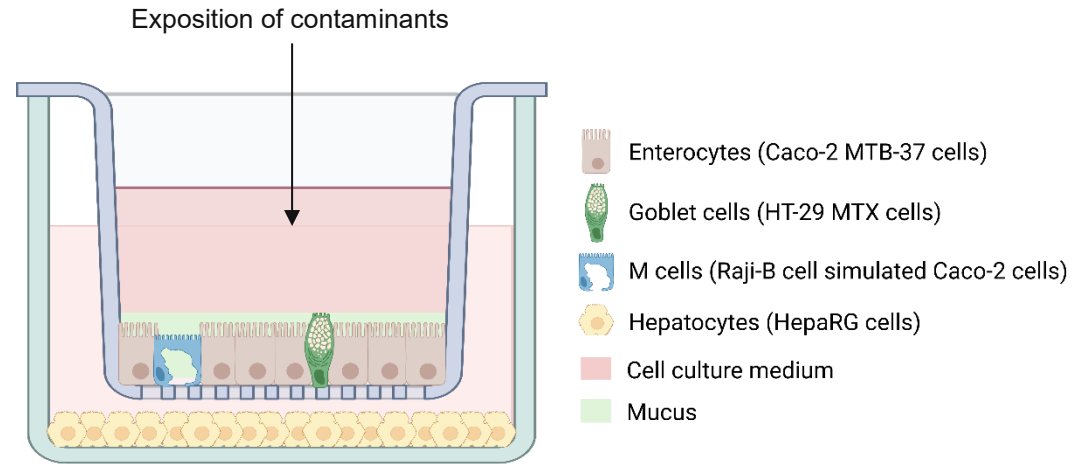
INVESTIGATION THE IMPACT CONTAMINANTS USING IN VITRO MODEL

Simulation of ingestion: focus on the intestinal cell barrier



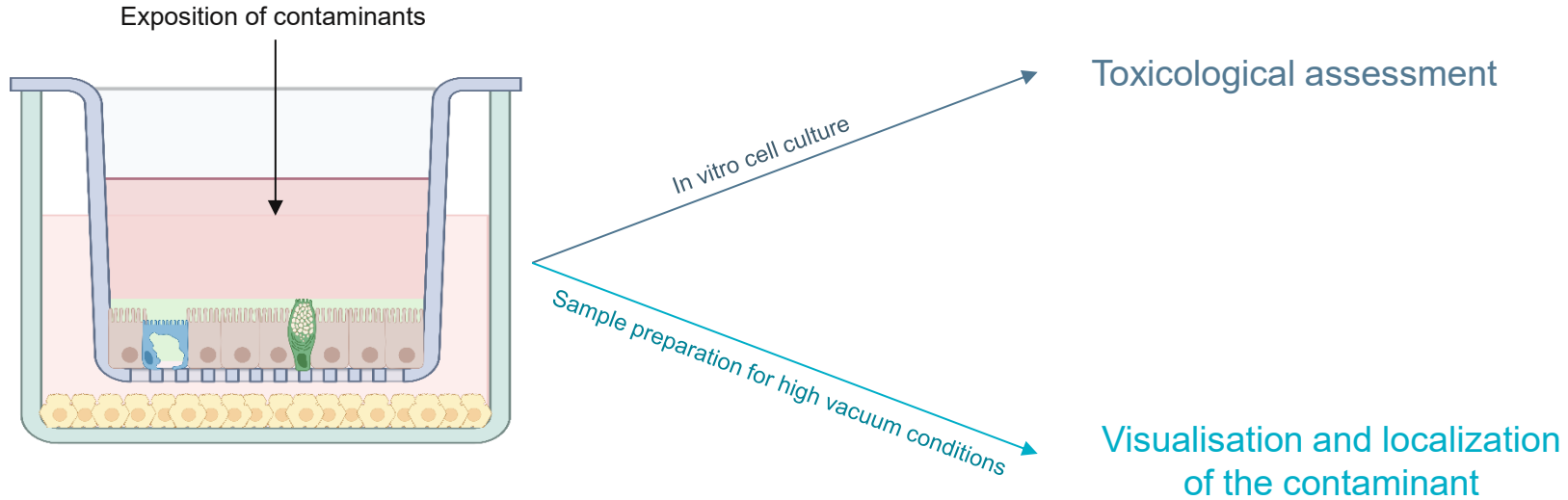
Created with BioRender.com

The intestinal cell barrier:
a highly **complex multilayered** system



Intestinal barrier **in vitro** cell model:
to mimic the complexity of the intestinal barrier

TOXICOLOGICAL ASSESSMENT AND LOCALIZATION OF THE CONTAMINANT



TOXICOLOGICAL ASSAY

Intestinal absorption, intracellular fate and toxicity

Assay	Measures ...	Higher signal indicates ...
Alamar Blue	Cell viability and metabolism	More viable cells, more or higher metabolic activity
ATP assay	Metabolic activity (respiration)	More cells or more metabolically active cells
LDH assay	Membrane damage	Greater cytotoxicity
DFCH-DA	Intracellular Reactive Oxygen Species (ROS)	Greater oxidative stress

- Toxicological assessment of the cell viability and response to the contaminants

THE CASE OF PFOA



Based on the work of Dr Charlotte Stoffels,
in the frame of the INTER-ANR-FNR FLUO-GUT project



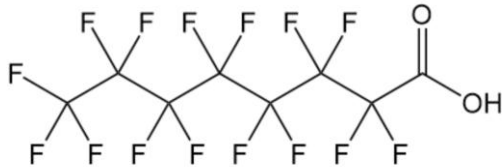
EMERGING CONTAMINANTS

PFAS: a persisting contaminant

PFAS

Family of over 10 000 man-made chemicals,
developed, used and known for their resistance to
water, heat and grease

- High chemical stability, the “forever chemicals”

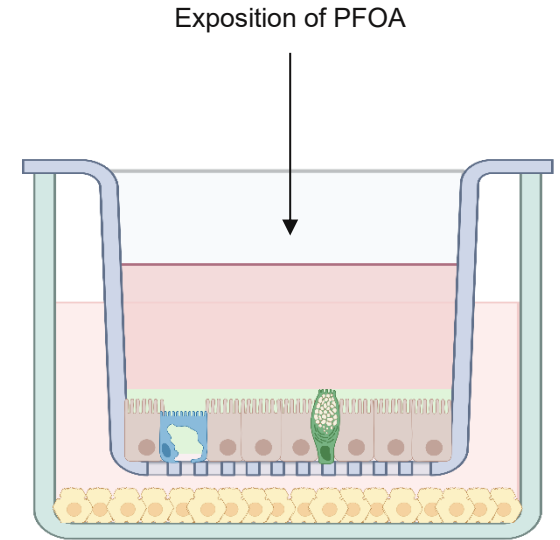


<https://www.fsawwa.org/page/PFAS>

INVESTIGATION OF THE IMPACT OF PFAS: INGESTION

Simulation of the ingestion of PFOA

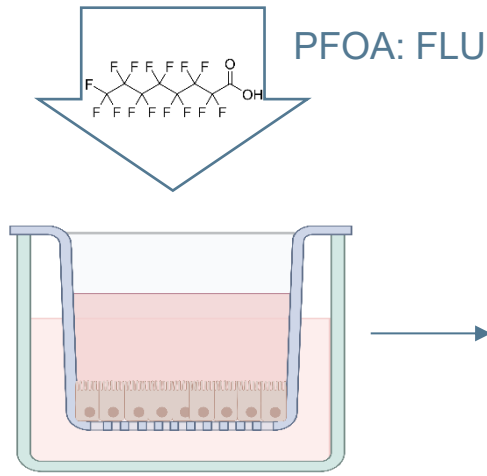
- Factory and treatment sites releasing PFAS in water
 - Migration of fire foam in soil and groundwater
 - Migration of coating from food packaging
 - ...
- Exposition to PFAS via **contaminated water or food**
- Scenario of **ingestion**, choice of **intestinal *in vitro* model**
- Exposition to **perfluorooctanoic acid (PFOA)**



Intestinal barrier *in vitro* cell model

LOCALIZATION OF THE PFOA IN THE CELLULAR MODEL

Toxicological assessment of the impact of PFOA

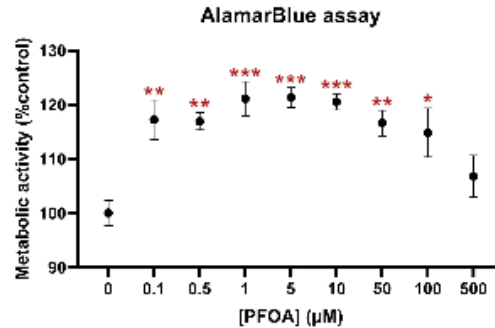


Enterocytes (Caco-2 MTB-37 cells)

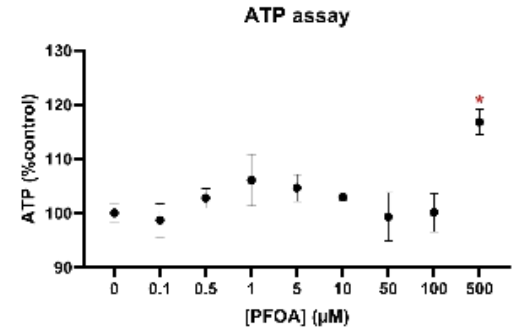
Cell culture medium

Stoffels C.B.A. et al. When subcellular chemical imaging enlightens our understanding on intestinal absorption, intracellular fate and toxicity of PFOA in vitro. JHazMat 480, 136205 (2024). <https://doi.org/10.1016/j.jhazmat.2024.136205>

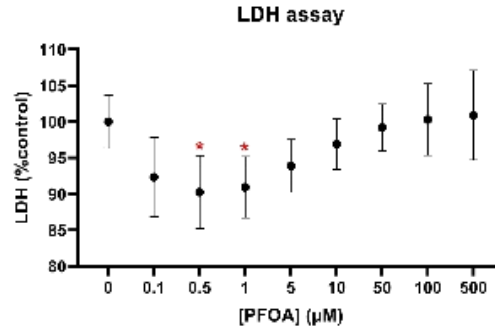
A



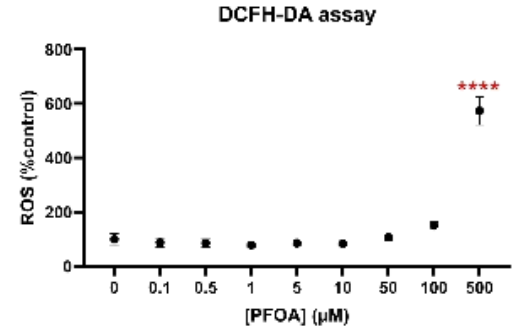
B



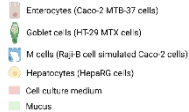
C



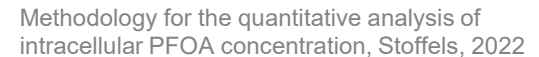
D



Localization of PFOA at subcellular scale cells using SIMS



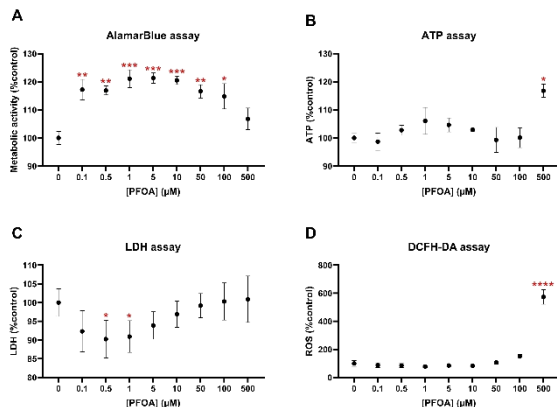
- Fixation with glutaraldehyde
- Post-fixation, staining with Osmium
- Dehydration
- Resin embedding
- Microtome cutting
- Mounting on stage
- EM + SIMS for correlative images



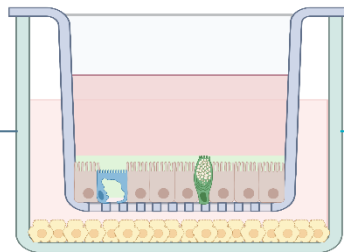
FLUO-GUT
Imaging toxicity

CORRELATION BETWEEN TOXICOLOGY AND LOCALIZATION OF THE CONTAMINANT

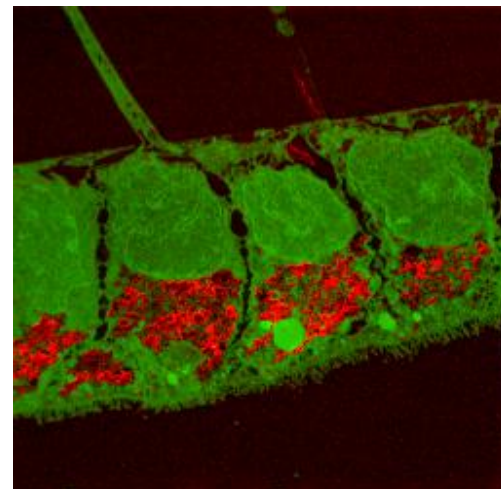
Toxicological assay



Insights of cell metabolic activity and viability



Secondary Ion Mass Spectrometry



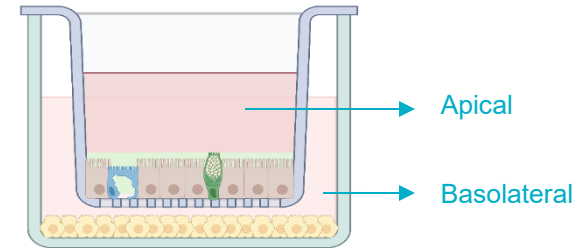
Localisation of the contaminants at the subcellular level

**Correlation
localization and
toxicological impact
of the contaminants**

CORRELATION BETWEEN TOXICOLOGY AND LOCALIZATION OF THE CONTAMINANT

Exposition of intestinal barrier *in vitro* cell model to PFOA: key find outs

- PFOA detected in the basolateral medium, indicating and absorption by the intestinal cells
- Accumulation within the intestinal cells, mainly in the cytosol
- Toxicological response from cells after exposition to PFOA



No clear toxic effect of PFOA showed by toxicological assessment, but uptake of PFOA by the intestinal cells and accumulation inside the cytosol demonstrated by mass spectrometry analysis

THE CASE OF MICRO- AND NANOPLASTICS



On-going work

In the frame of the INTER-ANR-FNR microPLAstox project



EMERGING CONTAMINANTS

Microplastics: a new focus of persisting contaminants

Microplastics and nanoplastics

Plastics particles smaller than 5 mm (micro) or smaller than 1 μm (nano), either manufactured or resulting from the degradation of bigger plastics

- Plastics are widely spread due to their resistance and durability



Microbeads in cosmetics products



Polymer pellets used for food packaging

Primary microplastics
(Intentional size)



Microfibers released during the washing



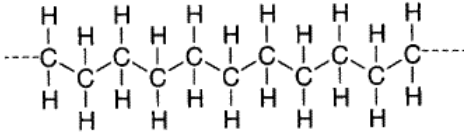
Plastics waste degrading in the environment

Secondary microplastics
(Resulting from degradation)

LOCALIZATION OF THE NANOPLASTICS IN THE CELLULAR MODEL

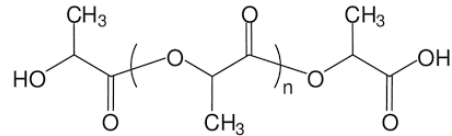
How plastics can be localized in cellular model ?

Polyethylene (PE)



Sciencehistory.org, 2026

Polylactic acid (PLA)



Bioplasticsnews.com, 2026

Chemical composition:
C, H, O ...

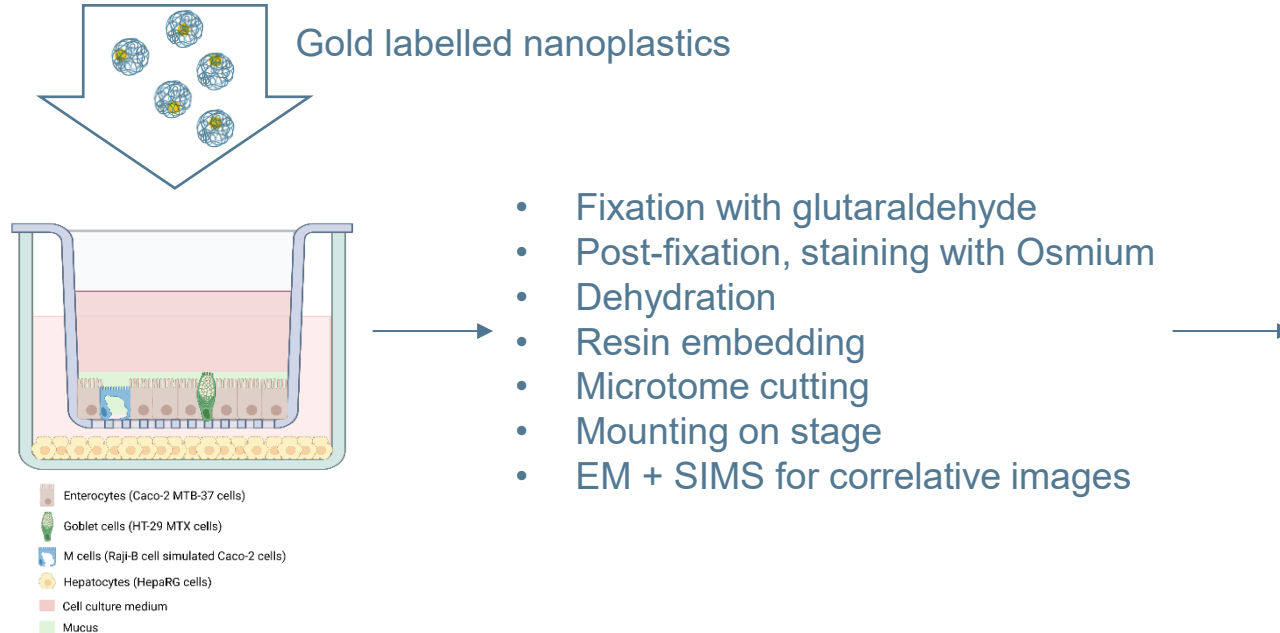
- No chemical difference with the biological background
- Looking for carbon specie inside a carbon matrix
- No native label in the contaminant

➤ Need for a label to localize the nanoplastics

Gold labelling of the nanoplastics

LOCALIZATION OF THE NANOPLASTICS IN THE CELLULAR MODEL

Localization of nanoplastics in the cells using gold labels



**Gold labelling:
localization of the
nanoplastics in the *in
vitro* cell models**

- Enterocytes (Caco-2 MTB-37 cells)
- Goblet cells (HT-29 MTX cells)
- M cells (Raji-B cell simulated Caco-2 cells)
- Hepatocytes (HepaRG cells)
- Cell culture medium
- Mucus

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et alimentaire



Université du Luxembourg



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- Dr Sébastien Cambier
- Dr Charlotte Stoffels
- Dr Jean-Sébastien Thomann
- Dr Antje Biesemeier

Université du Luxembourg supervision

- Dr Emma Schymanski

Toxalim (Toulouse, France)

- Dr Muriel Mercier-Bonin
- Dr Hervé Robert

THANK YOU FOR YOUR ATTENTION !

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