



Innovative *in vitro* tests to evaluate the impacts of nanomaterials

A SAFE BY DESIGN CONCEPT

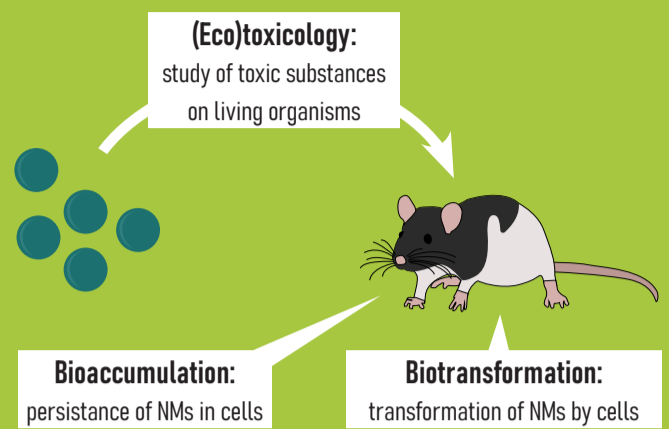
1 During the design of a new nano-based product **its performances** are tested at each step. If they are **efficient**, toxicology tests can be performed **2**. Otherwise, the product has to be modified **3**.

If the product passes toxicology tests, it proceeds to the next step. Otherwise, it has to be modified **4**.



TOXICOLOGICAL EVALUATION

The safe by design approach implies a solid knowledge of the **interactions** between **nanomaterials (NMs)** and **living organisms**:



HOW TO EVALUATE TOXICOLOGICAL IMPACTS OF NANOMATERIALS?

In vivo tests

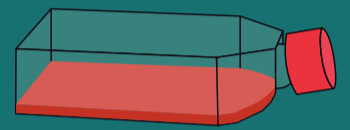
A substance is injected in mice/rats. **After an exposure of several weeks**, its effects on animals are analyzed.



- ✓ Impacts of the tested substances on **an entire organism**
- ✓ **Relevant** to predict human responses

In vitro tests

Human cells are developed and **exposed for several hours** to a substance, its impacts are then analyzed.



- ✓ **More ethical**
- ✓ **Faster**
- ✓ **Less expensive**
- ✗ **Potential problems with relevance** (nature and lengths of exposure)

OBJECTIVES

Limiting *in vivo* tests, doing more representative *in vitro* tests, expose cells to tested substances and analyze their effects **over several days/weeks**.

Exploring how *in vitro* systems can be used to investigate:

- ▣ the **persistence of biological effects**,
- ▣ the **bioaccumulation** of nanomaterials in cells,
- ▣ the **biotransformation** of nanomaterials by cells.

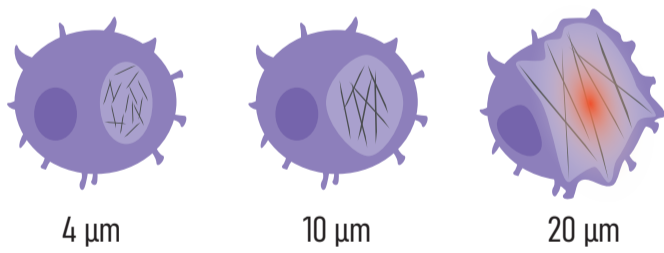
SOME RESULTS OF THE PROJECT

Representative *in vitro* tests for pro-inflammatory effects

In the literature, ***in vivo* tests** have been experimented. Mice have been exposed to silver nanowires of various lengths (4, 10 and 20 μm) during **28 days**.

These experiments have been compared to ***in vitro* tests**: mice macrophages (a cell responsible for the destruction of foreign substances) have been exposed to the same nanowires.

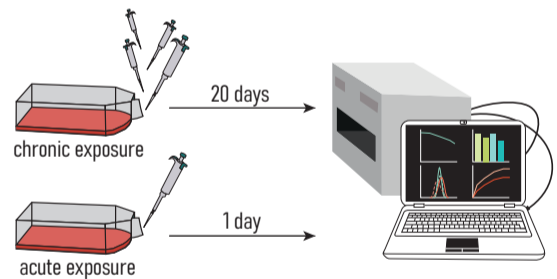
The **pro-inflammatory responses** of the cells have been investigated **immediately and 36 and 72 hours** after exposure.



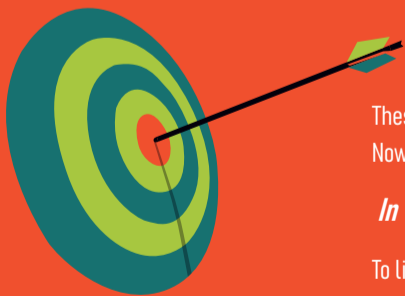
The obtained results are in line with those obtained using *in vivo* system and suggest that ***in vitro* models** can be **predictive for the pro-inflammatory potential of nanomaterials**.

Long term vs. short term effect of nanoparticles (NPs)

Macrophages have been exposed to either **1 $\mu\text{g}/\text{mL}$ silver nanoparticles daily for 20 days**: a chronic exposure scheme, or to **20 $\mu\text{g}/\text{mL}$ silver nanoparticles once for one day**: an acute exposure scheme.



Chronic exposure to silver NPs **induces stronger effects** than acute exposure on the metabolic and functional parameters of macrophages.



These results show that **innovative *in vitro* experiments can be predictive and can limit tests on animals**. Now, *in vitro* tests can efficiently reduce *in vivo* tests.

***In vitro* tests have been successfully extended** to longer exposure periods.

To limit the pro-inflammatory effects on macrophages, **nanowires under 20 μm appear to be a better choice**.

LIFE CYCLE STAGES STUDIED

