# Towards an integrated risk assessment of micro- and nanoparticles

## Planarians as an alternative *in vivo* model to link particle characteristics and uptake dynamics to adverse outcomes

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## CONTEXT & BACKGROUND

- **We cannot escape from micro- and nanoparticles (MNPs)!**
- Within our current world these tiny particles are **widely spread in consumer products**, e.g. added in paints, cosmetics, textiles, wound bandages or **originating from degrading plastics.**
- Due to **poor waste management** they end up in our environment, exposing humans and other organisms, resulting in several adverse effects, including **neurotoxicity**.
- There is a **wide variety in MNPs characteristics**: variation in type (i.e. silver, titanium, silica or plastics such as polystyrene, propylene, polyethylene…), size (i.e. micro vs nanoscale), shape (i.e. sphere, fiber, fragment…), surface group (i.e. positive, negative or neutral) …

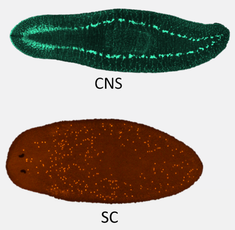
## PROBLEM DEFINITION & STUDY AIM

- Proper risk assessment of MNPs is difficult due to the wide variety of physicochemical properties, leading to complex particle behavior, resulting in different toxicological effects.
- Currently, it is unknown (1) which particle characteristics determine cellular uptake and tissue localization, (2) which of these properties induce specific adverse outcomes and (3) how they can be correlated with detailed mechanistic insights into their toxicity.
- **Therefore, we aim to obtain an integrated risk assessment of MNPs, linking particle characteristics and uptake dynamics to adverse outcomes, with a specific focus on neurotoxicity.**

## MODEL ORGANISM

**Planarians as an alternative *in vivo* toxicological model**

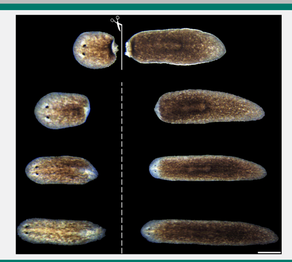
*Schmidtea mediterranea*




**Unique properties:**

**Extremely high regenerative capacity**: a large pool of **pluripotent stem cells** (SC) allows to regenerate a fully functional body within 7-10 days, including the **central nervous system** (CNS).

➔ We are able to study (neuro)developmental toxicity **in an *in vivo* context**.



## EXPERIMENTAL DESIGN

**Spherical polystyrene particles (PS)** of different sizes:

- PS50: 50 nm
- PS200: 200 nm
- PS01: 1 µm
- PS02: 2 µm

Exposure via planarian medium →

- **Particle characterization** | TEM – DLS – SLS
- **Particle fate** | Colocalization via confocal imaging
- **Particle effects: neurodevelopmental toxicity** | Developmental success, CNS structure, involved cell types, underlying SC dynamics and affected pathways.

**Integrative approach**

We also studied the toxicity of **silver** and **titaniumdioxide NPs** using planarians as an *in vivo* model.

*Interested?*
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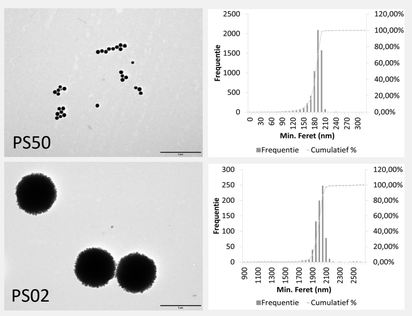
## PARTICLE CHARACTERIZATION



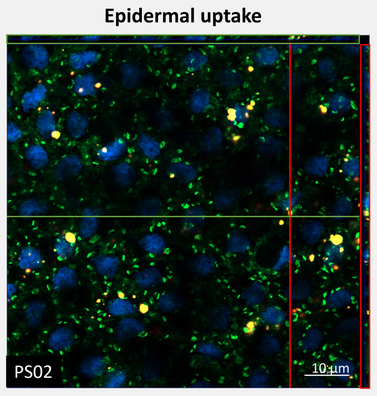

**Size distribution of PS-MNPs based on TEM**
Left: representative TEM images, Right: size distributions

| | TEM | DLS (Ultra-pure water) | | | DLS (Planarian medium) | | |
|---|---|---|---|---|---|---|---|
| **PS nano-particles** | **Feret min (µm)** | **Z-average (nm)** | **PDI** | **Z (mV)** | **Z-average (nm)** | **PDI** | **Z (mV)** |
| PS50NF | | 62.66 | 0.077 | -29.92 | 62.77 | 0.137 | -7.302 |
| PS50RF | | 49.42 | 0.09 | -23.79 | 53.485 | 0.142 | -5.55 |
| PS200NF | 0.182 | 220.4 | 0.025 | - 48.86 | 217.25 | 0.014 | -21.14 |
| PS200RF | 0.180 | 216.5 | 0.014 | -40.39 | 214.25 | 0.028 | -23.765 |
| | **TEM** | **SLS** (Ultra-pure water) | | | **SLS** (Planarian medium) | | |
| **PS micro-particles** | **Feret min (µm)** | **D10 (µm)** | **D50 (µm)** | **D90 (µm)** | **D10 (µm)** | **D50 (µm)** | **D90 (µm)** |
| PS01NF | | 0.856 | 1.08 | 1.39 | 0.809 | 1.22 | 3.08 |
| PS01RF | | 0.796 | 0.977 | 1.20 | 0.778 | 1.05 | 8.35 |
| PS02NF | 1.988 | 1.67 | 2.01 | 2.42 | 1.58 | 2.10 | 2.79 |
| PS02RF | 2.073 | 1.66 | 2.64 | 4.40 | 1.73 | 2.23 | 2.87 |

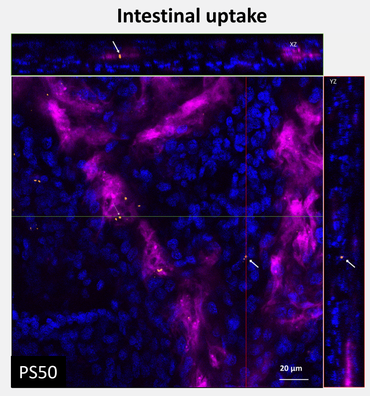
**Physical characteristics of PS-MNPs based on TEM, DLS and SLS measurements**
Z-average: mean-intensity based diameter, PDI: polydispersity index, Z: zeta potential

## PARTICLE FATE

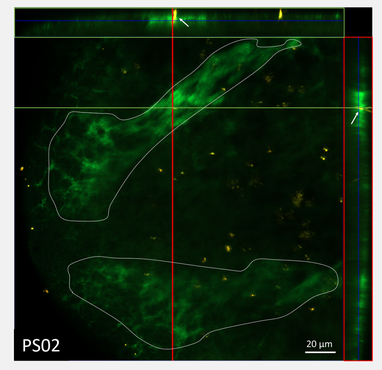
**Epidermal uptake**




**Intestinal uptake**

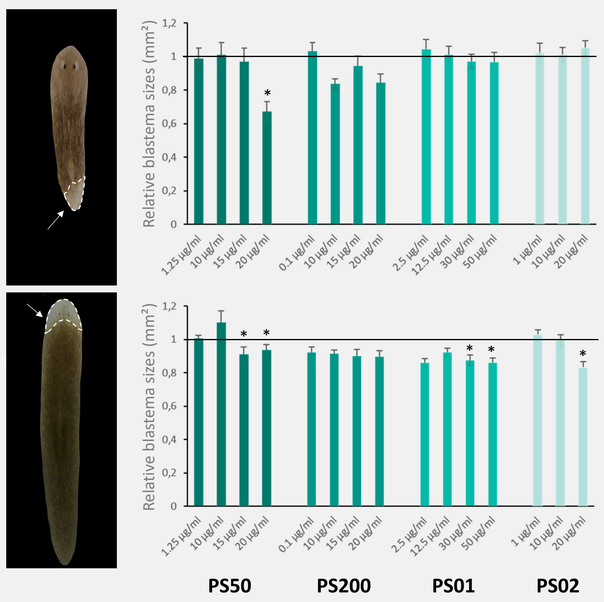



**Uptake close to neuronal structures**



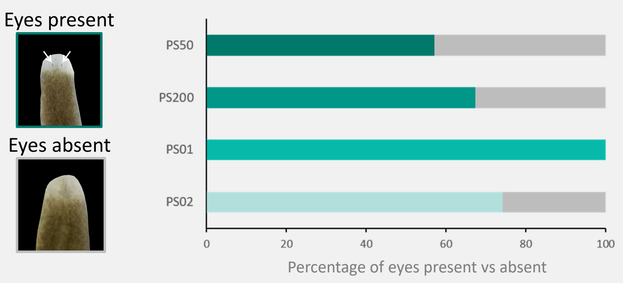

## PARTICLE EFFECTS

### 1. Impaired developmental succes: decreased tissue growth



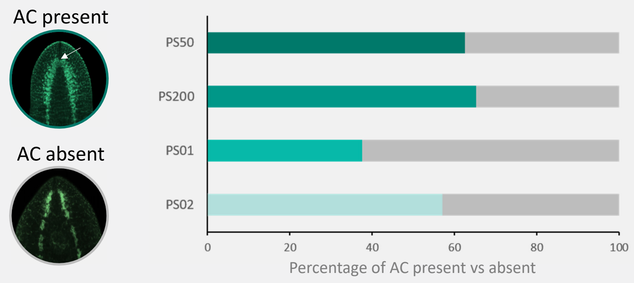

**The size of tissue growth (blastema) was determined in heads and tails at 7 dpa.** The concentrations used are indicated on the x-axis. Error bars represent standard errors. Statistical significance: * $p < 0.05$.

### 2. Impaired developmental succes: delayed eye development



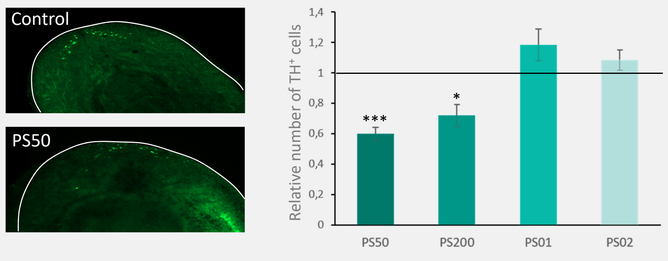

**The presence or absence of eyes was scored in the head region of 7 dpa tail fragments.** The concentrations used were 20 µg/ml for PS50, PS200 and PS02 and 50 µg/ml for PS01.

### 3. Affected CNS structure: delayed AC formation



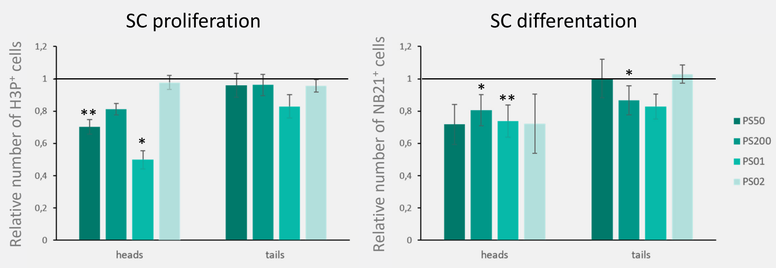

**The presence or absence of an anterior commissure was scored in the head region of 10 dpa tail fragments.** The concentrations used were 20 µg/ml for PS50, PS200 and PS02 and 50 µg/ml for PS01.

### 4. Decreased formation of dopaminergic neurons




**The number of TH-positive cells was quantified in the head region of 5 dpa tail fragments.**
The concentrations used were 20 µg/ml for PS50, PS200 and PS02 and 50 µg/ml for PS01. Error bars represent standard errors. Statistical significance: * $p < 0.05$ and *** $p < 0.001$.

### 5. Impaired SC dynamics




**The number of H3P and NB21-positive cells was quantified in heads and tails at 7 dpa.** The concentrations used were 20 µg/ml for PS50, PS200 and PS02 and 50 µg/ml for PS01. Error bars represent standard errors. Statistical significance: * $p < 0.05$ and ** $p < 0.01$.

## CONCLUSION AND OUTLOOK

**STUDY HIGHLIGHTS:**

- A detailled physicochemical characterization for the studied PS particles was obtained.
- PS particles were taken up in the epidermis, intestine and near neuronal structures.
- PS particles of different sizes impaired developmental success and the CNS system.
- Larger particle sizes led to a delay in eye formation, while smaller particle sizes affected the dopaminergic neurons.
- Underlying SC proliferation and differentation were decreased.

**An in-depth understanding of MNPs toxicity is only possible by studying their induced effects, while taking the wide range of MNPs properties into account.**

**➔ This study provides a first step towards proper risk assessment of MNPs, using planarians as *in vivo* alternative model to integrate particle characteristics with uptake dynamics and adverse outcomes to obtain detailed toxicological insights.**

## INTERESTED?

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