

What do we know about the early life biological effects of MNPs?

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Tiered toxicity testing in placenta and developmental models

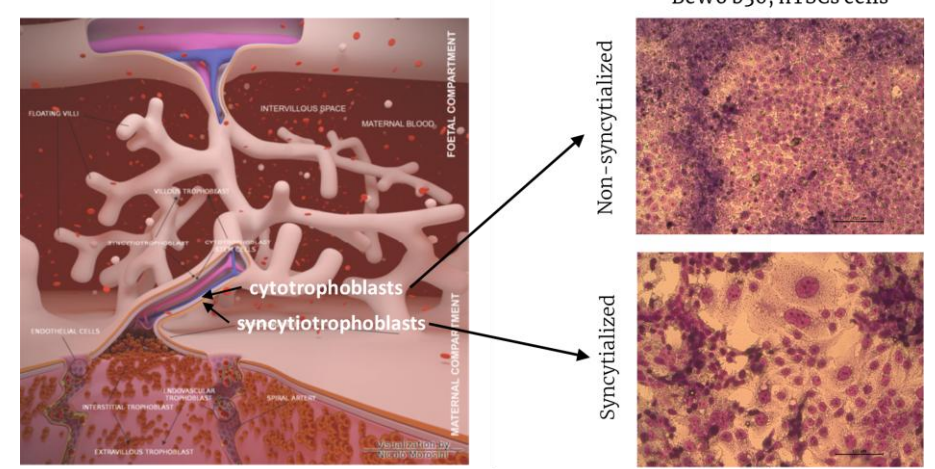
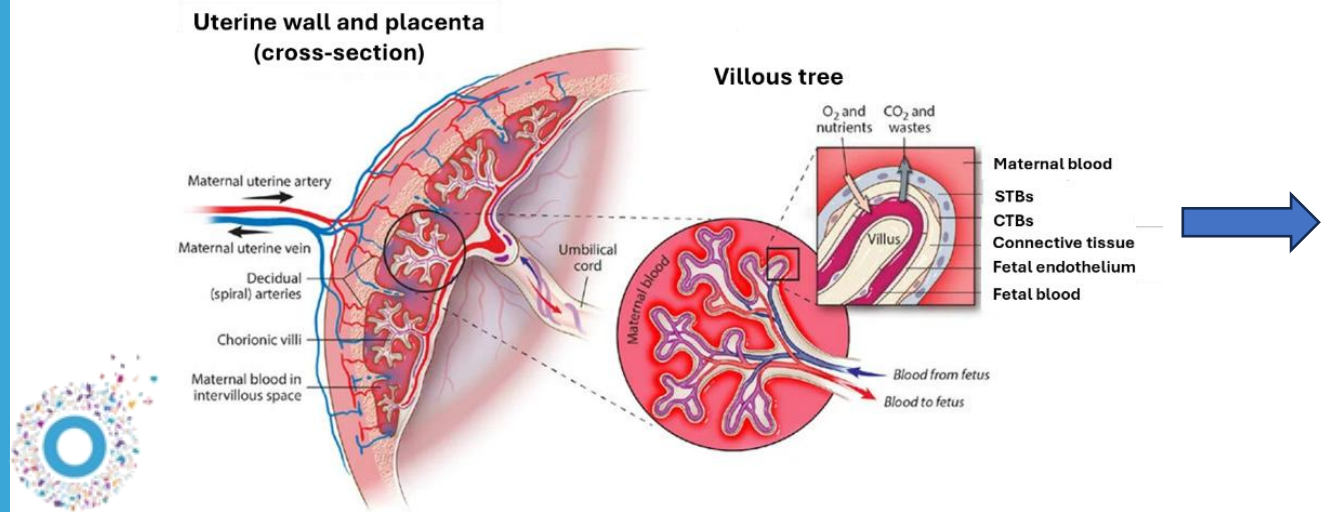


- Uptake
- Transport
- Toxicity
 - Cytotoxicity
 - Membrane integrity
 - Oxidative stress
 - Genotoxicity

- (Pro)inflammatory markers
- Transcriptomic profiles
- Cellular senescence
- Transport in co-cultures
- Steroidogenesis
- Functional markers

- Transplacental transfer
- Placental tissue toxicity markers

- Zebrafish embryo
- C. elegans
- Mouse embryonic stem cells



TIER 1 MNPs

- 33 unique particles
- Fluorescent
 - PS, PVC, PET, PA, PMMA
- Pristine
 - PS, PVC, PET, PA, PMMA, HDPE, PE, PS-AMINO, PS-CRX
- UV-weathered
 - PS, PVC, PET, PA
- Size-range
 - From 50 nm to 10 μm

Source	Identification Code	Polymer abbrev.	Pristine or fluorescent	Shape	Size/size-range (D10-D90, μm)	Synthesis method	Endotoxins (LOD=0.01 ng/mL)	Additives/ surfactans
Commercial	PS_0.05	PS	Pristine	sphere	0,05 [^]	bottom-up	<LOD	0.6 μM *
	PS_0.2	PS	Pristine	sphere	0.2 [^]	bottom-up	<LOD	4 μM *
	PS_1.0	PS	Pristine	sphere	1 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	PS_10.0	PS	Pristine	sphere	10 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	F_PS_0.05	PS	Fluorescent	sphere	0.05 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	F_PS_0.2	PS	Fluorescent	sphere	0.2 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	F_PS_1.0	PS	Fluorescent	sphere	1 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	F_PS_10.0	PS	Fluorescent	sphere	10 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
	PS_1.0_CRBX	PS	Carboxy-groups	sphere	1 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20
PS_1.0_AMINO	PS	Amnio-groups	sphere	1 [^]	bottom-up	<LOD	<0.1% of SDS or Tween-20	
HDPE	HDPE	Pristine	sphere	0.2-10.0	bottom-up	<LOD	<30% trade secret	
AURORA synthesized	UU_PMMA	PMMA	Pristine	irregular	0.3-0.7	bottom-up	<LOD	32 μM *
	UU_PVC	PVC	Pristine	irregular	1.9-5.1	bottom-up	<LOD	113 μM *
	UU_PET	PET	Pristine	irregular	0.9-3.0	bottom-up	<LOD	9 μM *
	UU_PS	PS	Pristine	irregular	1.7-4.9	bottom-up	<LOD	110 μM *
	UU_PA6.6	PA	Pristine	irregular	0.3-0.6	bottom-up	<LOD	tbd
	UU_PA11	PA	Pristine	irregular	0.3-0.6	bottom-up	<LOD	tbd
	UU_PE	PE	Pristine	irregular	tbd	bottom-up	<LOD	tbd
	UU_F_PMMA	PMMA	Fluorescent	irregular	0.6-1.2	bottom-up	<LOD	56 μM *
	UU_F_PVC	PVC	Fluorescent	irregular	1.7-5.6	bottom-up	<LOD	146 μM *
	UU_F_PET	PET	Fluorescent	irregular	1.3-4.6	bottom-up	<LOD	1820 μM *
	UU_F_PS	PS	Fluorescent	irregular	tbd	bottom-up	<LOD	93 μM *
	UU_F_PA	PA	Fluorescent	irregular	0.3-0.5	bottom-up	<LOD	tbd
	UU_PA_UV_6D	PA	UV-weathered	irregular	tbd	bottom-up	<LOD	tbd
	UU_PET_UV_6D	PET	UV-weathered	irregular	tbd	bottom-up	<LOD	tbd
UU_PVC_UV_6D	PVC	UV-weathered	irregular	tbd	bottom-up	<LOD	tbd	
UU_PVC_H2O2	PVC	UV-weathered	irregular	tbd	bottom-up	<LOD	tbd	
UU_PVC_H2O2_UV_6D	PVC	UV-weathered	irregular	tbd	bottom-up	<LOD	tbd	
CUSP synthesized	PS_UV_16D	PS	UV-weathered	sphere	0.1-0.3	bottom-up	<LOD	<0.1% of SDS or Tween-20
	PS_UV_24D	PS	UV-weathered	sphere	0.1-0.3	bottom-up	<LOD	<0.1% of SDS or Tween-20
	CSIC_PET	PET	Pristine	irregular?	0.02-0.09	bottom-up	tbd	na
	G_PA_disc	PA	Pristine	discs	10x3	top-down	tbd	na
	G_PA_fibers	PA	Pristine	fibers	10x30	top-down	tbd	na

Note: Size-range (D10-D90 μm) determined with Mastersizer; [^] size-range as reported by the vendor

* SDS concentration as measured in in 10 mg/mL MNP stock

fluorescent

UV weathered



TIER I

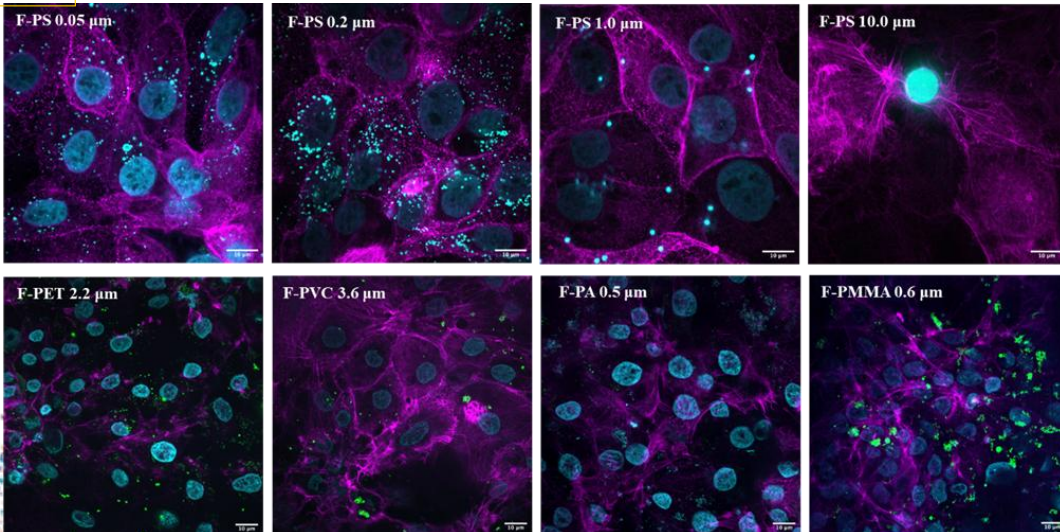


Screening and prioritization

- Uptake of fluorescent MNP
 - PS, PET, PVC, PA, PMMA taken up
 - all placenta cell models (BeWo b30, hTSCs)
- Toxicity of weathered or pristine MNPs
 - 1-100ug/mL at max 72h exposure
 - No acute cytotoxicity
 - except for PA
 - Membrane integrity affected
 - only at the highest conc. tested
 - Oxidative stress
 - No effects

Key:

- Magenta = F-actin
- Cyan = Nuclei
- Green = MNPs



		TIER 1											
		Cytotoxicity			Membrane integrity			Oxidative potential (CellRox)			Oxidative stress (SRXN1-GFP)		
Exposure time (h)		24			24 48 72			24			24		
Concentration (μg/mL)		1	10	100	100			1	10	100	1	10	100
Commercial	PS_0.05												
	PS_0.2												
	PS_1												
	PS_10.0												
	PS_1_CRBX												
	PS_1_AMINO												
	HDPE												
AURORA synthesized	UU_PMMA												
	UU_PVC												
	UU_PET												
	UU_PS												
	UU_PA6.6												
	UU_PA11												
	UU_PE												
	UU_PA_UV_6D												
	UU_PET_UV_6D												
	UU_PVC_UV_6D												
	UU_PVC_H2O2												
	UU_PVC_H2O2_UV_6D												
	CUSP	PS_UV_16D											
PS_UV_24D													
CSIC_PET													
G_PA_disc													
G_PA_fibers													
Solvent control	0.5% 1-propanol												
Particle control	TiO2												

Green: no effect; Red: statistically significant effect (One-way Anova, p<0.05); Empty: not measured

TIER 1



Screening and prioritization

TIER 2



In vitro FetoPlacental toxicity markers of MNPs

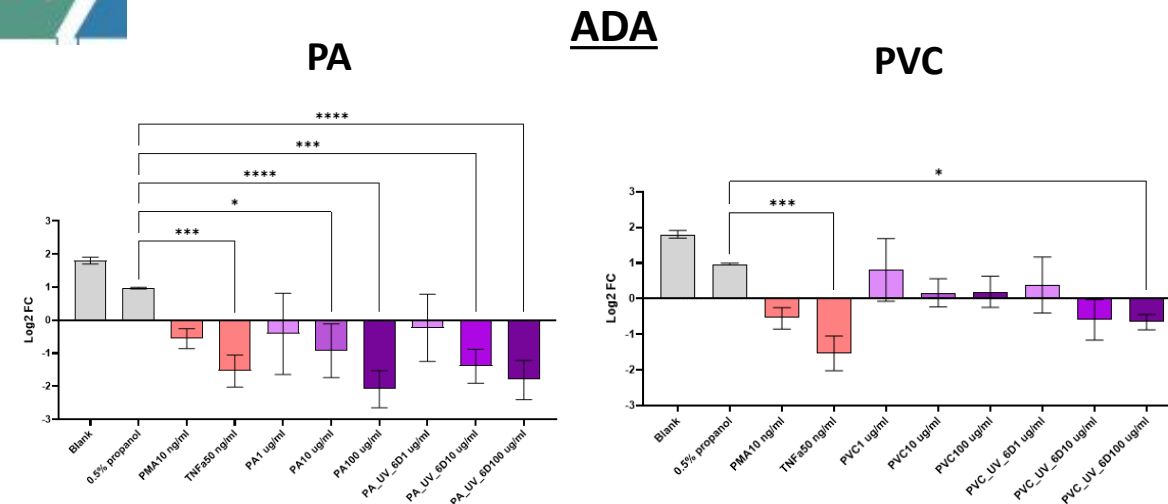
■ (Pro)inflammatory markers

- hTSCs-STs exposed to weathered and non-weathered PA, PET and PVC (1-100 $\mu\text{g}/\text{mL}$, 24h)
- Weak effects on classical markers (IL-6, IL-8, TNF- α)
- 92 proteins analysed with the **Olink® Inflammation T96** panel
- Up to **35 proteins detected** above LOD
- Two proteins, **ADA** and **CXCL11**, were significantly altered (PA mainly)

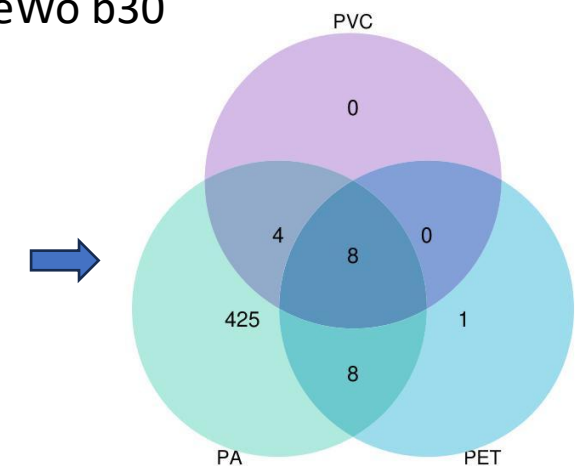
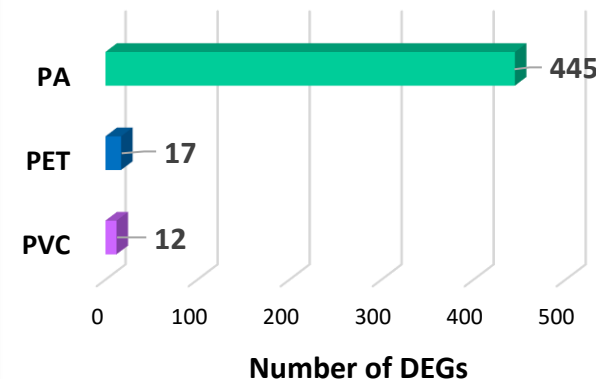
■ Transcriptomic profiling

- BeWo b30 & hTSCs (PA, PET and PVC, 10 $\mu\text{g}/\text{ml}$, 24h)
- **PA** showed the **highest number of DEGs**
- Effects on genes involved in trophoblast **differentiation, invasion, cell survival, stress responses**, etc.
- **Cell-type specific**
 - BeWo b30 > hTSCs
 - CTs > STs

Human trophoblast stem cells



NS BeWo b30



TIER 1

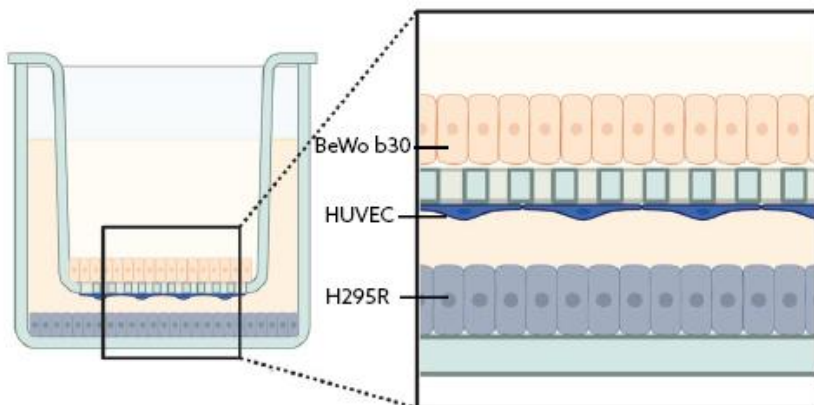


Screening and prioritization

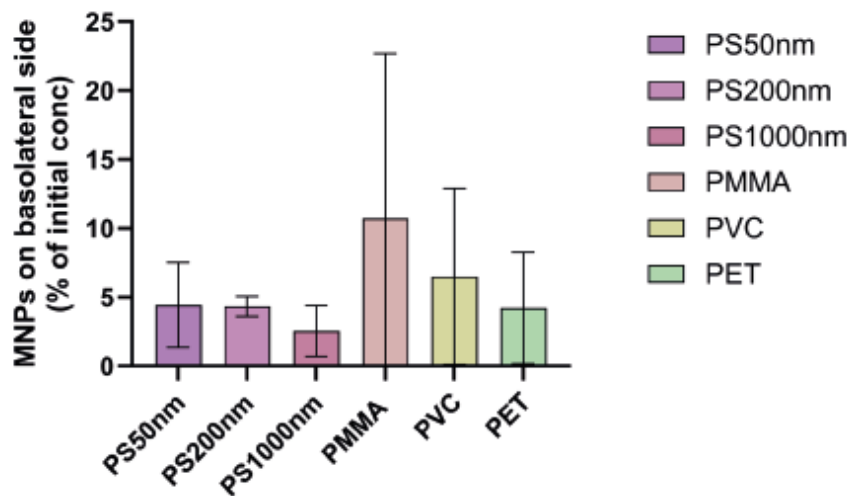
TIER 2



In vitro FetoPlacental toxicity markers of MNPs



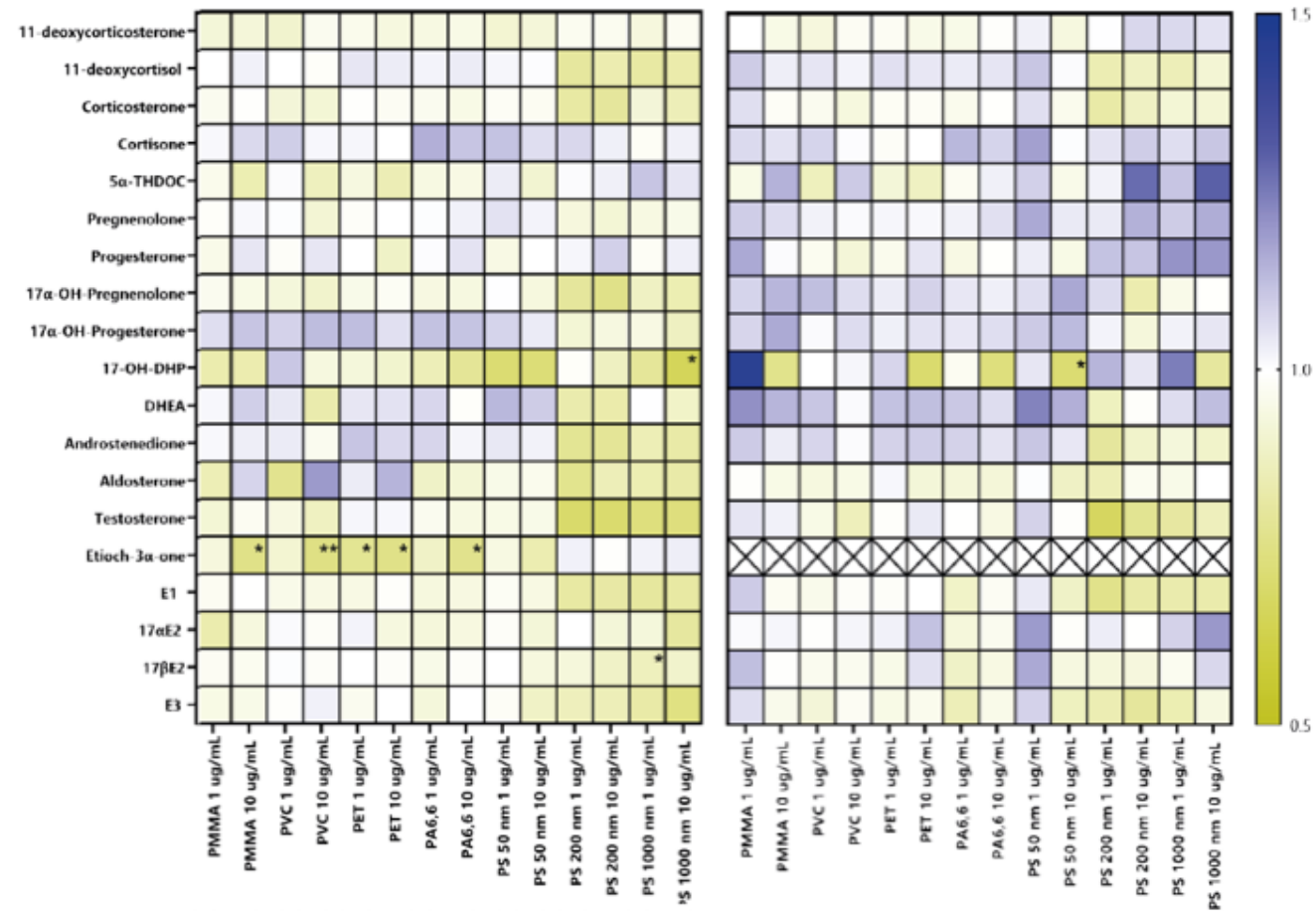
Schematic overview of the BeWo b30/HUVEC-H295R tri-culture



Translocation of fluorescently labelled MNPs

Apical side

Basolateral side



Steroid hormone levels in the BeWo b30/HUVEC-H295R triculture



TIER 1



Screening and prioritization

TIER 2

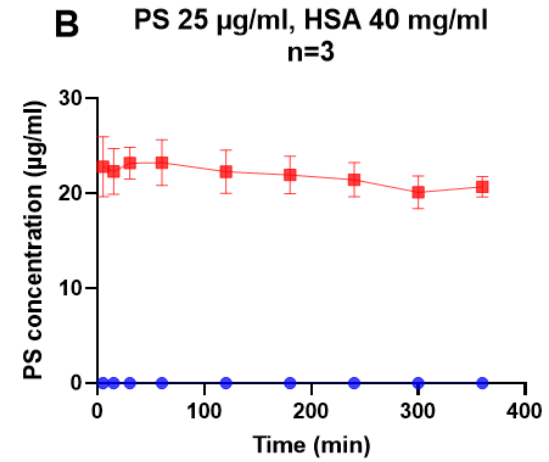
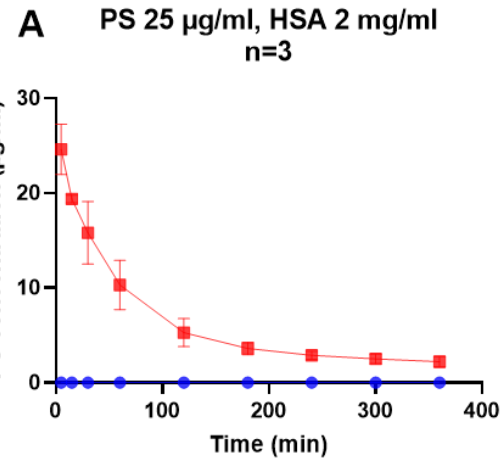
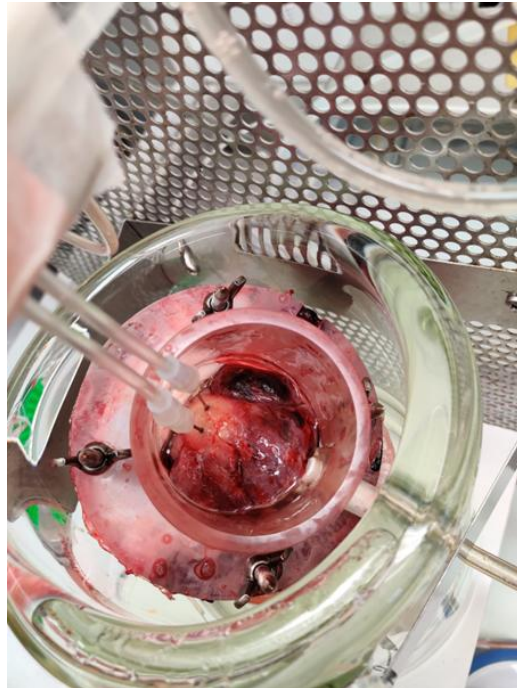
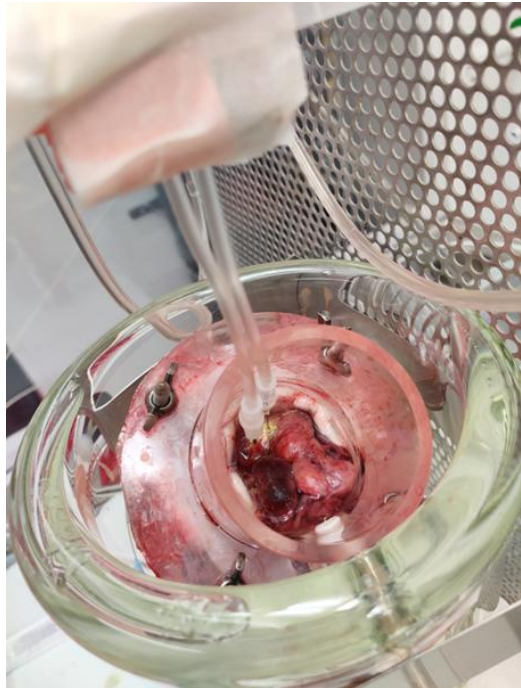


In vitro FetoPlacental toxicity markers of MNPs

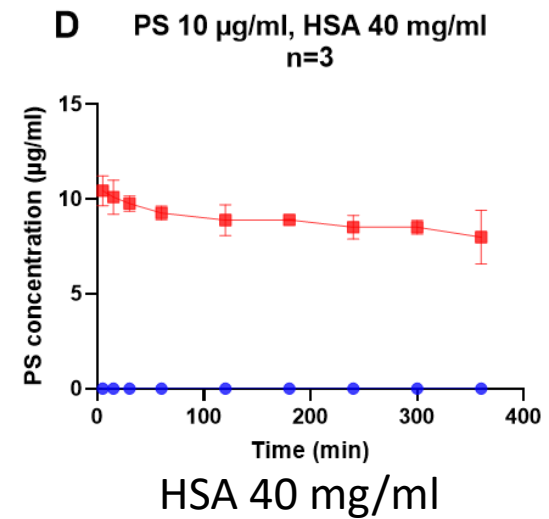
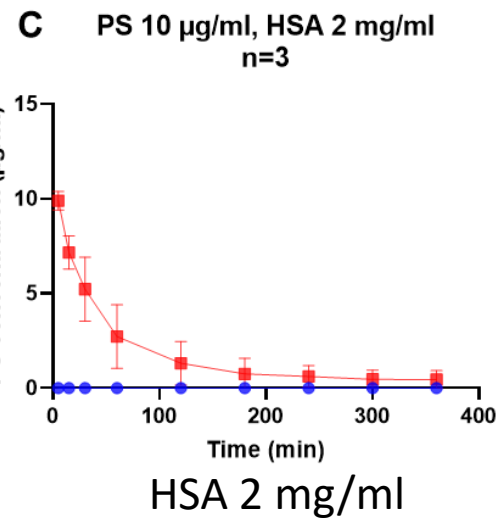
TIER 3



placental perfusion



● Fetal
■ Maternal



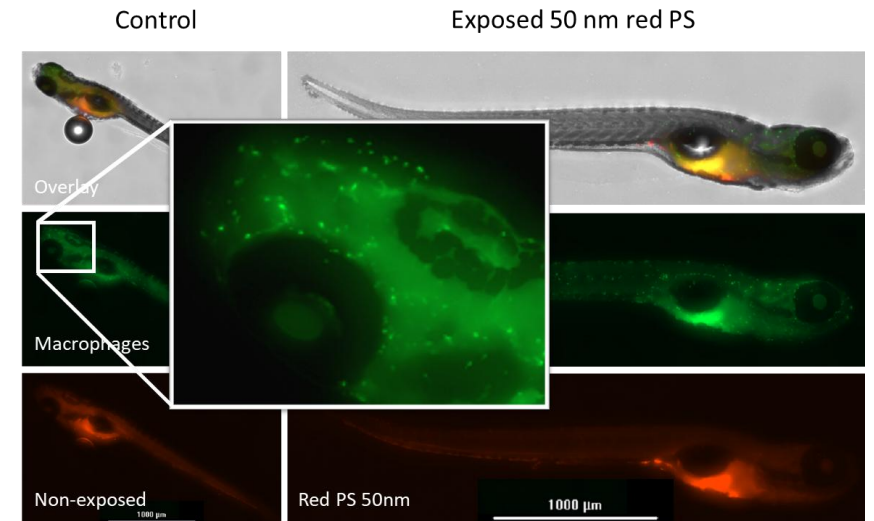
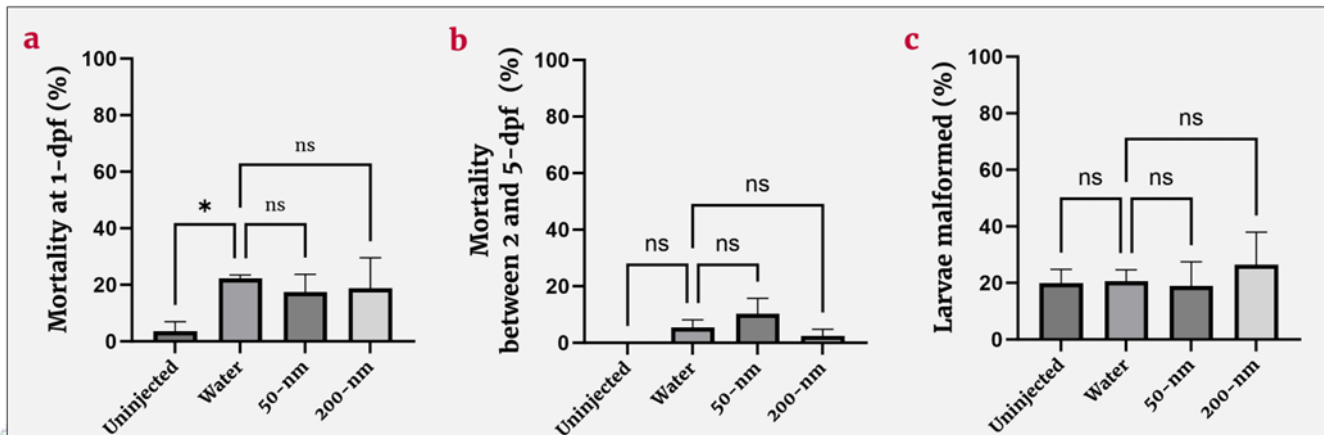
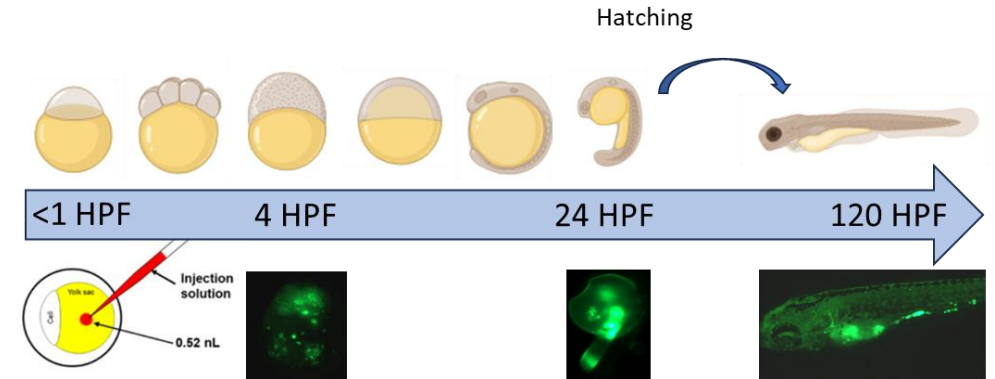
➤ Minor transplacental transfer of Fluo-PS





▪ **Zebrafish embryo toxicity**

- zebrafish embryos exposed to PS and PA (2.5 ng)
 - **MNPs internalised** and found throughout the zebrafish larvae at 5dpf
 - **no significant developmental effects**
 - RNA seq and macrophage analysis ongoing



Summary

- **Placenta trophoblast models**
 - All studied fluorescent MNPs were internalised
 - No acute toxicity was observed, except for PA particles
 - Several MNPs induced membrane damage.
 - No intracellular oxidative stress responses
 - Cytokine profiling: specific proteins affected by MNP exposure.
 - RNA sequencing: PA induced highest number of differentially expressed genes
- **BeWo b30/HUVEC-H295R tri-culture model:**
 - Limited polymer-specific effects on steroidogenesis.
- **Placenta perfusion model**
 - Fluo-PS particles accumulated in placental tissue
 - minor transplacental transfer of Fluo-PS
- **Developmental models**
 - No acute developmental effects on zebrafish embryos
 - No effects in embryonic stem cell test or C. elegans (data not shown)



What do we know about the early life biological effects of MNPs?

- MNPs tested are taken up in cells and tissues
- No or minimal acute toxicity, except for PA
- Subtle effects on steroidogenesis, gene and protein expression

What do we not know yet?

- Effects of chronic exposure
- Effects of mixtures and multiple stressors
- Effects on human models of dev tox
- ...



Dr Jeske van Boxel



Dr Hanna Dusza

