Well-Characterized Nanoplastics for Oral Exposure Studies in vivo

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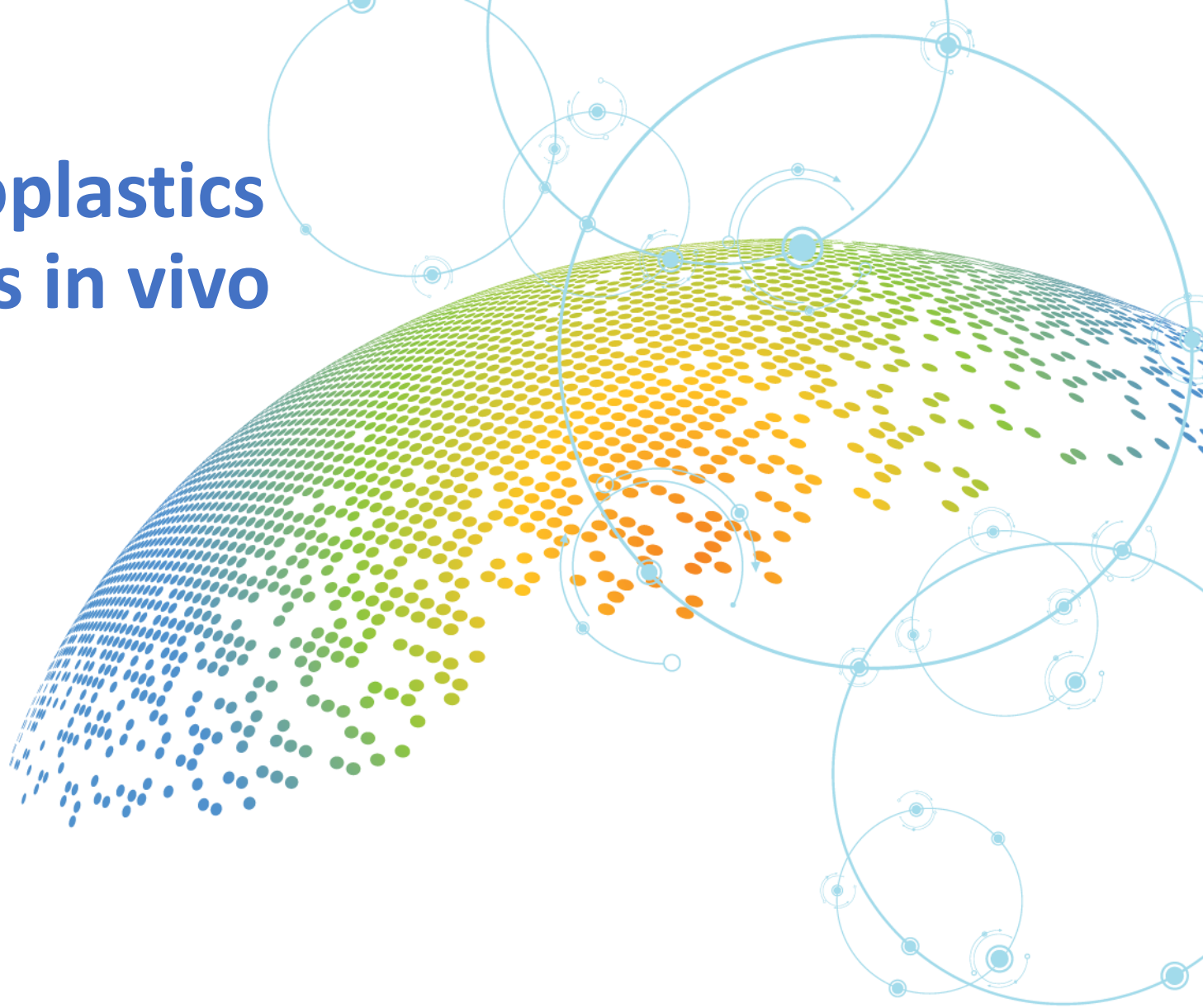

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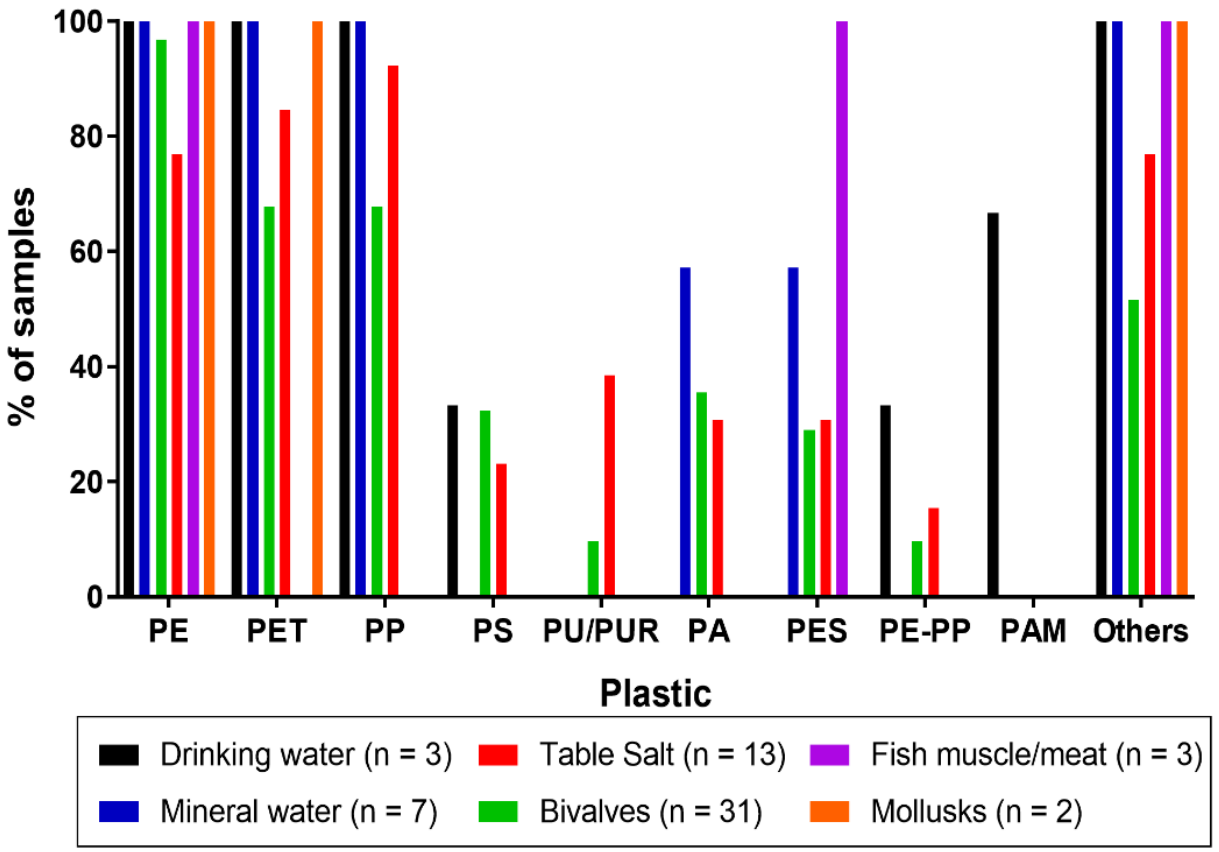
Outline: Well-Characterized Nanoplastics for Oral Exposure Studies in vivo

- Brief Background on oral exposure of nanoplastics
- Fabrication/Characterization of in-house fabricated nanoplastics
- Biological Studies at RTI
  - In vitro
  - In vivo oral exposure
- Important Questions/Next Steps

Image from Nat. Geo. exhibit, 'Plant or Plastic' Norfolk, VA.

What Plastics Are We Exposed To?

- Review of small-scale plastics detected in drinking water, Beverages, Food Sources (<50 µm)
- Mixtures in samples
  - >35 types of polymers reported in drinking water, beverages, and food sources.
  - Common polymers reported include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyethersulfone (PES), polyamide (PA), polystyrene (PS).



Mortensen NP, TR Fennell, LM Johnson (2021) NanoImpact.

Oral Exposure of Polystyrene (PS) Particles & Gastrointestinal Absorption in Rats

Jani et al '89

Fluorescent PS particles; oral gavage; 100nm – through 1 µm detected in Peyer's patches, villi, liver, lymph nodes, spleen.[1]

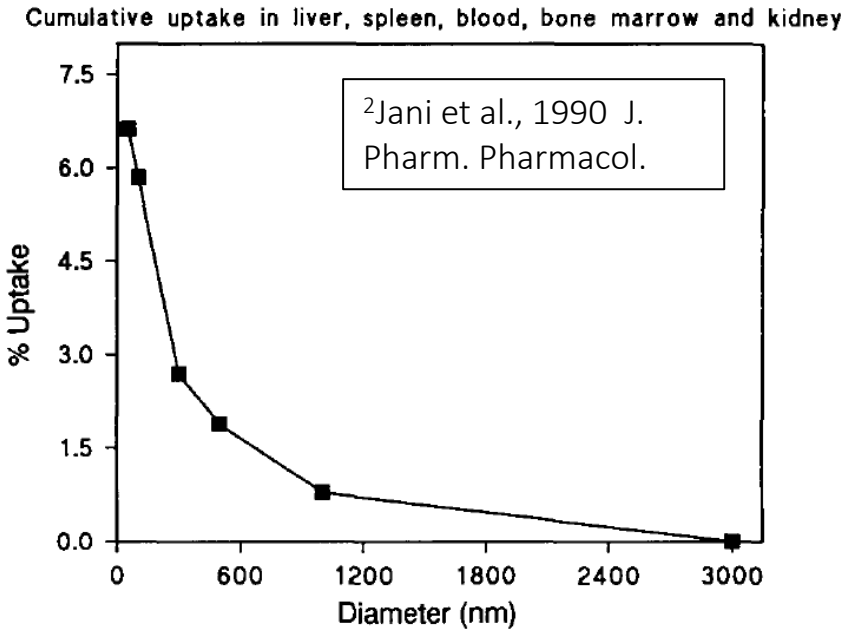
Jani et al '90

Quantify oral absorption/location of PS different sizes; increases with decreasing size for PS spheres (50 nm - 3 µm), in rats. [2]

Jani et al '96

Biliary elimination of FITC labeled PS spheres (50, 500 nm, 1, 3 µm); size-related excretion of PS spheres into the bile.[3]

Translocation of PS across gastrointestinal wall and uptake of PS (<50 nm) within systemic organs.

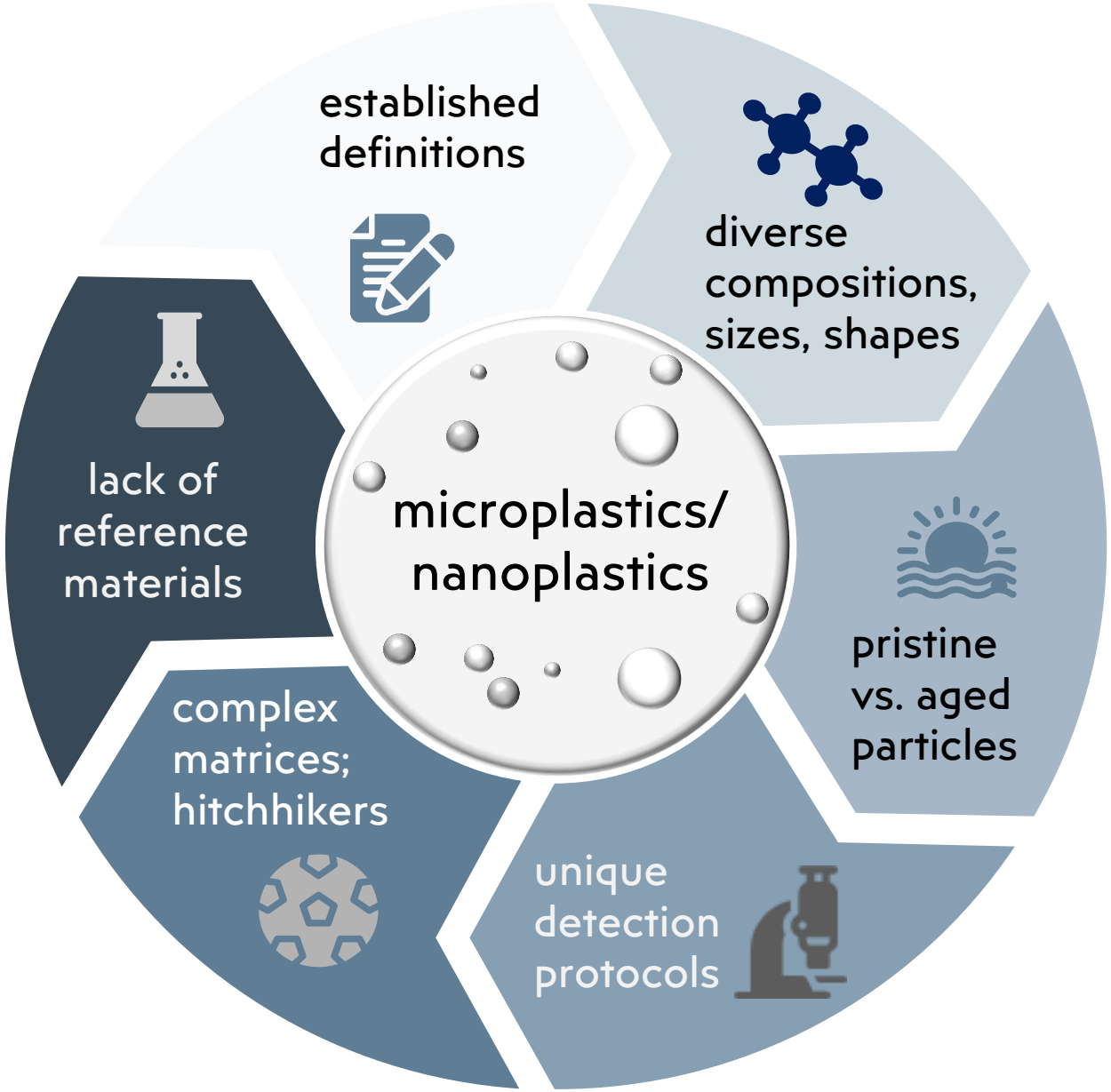


[1]Jani et al. 1989 J. Pharm. Pharmacol. DOI: 10.1111/j.2042-7158.1989.tb06377.x
[2]Jani et al., 1990 J. Pharm. Pharmacol. DOI: 10.1111/j.2042-7158.1990.tb07033.x
[3]Jani et al., 1996 J. Drug Targeting. DOI: 10.3109/10611869609046266

Factors to Consider for Micro/Nanoplastics Research

Diversity & unique attributes of microplastics & nanoplastics require new approaches for conducting studies

How do we deal with these variables?



Adopted from Paul, Maxi B. et al. Nanoscale Advances 2 (2020): 4350.

Approach: Systematic Testing with Well-Characterized NPs

Fabrication

Fabricate nanoplastics: aqueous suspension or aerosolization

Characterization

Characterize PCP; quantify solvent traces; test for sterility, endotoxins, surface adsorption

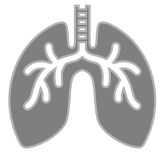
Biological Studies

NPs must be sterile, solvent/endotoxin-free to test the biological responses of composition, size, shape

Exposure Routes

Ingestion

Inhalation



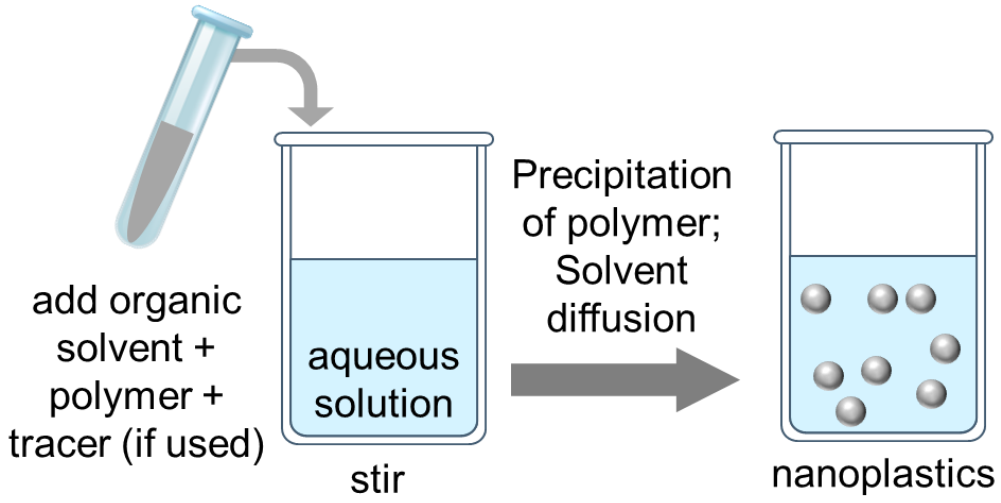
Particle Exposures

Expose particles to defined perturbations (UV, mechanical, heat) or chemicals (“hitchhikers”)

Fabrication of Nanoplastics

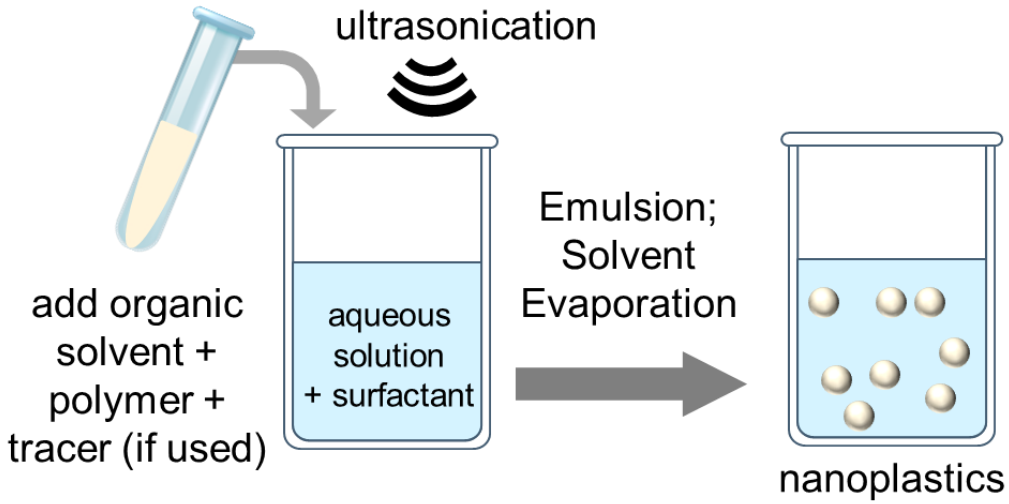
General Approach: Dispersion of Preformed Polymers

Nanoprecipitation



Advantages: rapid, single-step, low-energy, reproducible, amenable to scale-up

Emulsion/Solvent Displacement



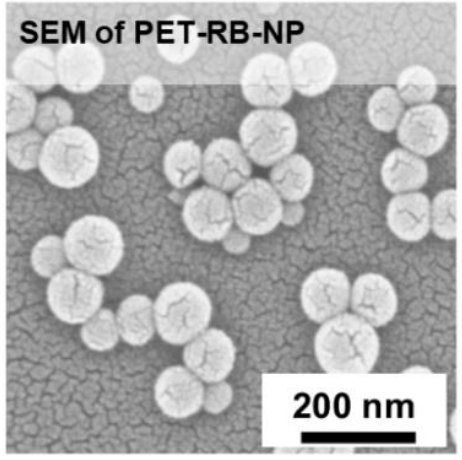
Advantages: rapid and useful for more hydrophilic polymers, but requires a high shear

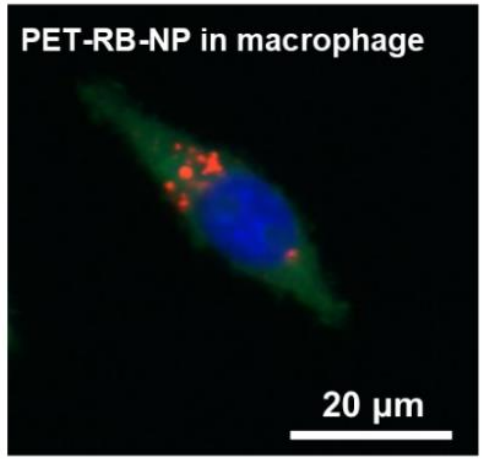
Fluorescently-Labeled NPs- Spatial Distribution In Cells

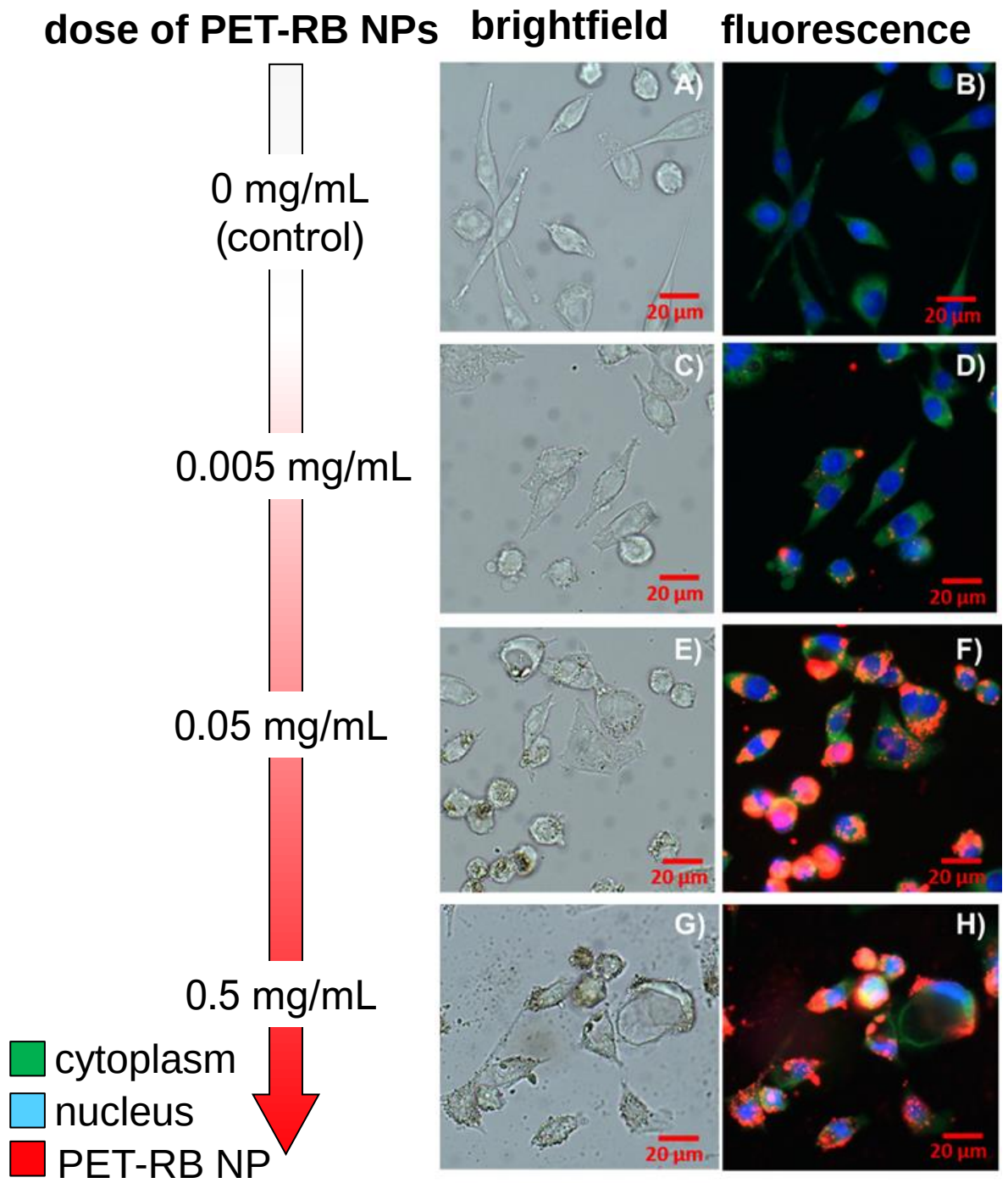
Exposure of PET NPs to Macrophages

- PET-NPs taken up by Macrophage Cells (Raw 264.7 cells) in dose-dependent manner

- Clear morphological changes occurred at higher concentrations of PET-RB NPs (0.5 mg/mL), showing enlarged phagosomes and elongated nuclei.





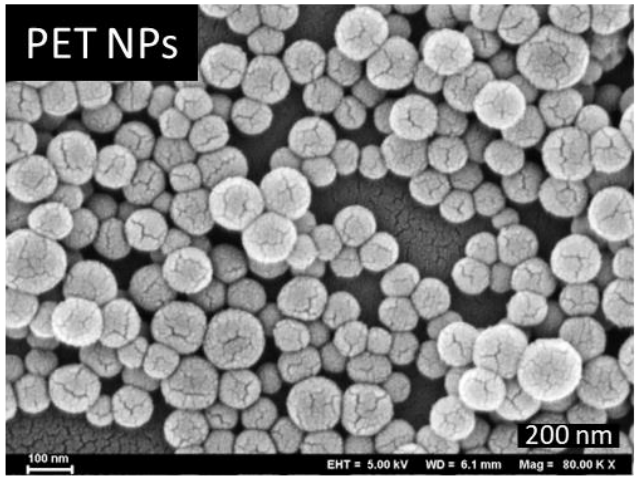


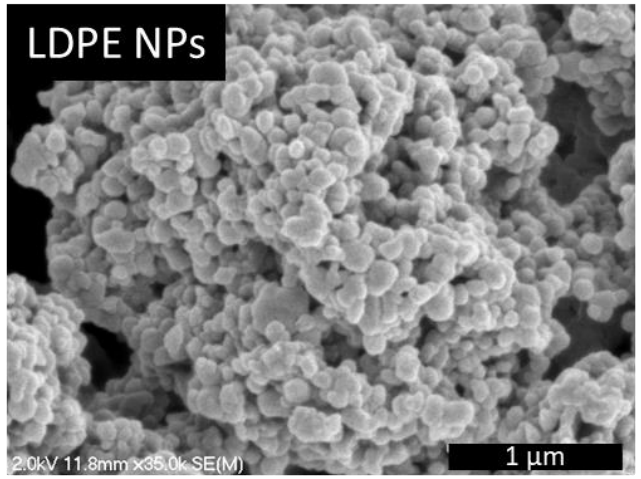
Johnson et al. Nanoscale Adv., 2021, 3, 339-346

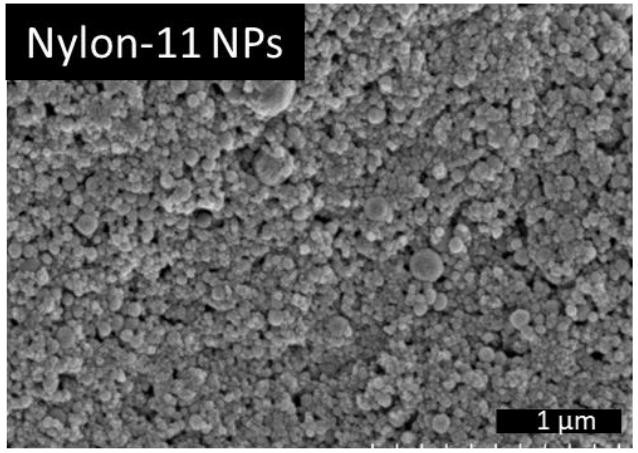
Characterization of NPs

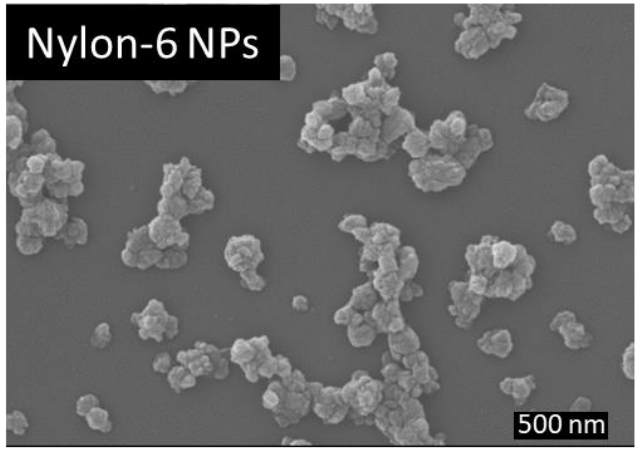
Thorough characterization of nanoplastics prior to initiating testing in biological systems

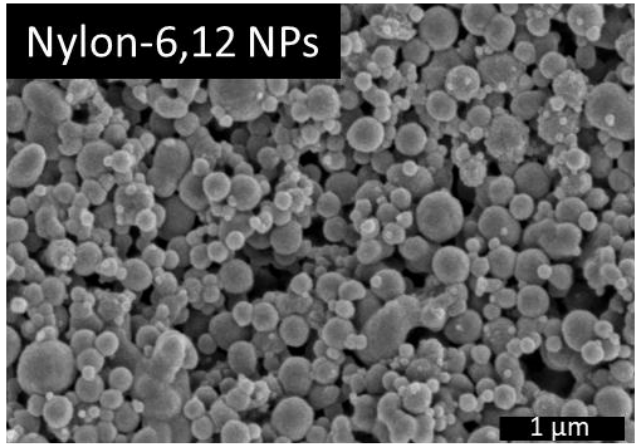
- ✓ ***Microscopy-*** *morphology, fluorescence*
- ✓ ***FT-IR****-characteristic absorption bands of bulk polymer*
- ✓ ***GC/MS****: characteristic peaks*
- ✓ ***DLS****: hydrodynamic diameters*
- ✓ ***Zeta Potential*** *in various solutions*
- ✓ ***Endotoxin****: > 0.5 EU/mL*
- ✓ ***Leaching studies****: ensure stability of tracer incorporation.*

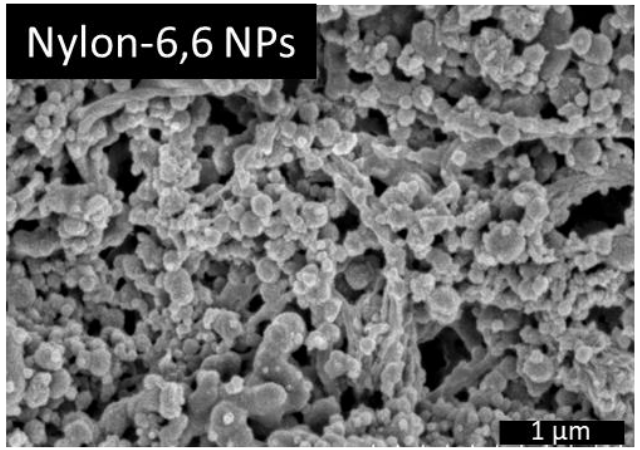




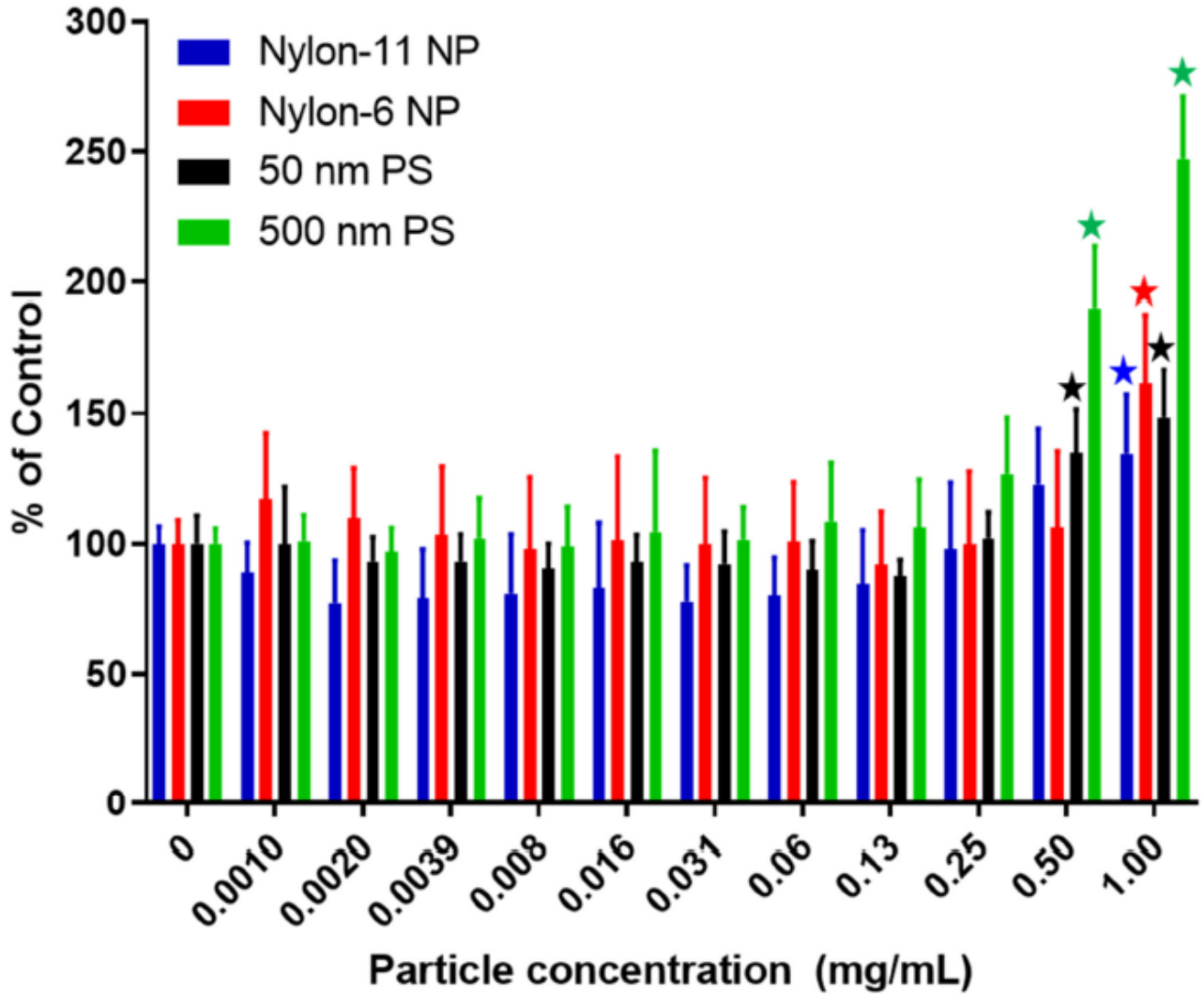








Nylon NPs in RAW264.7 Macrophages



Krovi et al. Nanomaterials, 2022, 12, 2699

Polyamide (nylon) Properties

- Diverse chemical family
- Susceptible to strong acid/alkaline
- High toughness

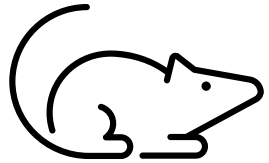
Products

- Films for Food Packaging
- Filaments (toothbrush bristles)
- Cosmetics
- Tubing for beverages
- Fabric and Fibers (clothing, flooring)

Cytotoxicity Assay

- Cytotox. via cell membrane integrity (LDH)
- PS NPs: no significant cytotoxic effects up to 0.25 mg/mL
- Nylon NPs: no cytotoxicity until high doses (1mg/mL)
- 500 nm PS at (1 mg/mL) interfered with LDH assay

In vivo Evaluation of NPs in Sprague Dawley Rats



Purpose: Investigate potential biological effects of NPs (nylon, PS) following oral exposure early in life

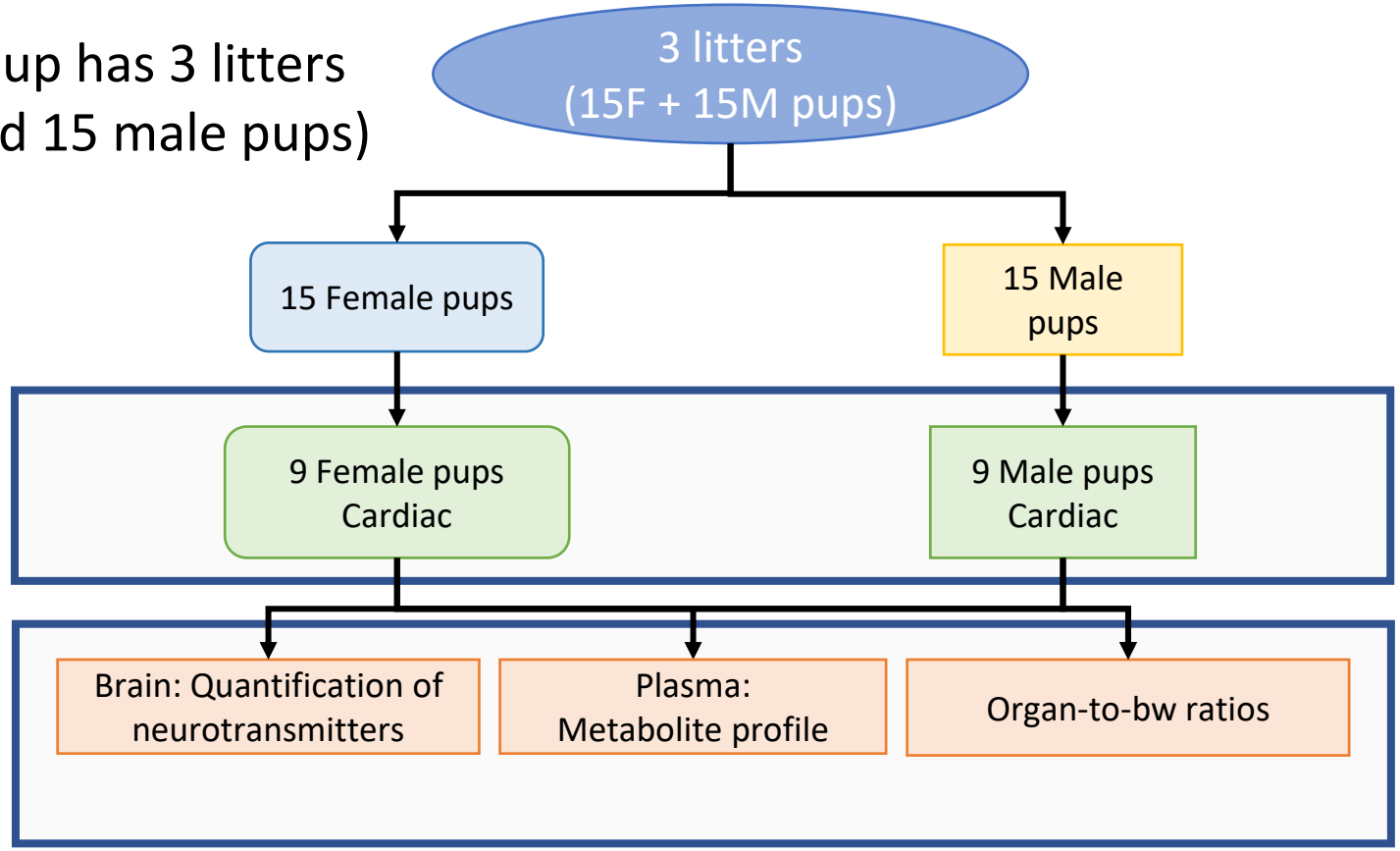
Study design:

Each dose group has 3 litters (15 female and 15 male pups)

PND 7 – 10: Gavage daily (4 doses)

PND 20: Non-invasive assay

PND 21: Necropsy



Dose Groups:

- Vehicle control (H_2O)
- 20 mg/kg bw/day Nylon-11 NP
- 20 mg/kg bw/day PS NP

Nanoplastic characterization:

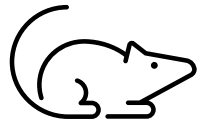
- Endotoxin
- Size and surface chemistry
- Composition

Analysis:

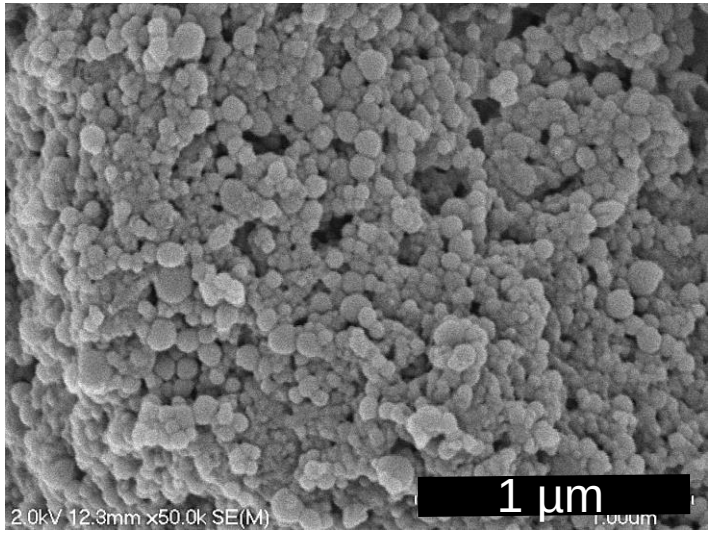
- Body weight (bw)
- Cardiac assessment: ECG
- Organ-to-bw ratio (brain, liver)
- Brain: Neurotransmitters
- Plasma: 186 metabolites

PND=post-natal day

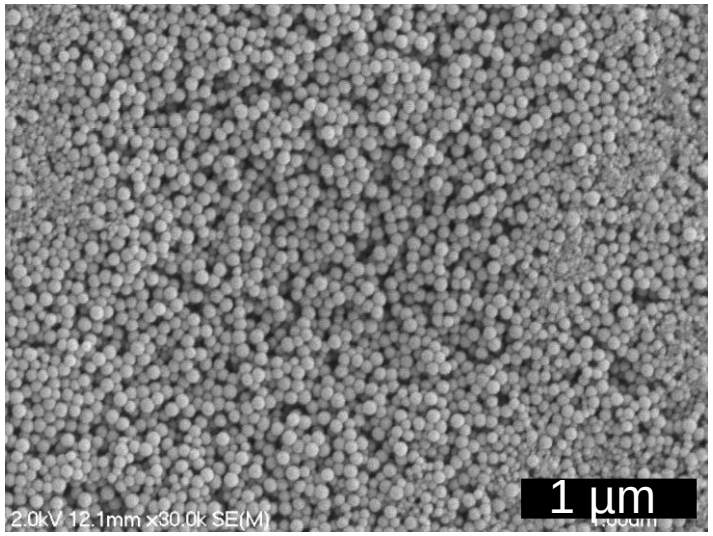
In vivo Evaluation of NPs in Sprague Dawley Rats: Preliminary Results



Nylon-11 (RTI)

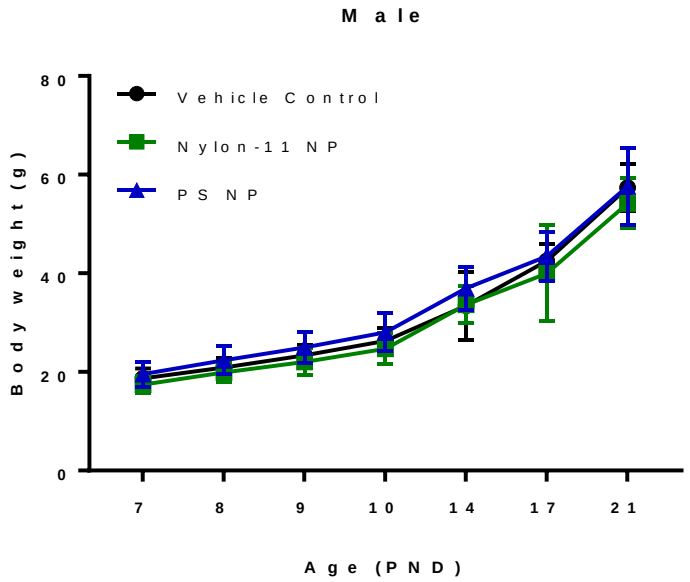


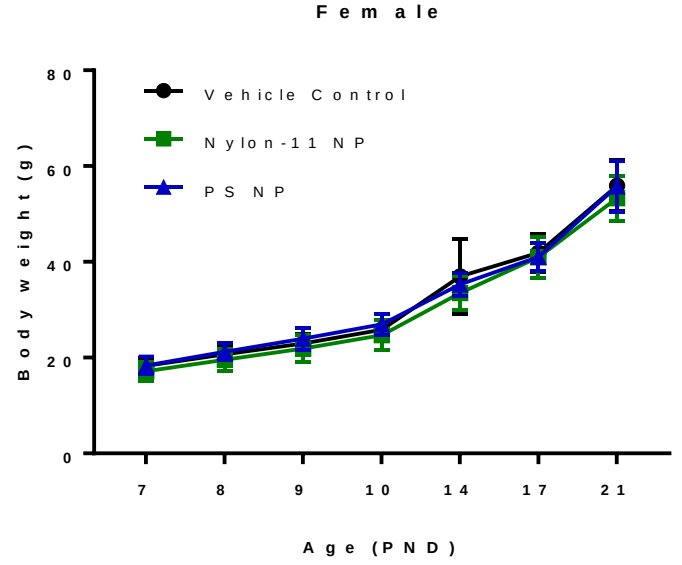
Polystyrene (Spherotech)



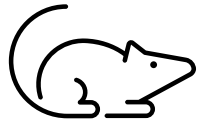
NP	Average size (nm)	Average PDI	Average zeta potential (mV)	Endotoxin (EU/mL)*
Nylon-11	139 ± 44	0.2 ± 0.04	28 ± 0.6	0.054
Polystyrene (PS)	96 ± 21	0.04 ± 0.02	-49 ± 0.3	0.048

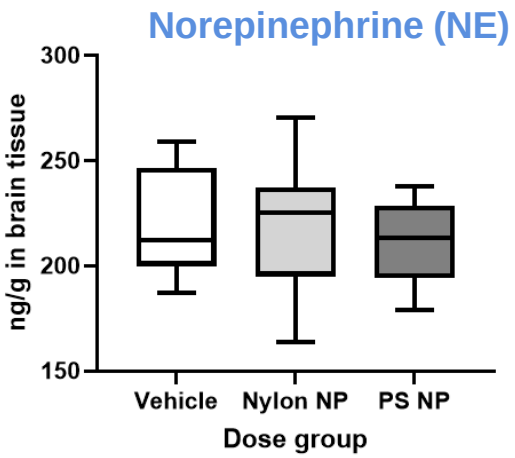
Endotoxin values below the recommended FDA limit of 0.5 EU per mL for medical devices

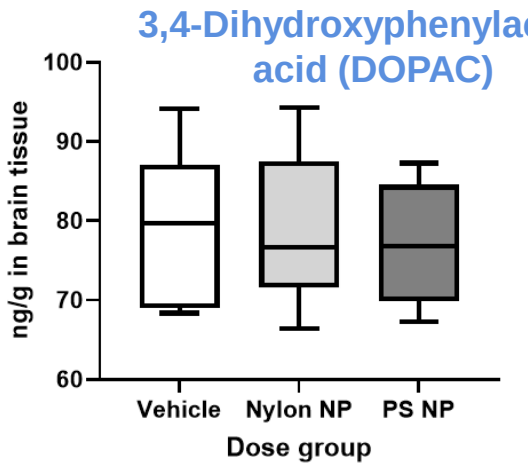


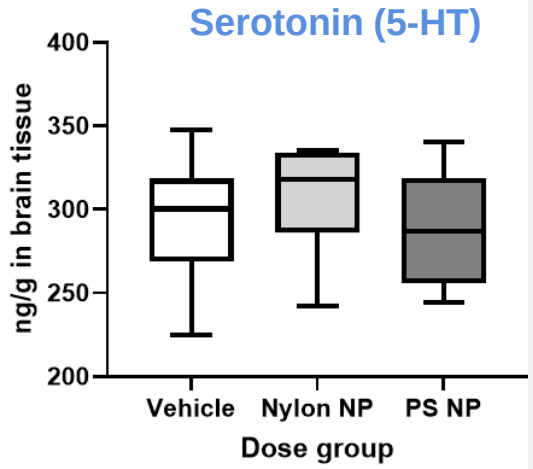


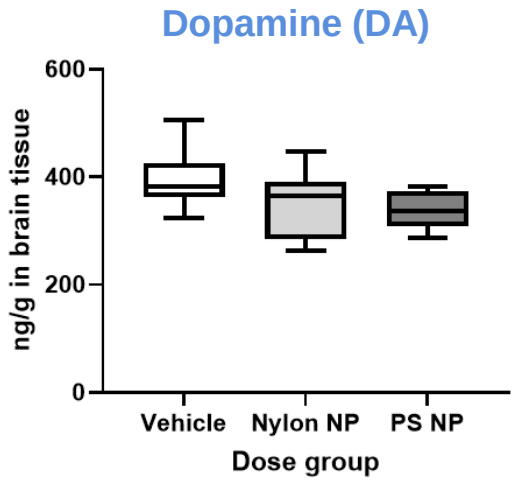
Neurotransmitter Concentration in Brain: Female Sprague Dawley Rats

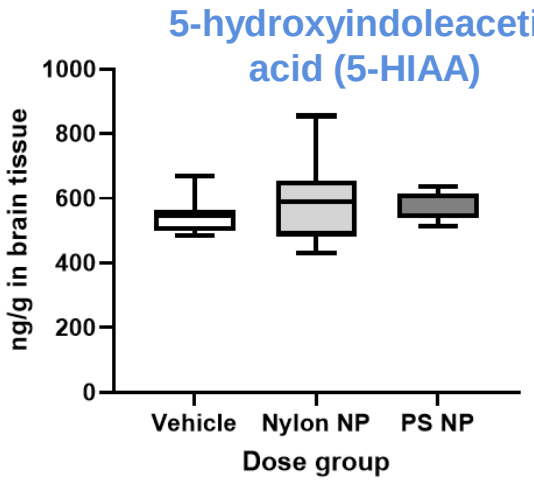


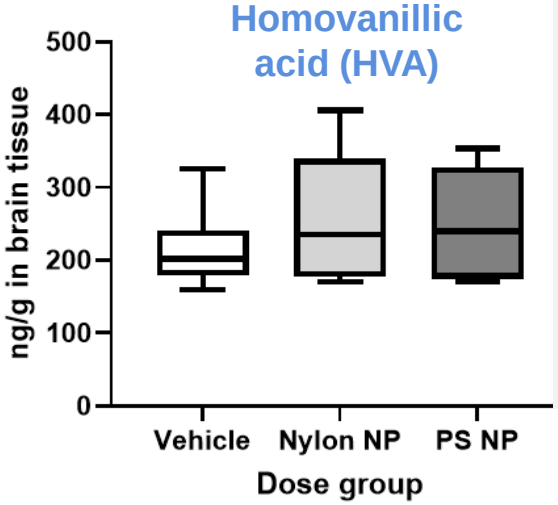








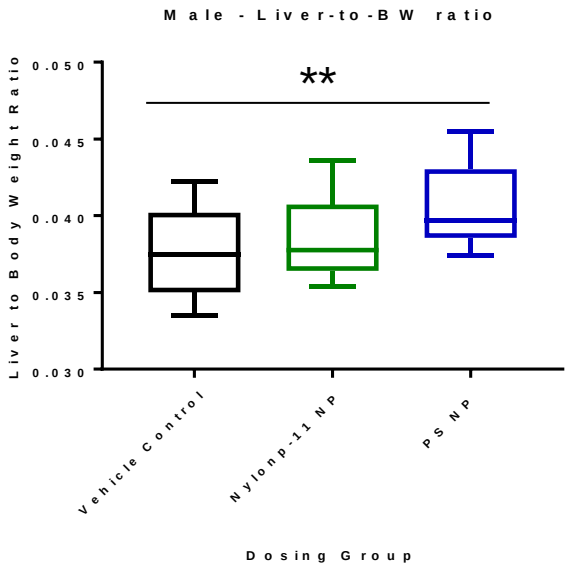


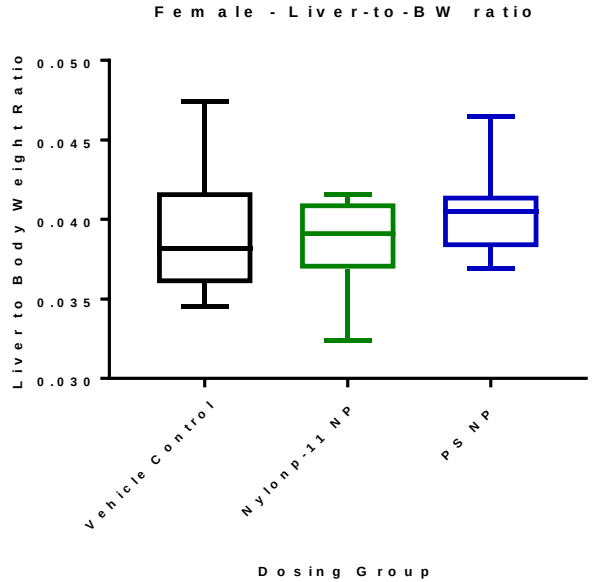


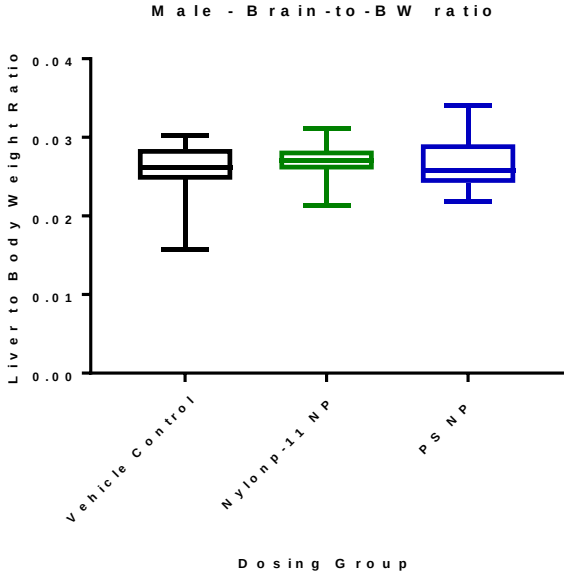
Preliminary Results

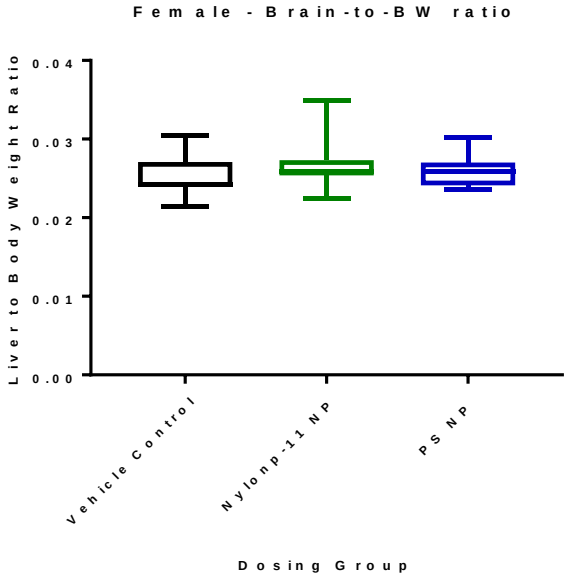
- Concentration of 6 monoamine neurotransmitters/ metabolites determined in right-brain for male and female pups at PND 21
- No significant changes were found in either sex for any analytes.

In vivo Evaluation of NPs in Sprague Dawley Rats





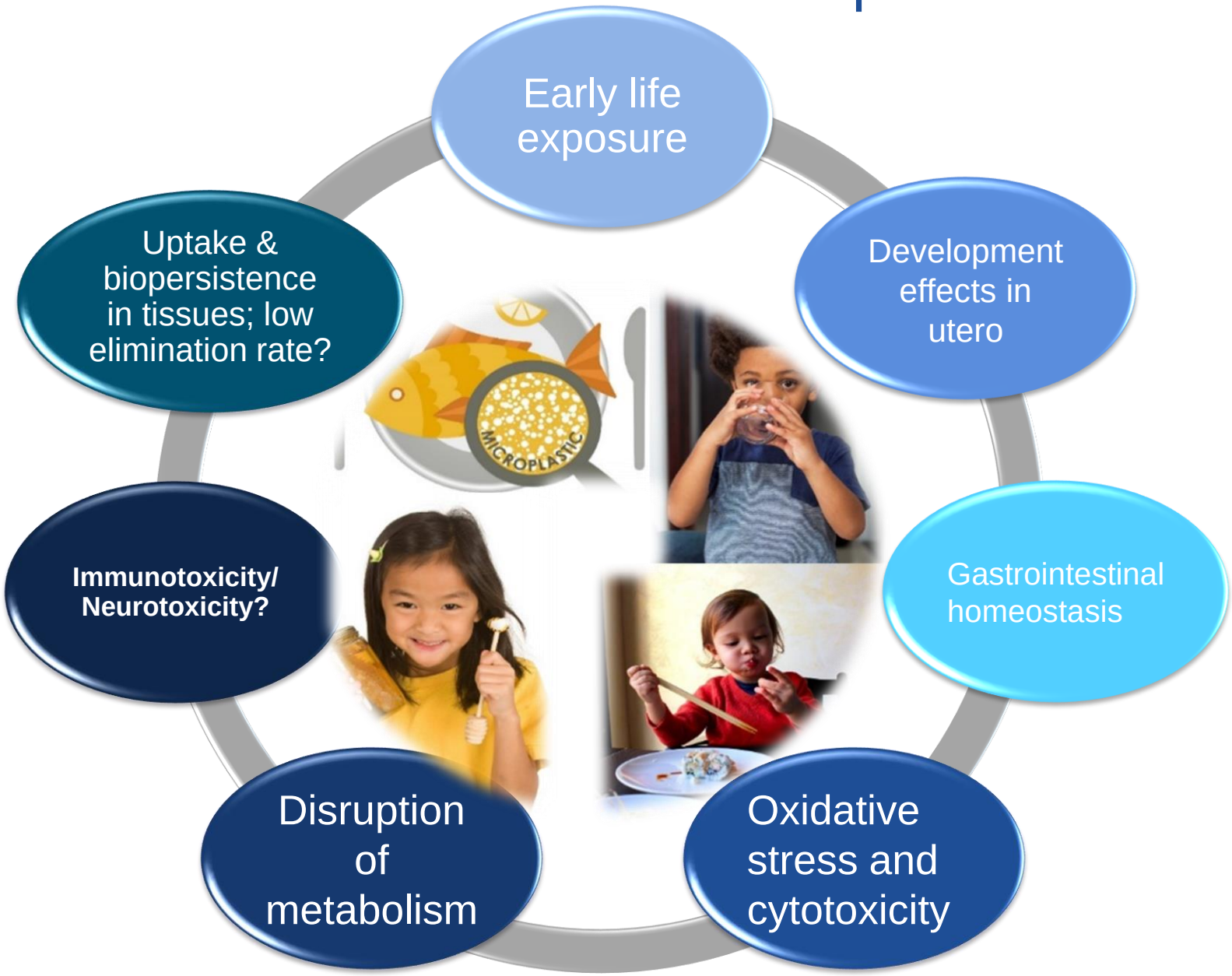




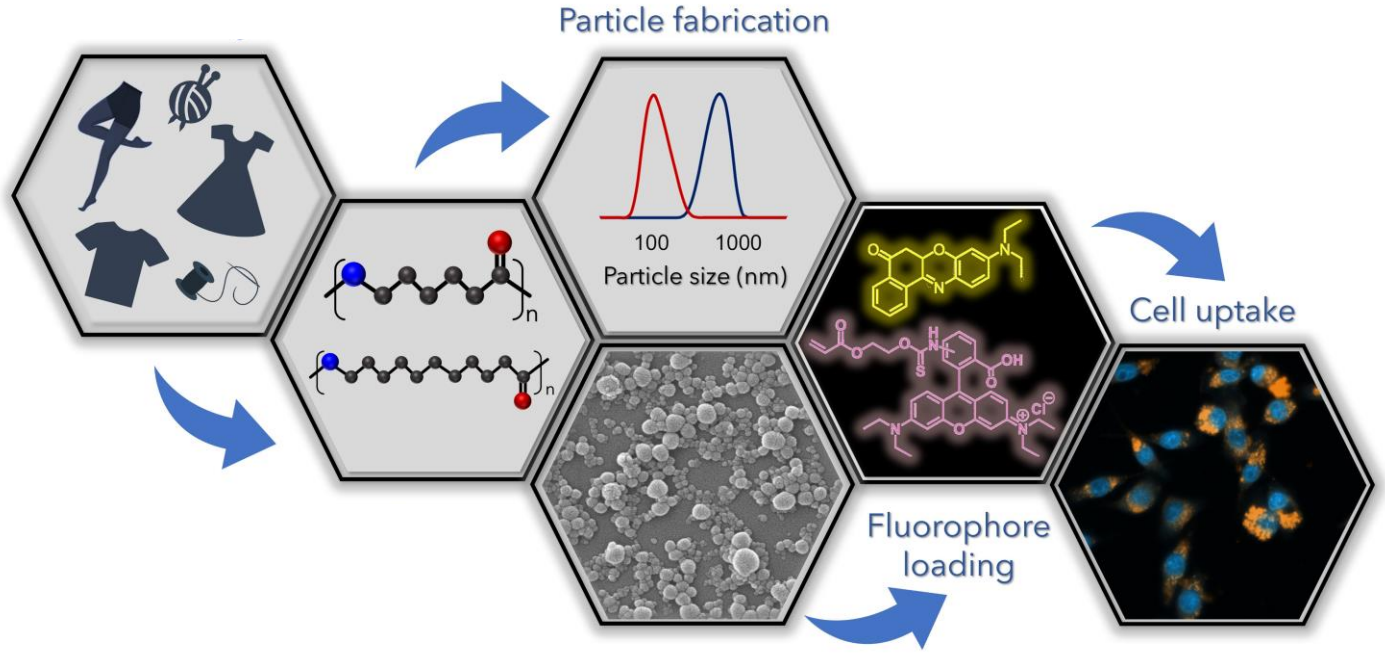
Liver/BW Ratio: useful endpoint to detect organ toxicity.
Brain/BW Ratio: Brain undergoes growth/development early in life; multiple events can impact biochem/homeostasis in the brain
Preliminary Results:

- Increased liver-to-BW ratio for males administered with PS NPs as compared to the vehicle control.
- No differences for other analytes

Questions: Potential Health Risks of Oral Exposure to NPs



Future Research Needs



- A better understanding of exposure and effects.
- Reference materials reflecting the plethora of NP and MP identified as sources of human exposure.
- Development of sampling strategies, extraction, characterization, and identification.
- Systematic studies of the role of PCP including size and polymer chemistry plays in uptake and interference with biological functions.
- Prioritization of which NP and MP should be studied.
- Removal of existing NP and MP from the environment and sources of human exposure.

Acknowledgements and Contributors

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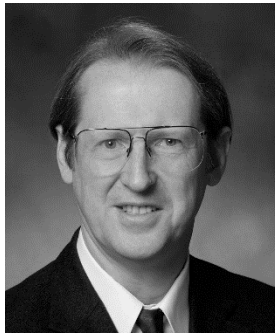

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Funding sources:

NIH NIEHS

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Research Chemist

Jeff Mecham, PhD
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Maria Moreno, BS
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Materials Characterization

Dr. Jean Kim, Dr. Sara Maloney, Ms. Brenda Fletcher, Mr. Rodney Snyder, Mr. Randy Ruthowske, Ms. Randi Carter, Mr. Alex Kovach, Ms. Sarah Harrison, Dr. Ian Stewart, Dr. Shyam Aravamudhan