Introducing AURORA

Actionable eUropean ROadmap for early-life health Risk Assessment of micro- and nanoplastics

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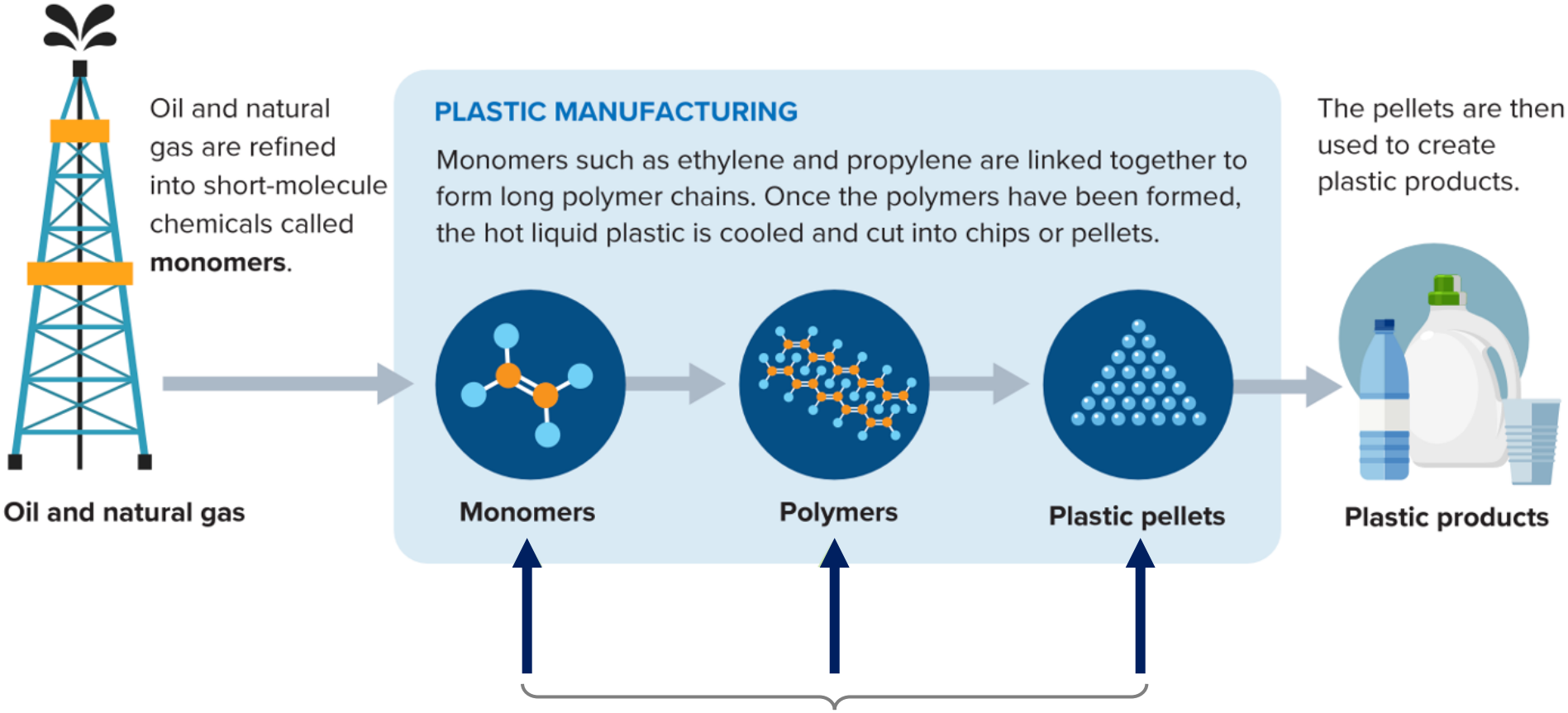
www.AuroraResearch.eu

What are plastics?



Additives

Plasticizers, flame retardants, stabilizers, antioxidants, UV stabilizers, slip agents, lubricants, anti-statics, curing agents, biocides, colorants, fillers, reinforcements

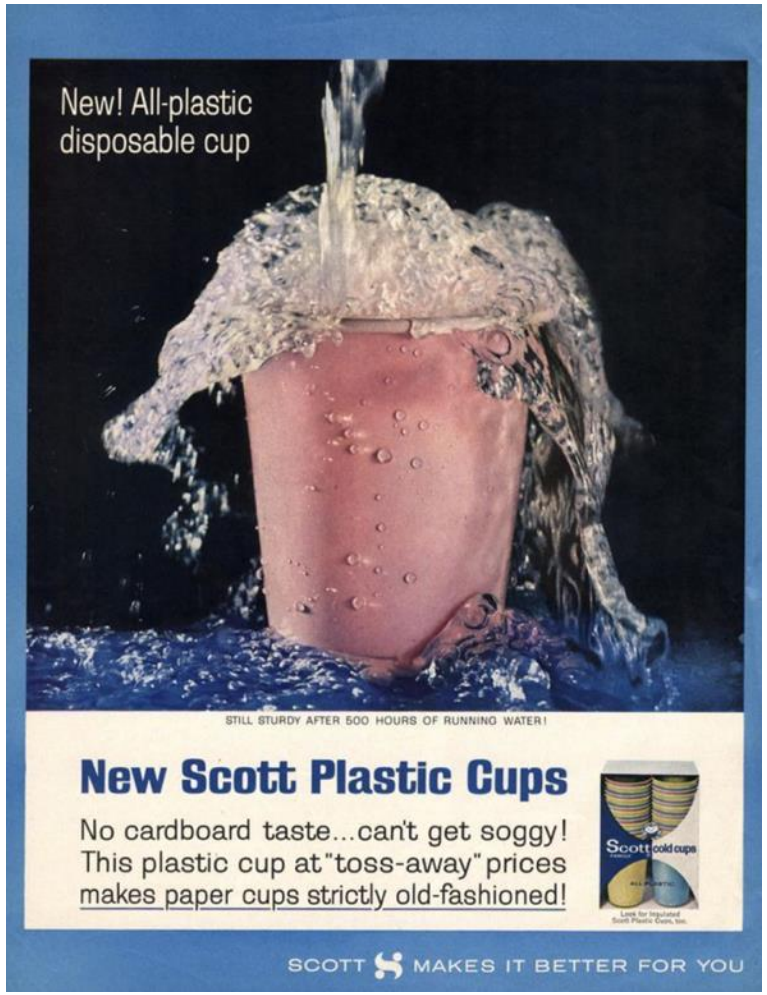
https://knowablemagazine.org/article/food-environment/2020/solving-growing-plastics-waste-puzzle. Accessed 28 April 2023

Hahladakis JN et al. 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater.

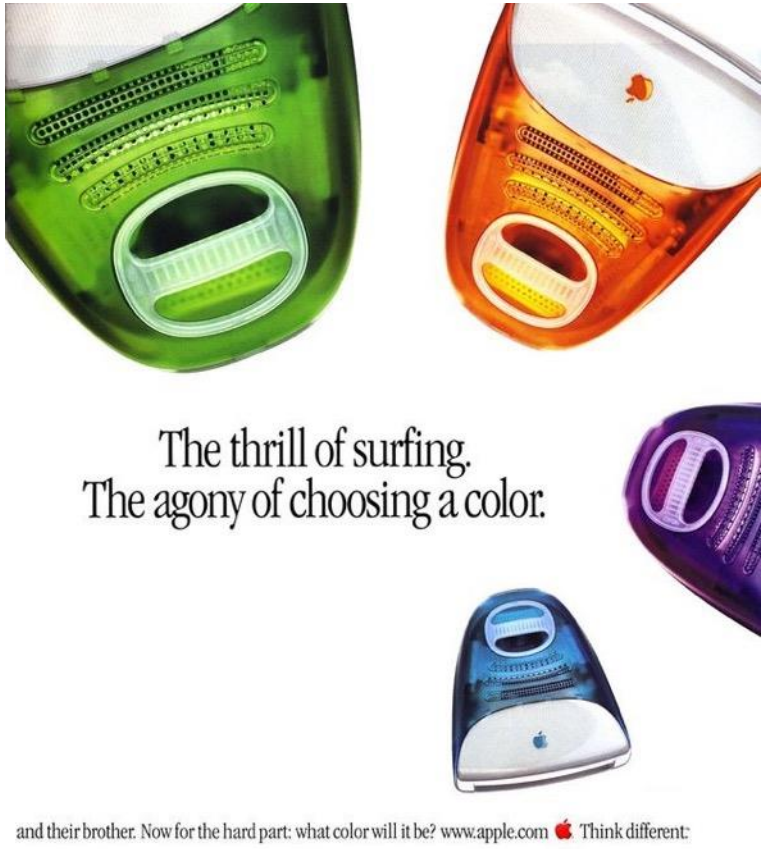
Fantastic plastic

1950s

1960s



1990s



Courtesy of Florian Meier

Large planetary health problem

June 3, 2023, APA/AFP/ISHARA S. KODIKARA

Overview of human MNP studies (by Aug 2023)

Authors	Year	Setting	Subject	Sample	Method
Pauly, et al.	1998	NY, USA	114 lung cancer patients	lung tissue	microscopy
Nayebare, et al.	2018	NY, USA	30 healthy adults	urine	HPLC-MS/MS
Schwabl, et al.	2019	EU and Asia	8 healthy volunteers	stool	FITR
Monteleone, et al.	2019	Furtwangen, DE	4 healthy volunteers	blood	FTIR
Zhang, et al,	2020	Beijing, CN	26 male students	feces	FTIR
Ibrahim, et al.	2020	Peninsular, Malaysia	11 adults	colectomy samples	stereo- and FTIR microscopy
Yan, et al.	2020	Nanjing, CN	human	feces	Raman microspectroscopy
Zhang, et al.	2021	NY, USA	3 new-born, and 6 infants, and 10 adults	meconium, feces	HPLC-MS/MS
Luqman, et al.	2021	Surabaya, IDN	11 healthy participants	stool	Raman spectroscopy
Amato-Lourenço, et al.	2021	São Paulo, BR	20 corpses	lung tissue	Raman spectroscopy
Wibowo, et al.	2021	Mojokerto, ID	11 healthy participants	stool	Raman spectroscopy
Braun, et al.	2021	Berlin, DE	2 women	placenta, meconium, and maternal stool	FTIR
Ragusa, et al.	2021	Rome, IT	4 women	placenta	Raman spectroscopy
Abbasi, et al.	2021	Iran	2000	hair, skin, saliva	Raman microspectroscopy
Liu, et al.	2022	Shanghai, CN	18 mother-infant pairs	placenta, meconium, infant feces, breast milk	8700 LDIR
Leslie, et al.	2022	AMS, NL	22 healthy volunteers	blood	Py-GC/MS
Yan, et al.	2022	Nanjing, CN	50 healthy and 52 IBD patients	feces	Raman spectroscopy
Huang, et al.	2022	Shantou, CN	22 patients with lung disease	sputum	FITR
Baeza-Martínez, et al.	2022	Alicante, ES	44 patients	bronchoalveolar lavage fluid (lower airway)	µ-FTIR
Liu, et al.	2022	Shanghai, CN	18 mother-infant pairs	placentas and meconium	LDIR
Jenner, et al.	2022	Hull, UK	11 patients	lung tissue	µFTIR
Ragusa, et al.	2022	Rome, IT	34 women	breastmilk 1 day after delivery	Raman spectroscopy
Horvatits, et al.	2022	Hamburg, DE	6 patients and 5 healthy	liver, kidney and spleen	fluorescence microscopy and µRaman
Ho, et al.	2022	Hongkong	8 adults	feces	Raman microspectroscopy
Ragusa, et al.	2022	Rome, IT	12	placenta	UHPLC-MS/MS
Baeza-Martínez, et al.	2022	Alicante, ES	44 adult patients	BALF	stereomicroscopy, µ-FTIR and SEM-EDS
Zhang, et al.	2022	Chengdu, CN	40 subjects	intestinal secretions	laser infrared imaging
Ragusa, et al.	2022	Rome, IT	10 pregnant women	placenta	VP-SEM and TEM
Chen, et al.	2022	Shanghai, CN	Non-small cell lung cancer patients	100 lung specimens with ground glass nodule	µ-FTIR, LDIR, and Raman, SEM/EDS
Zhu, et al.	2023	Wuxi, CN	17 healthy	placenta	LD-IR spectroscopy
Yang, et al.	2023	Beijing, CN	15 cardic patients	heart, blood, etc.	LDIR
Rotchell, et al.	2023	Hull, UK	5 surgery patients	saphenous vein tissue	µFTIR
Shahsavaripour, et al.	2023	Sirjan, Iran	19 workers	skin, saliva, hair	micro-Raman spectroscopy
Pironti, et al.	2023	Ancona, IT	6	urine	Raman spectroscopy
Montano, et al.	2023	Campania, IT	10 healthy men	semen	Raman microspectroscopy
Zhao, et al.	2023	Beijing, CN		30 semen and 6 testis	LD-IR and Py-GC/MS
Li, et al.	2023	Guangzhou, CN	37 newborns	meconium	3D microscope and micro-FTIR
Guan, et al.	2023	Nanjing, CN	104 patients	13 body fluids	Raman microspectroscopy
Wu, et al.	2023	Nanjing, CN	26 adult cardiovascular patients	thrombus	Raman spectroscopy
Cetin, et al.	2023	Erzurum, TR	16 colonrectal cancer patients and 15 controls	tumoral and non tumoral colon tissues	ATR-FTIR, Raman

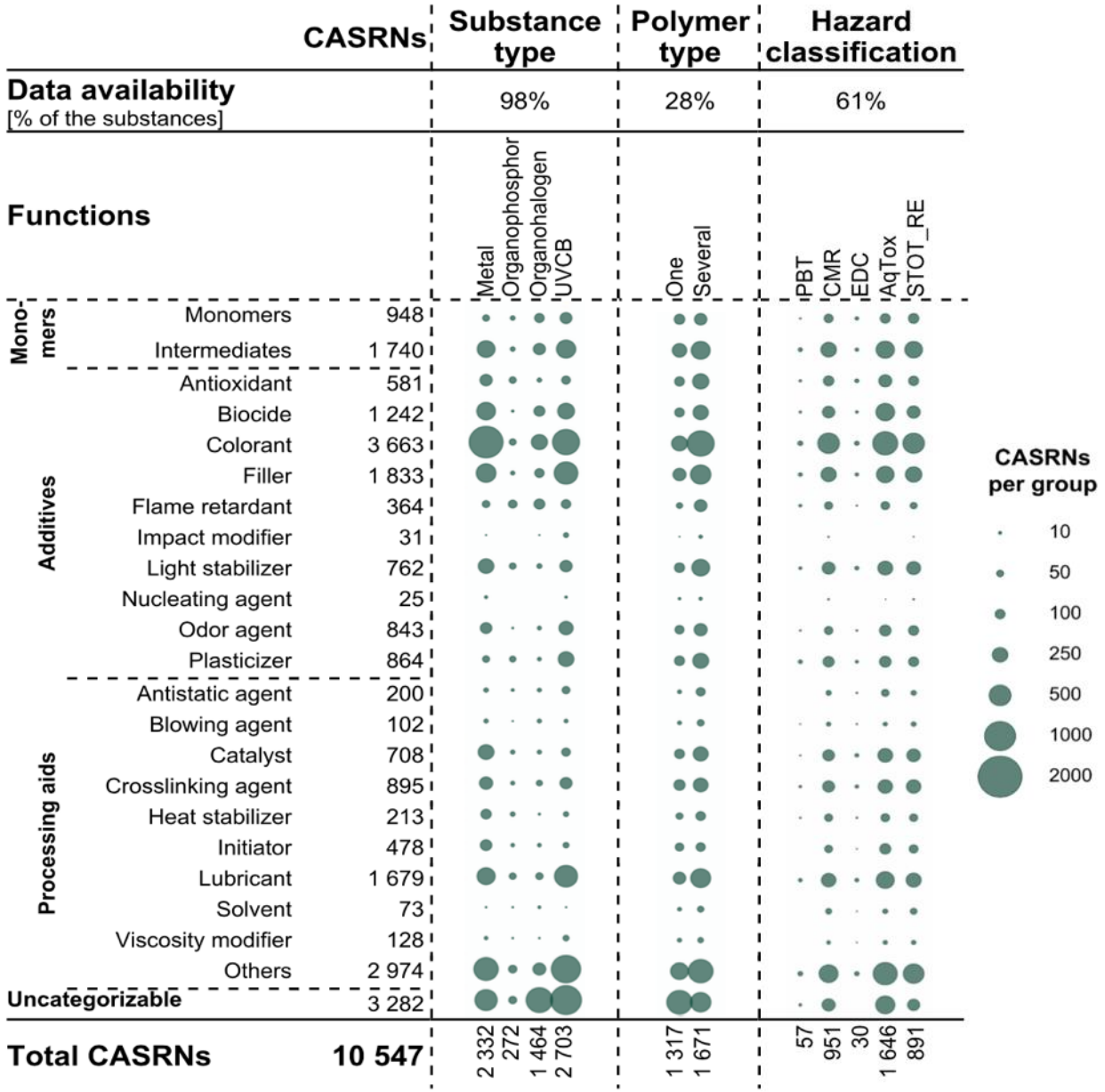
MNP is a complex exposure

- Size fractions (micro, nanoplastic)
- Morphology
  - Fibre, fragment, granule, film, foam, filament, flake

Complex exposure

- Size fractions
- Morphology
  - Fibre, fragment, granule, film, foam, filament, flake

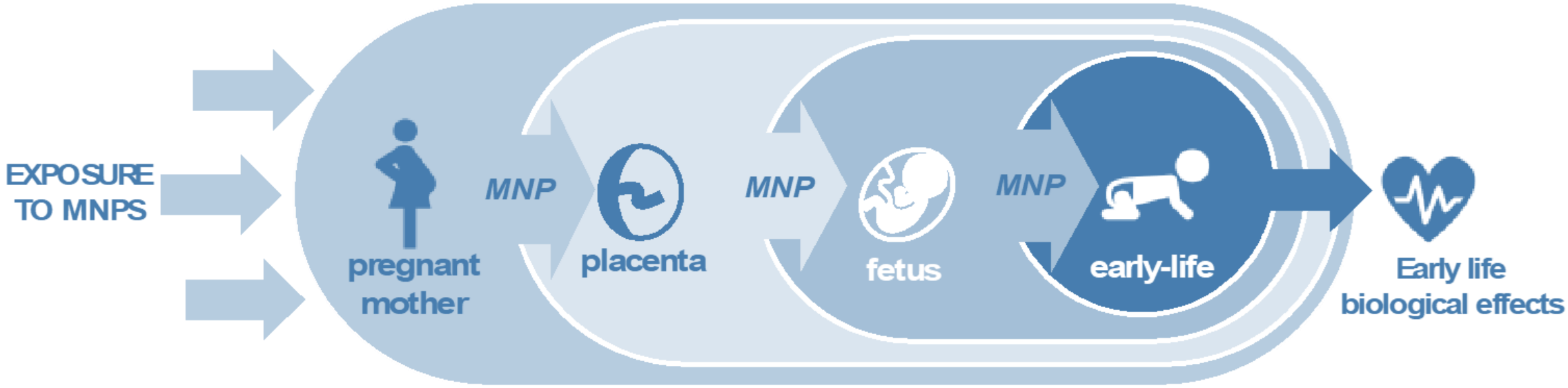
- Chemical composition
  - 5300 polymer formulations are commercially available
  - Monomers and oligomers
  - Chemical additives (e.g., plasticizers, flame retardants, stabilizers, pigments, biocides) → up to 50% weight
  - Non-intentionally added substances (i.e., impurities, reaction by-products, degradation products)
- Solubility
- Surface chemistry
- Adsorbed/absorbed
  - Microbes/bioflims, chemicals, metals



Adopted from Weithmann et al Science Advances 2018

AURORA

Goal: to develop a roadmap for assessing the health risks of micro- and nanoplastics for **early-life health**



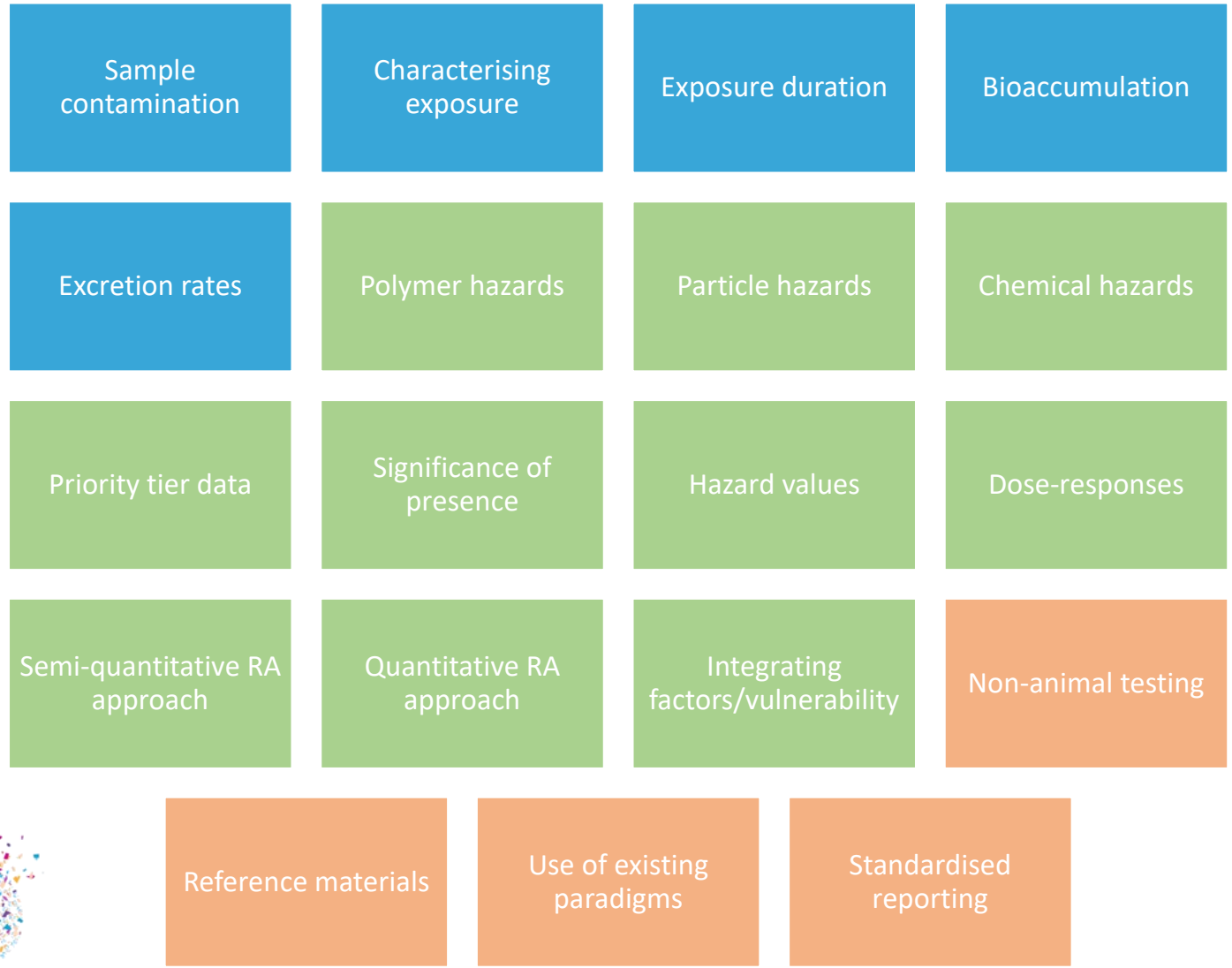
Why Early Life Health?

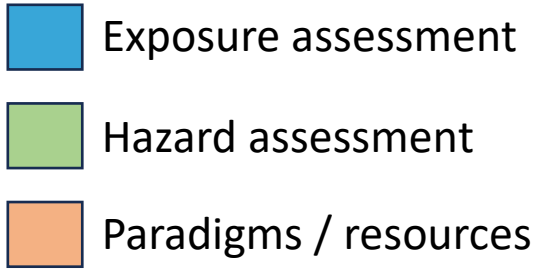
Vulnerable time period

Longlasting effects into adulthood

Previous evidence of placent transfer of particles and chemicals related to impaired child development

Current gaps in Human Health Risk Assessment Framework MNPs on early-life health

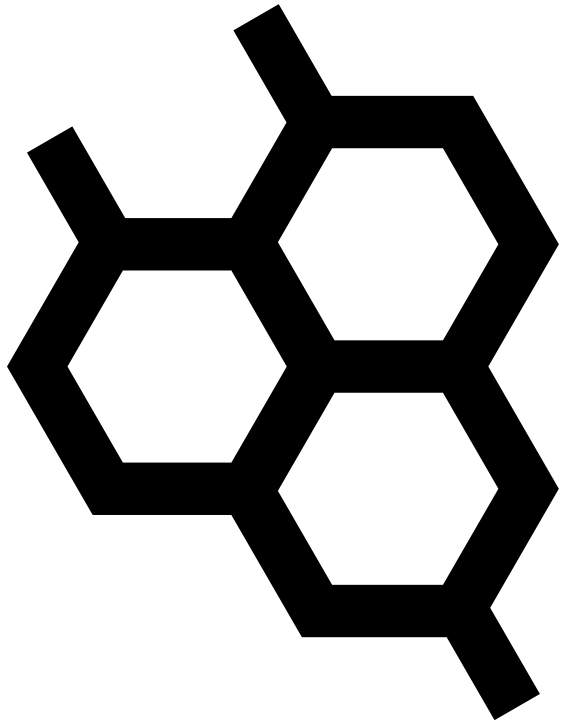




Christopher et al. Microplastics and Nanoplastics https://doi.org/10.1186/s43591-024-00089-3

MNP Quantification

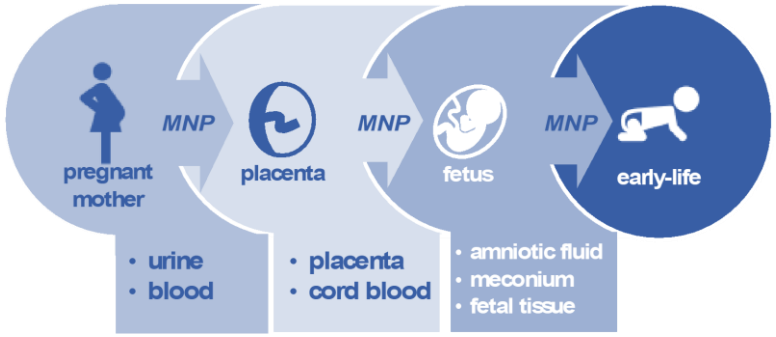
Spectro-Microscopic techniques

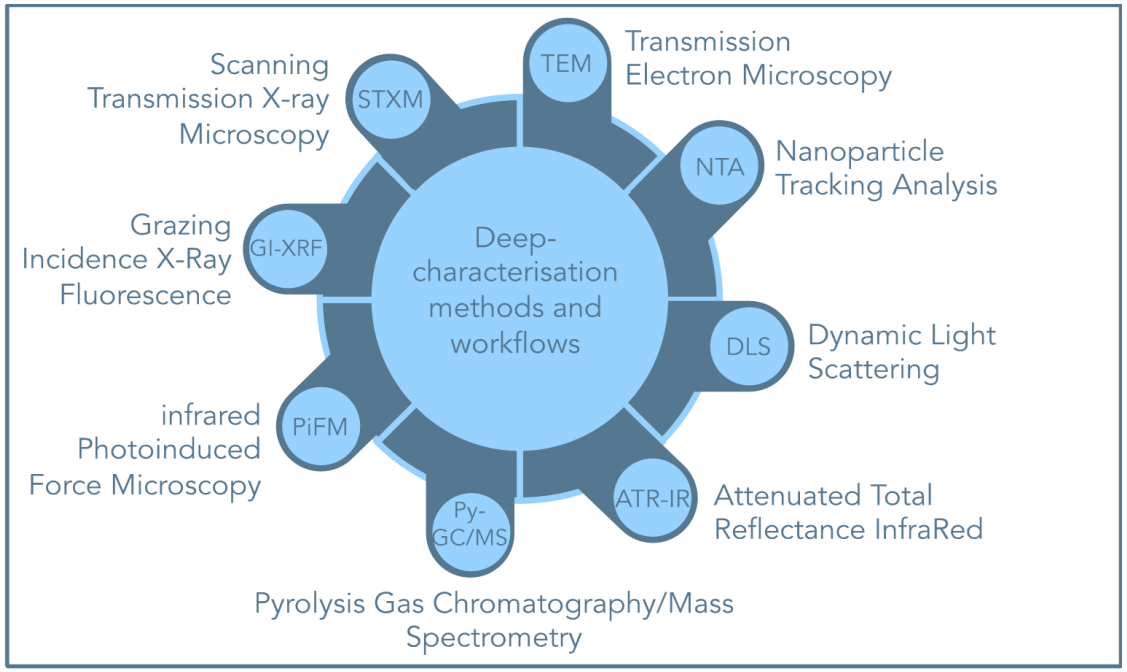


Chromatography/Mass-spectrometry

MNP Quantification – Slow but Deep

1. **develop new methods for in-depth characterization of micro- and nanoplastics in complex matrices** (human tissues: urine, blood, placenta)



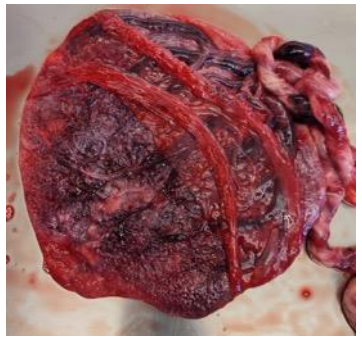


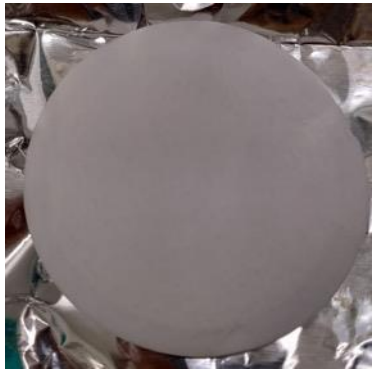
Microscopy, Spectroscopy, Chromatography

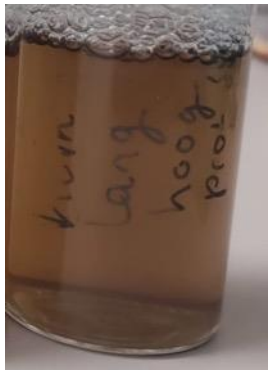
Particle count, Mass, Chemical Composition, Size, Morphology, Surface Chemistry

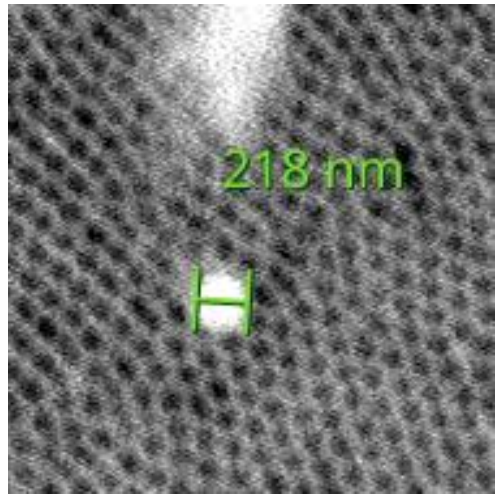
Develop SOP for MNPs in complex sample matrices

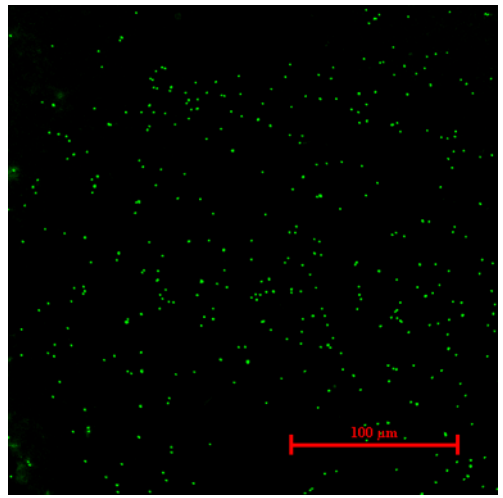
Laura Zoutendijk











- Recovery rate = particles detected in droplet/theoretical number of particles in droplet in [±90%]

MNP in placenta samples


Laura Zoutendijk & Britt Juffer

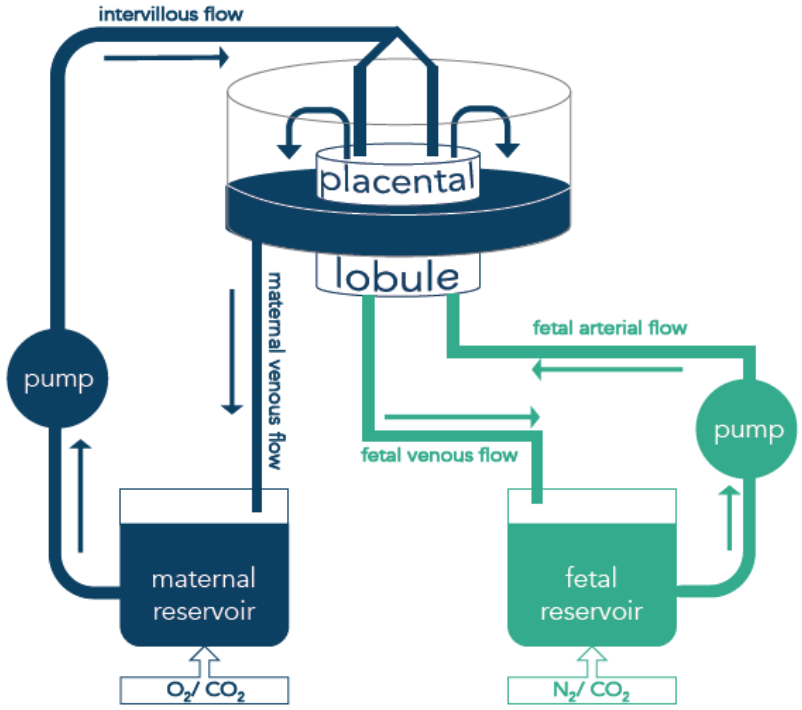
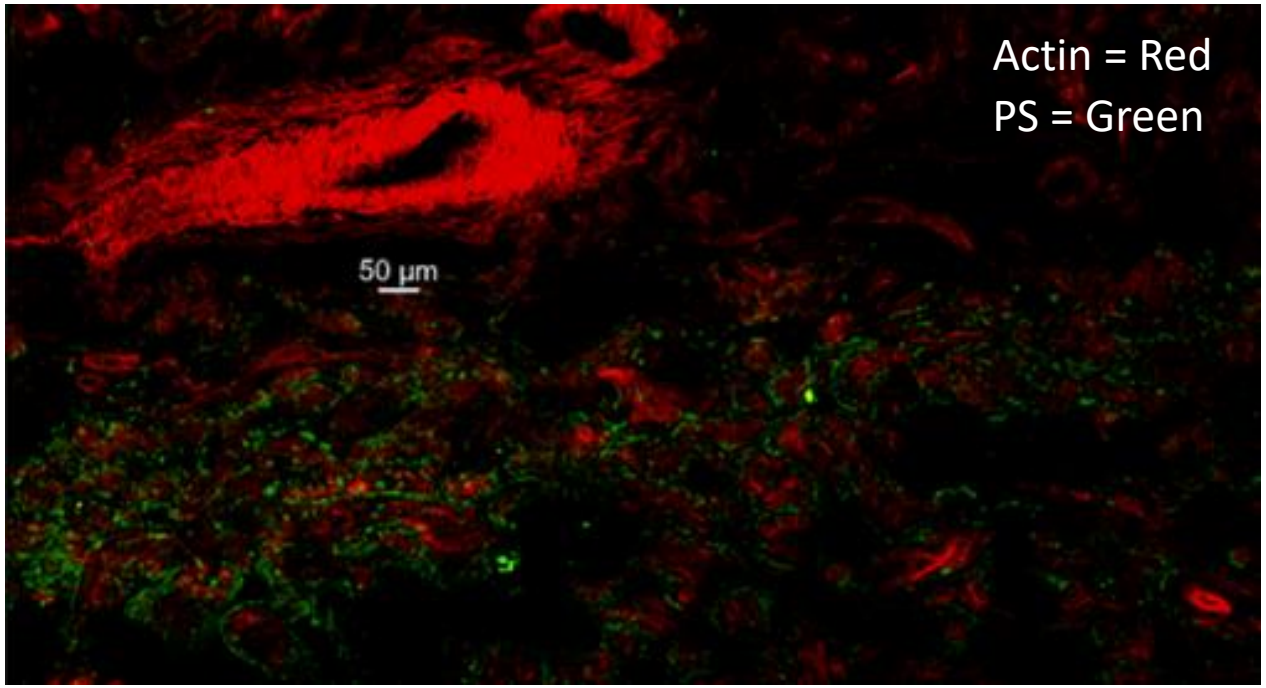


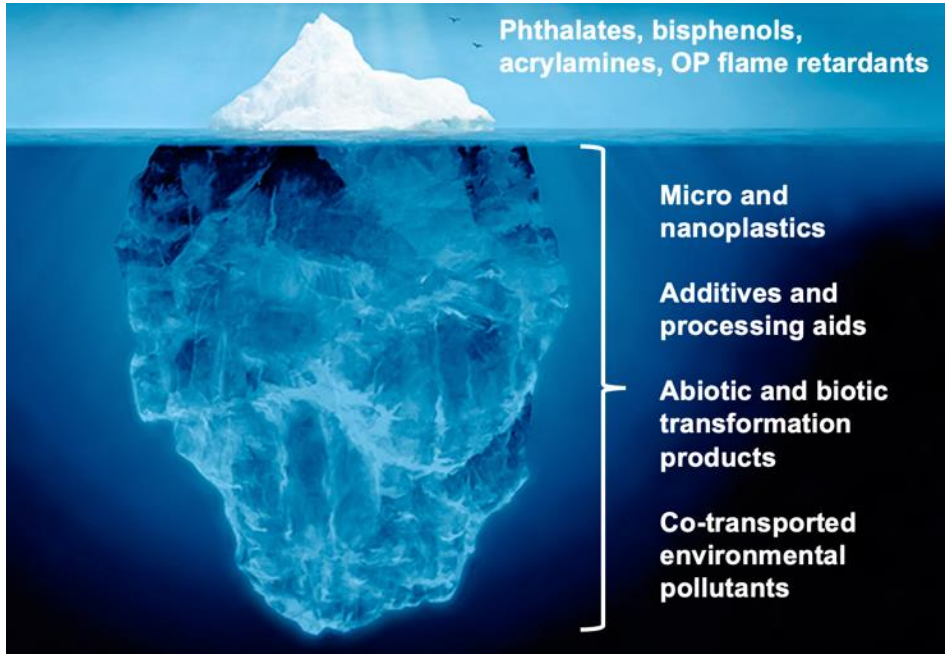
Fig. 17: The placental perfusion model.

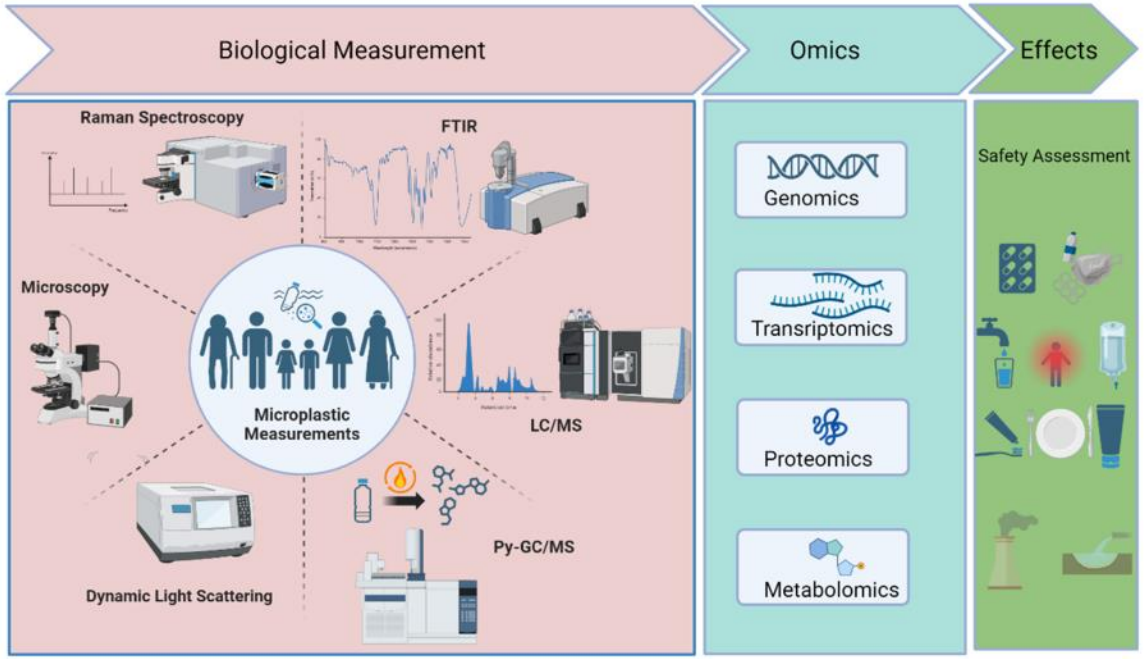


Placenta perfusion with fluorescent polystyrene particles (50 nm). Image through chemical force microscopy (CFM).

MNP Quantification – Scalability and complexity

Advancements in Assays for Micro- and Nano-Plastic Detection: Paving the Way for Biomonitoring and Exposomics Studies



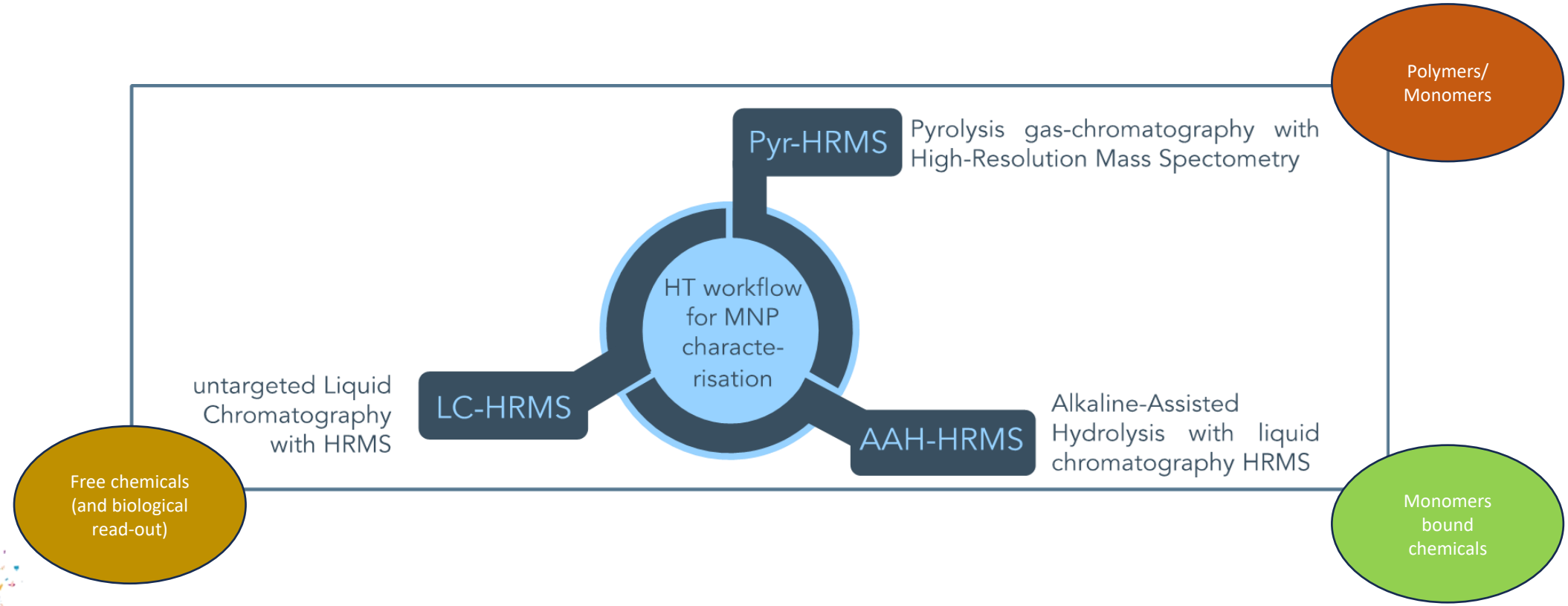


Annual Review of Pharmacology and Toxicology

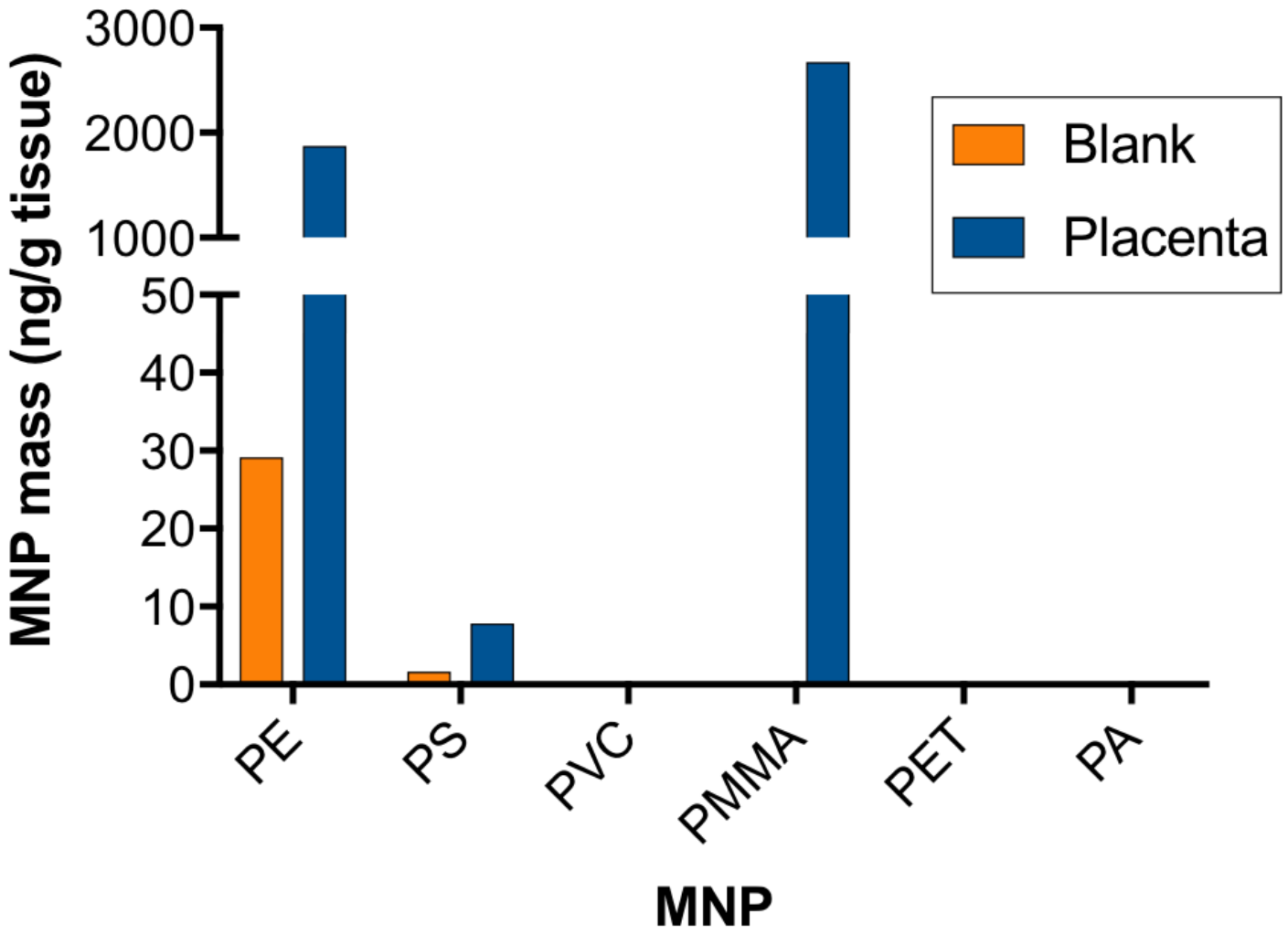
Kuanliang Shao, et al. In Press

MNP Quantification – Fast and Wide

Innovate high-throughput methods for use in large scale health (biomonitoring) studies (FAST-ME)

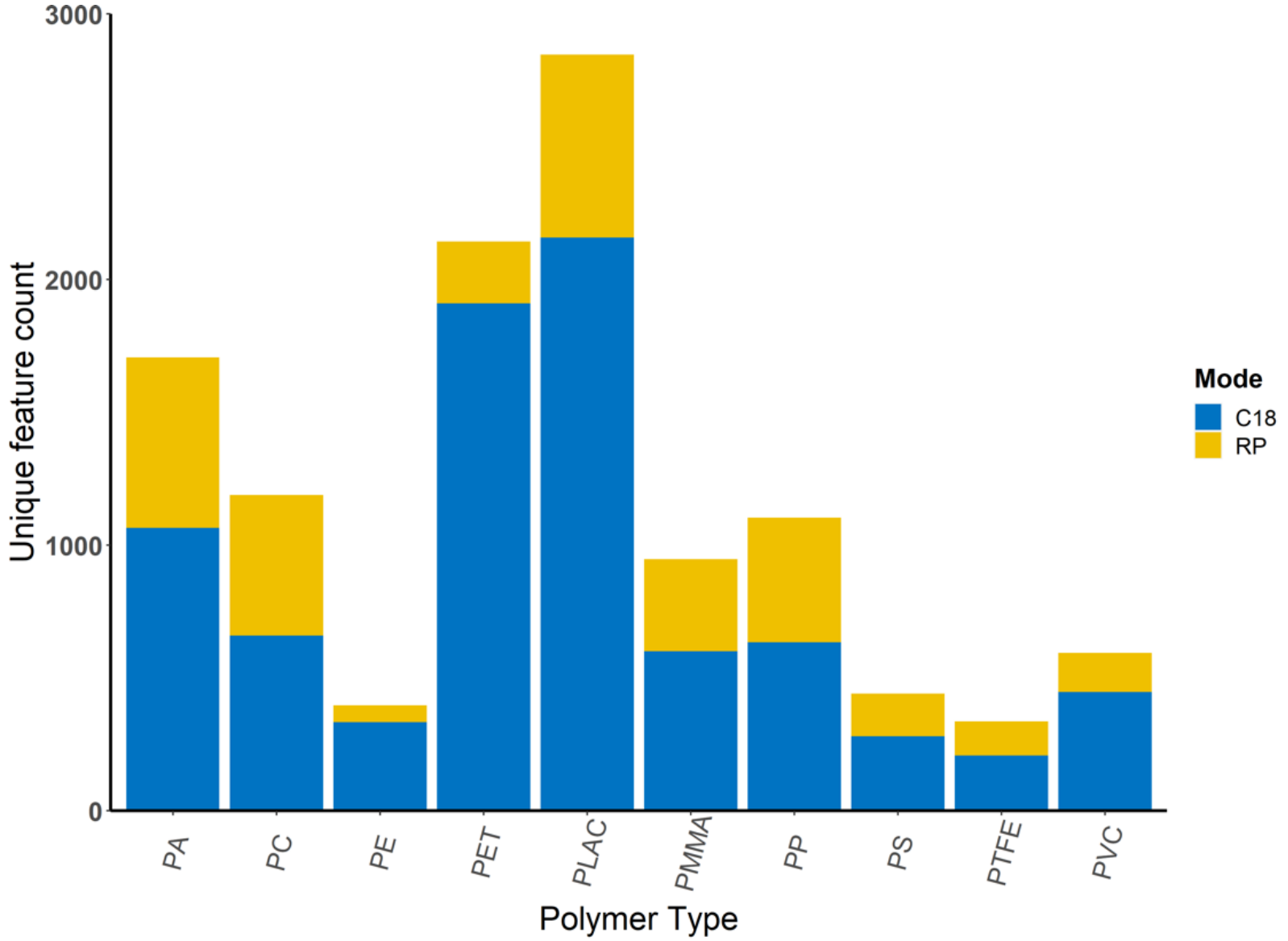


Pyrolysis with GC-HRMS mass-based measurements of select polymers in human placenta



Courtesy Doug Walker, unpublished

Alkaline-assisted hydrolysis and dissolution provides unique chemical profiles for each polymer

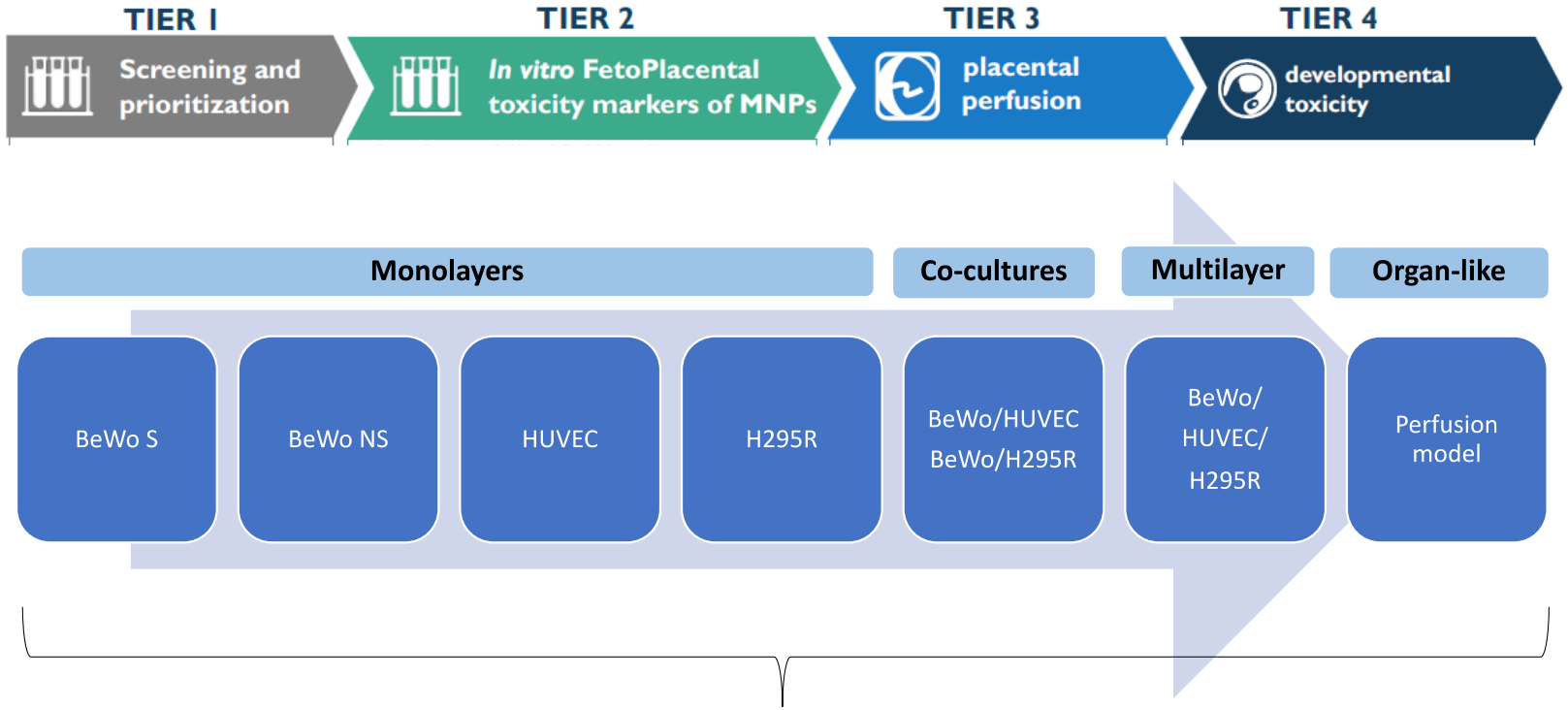


Courtesy Doug Walker, unpublished



Hazard, in-vitro

Assess health effects in placenta and the developing fetus of common polymers, bioplastics, common mixtures (considering size, shape, degradation)



MNP uptake/transport and effects on placental integrity and function:

endocrine function, metabolism, immune responses, premature aging…

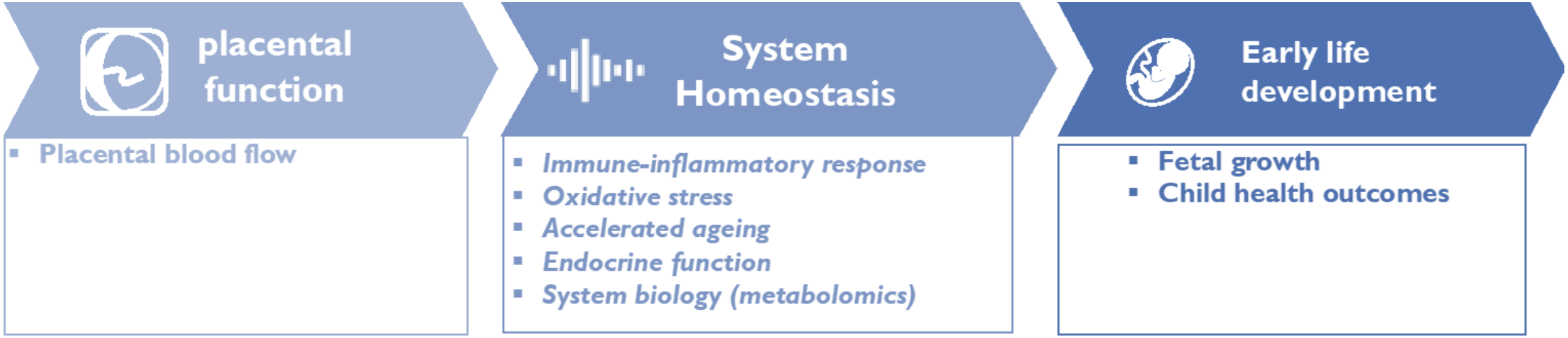
TIER 1 and 2 Summary – To date (~25 polymers tested)

	Assay type	Method	Endpoint	Indicative of
TIER 1	Cytotoxicity	CellTiter Glo	**ATP** production	metabolic activity
	Membrane integrity	LDH leakage	extracellular **LDH**	membrane disruption
	Oxidative stress	CellROX™ fluo probe	**ROS** production	oxidative stress
TIER 2	Gene expression	qPCRs	interleukin 6 (**IL-6**)	inflammation
			interleukin 8 (**CXCL8**)	inflammation
			nuclear factor eryth	defense against oxidative stress
			nuc	inflammation/apoptosis
			(**BAX**)	activator of apoptosis
			ycin Dependent Kinase Inhibitor 1A (**CDKN1A**)	cell cycle arrest
	Cytokine secretion	ELISA	interleukin 6 (**IL-6**)	pro-inflammatory
			interleukin 8 (**IL-8**)	pro-inflammatory
			tumor necrosis factor-alpha (**TNFα**)	pro-inflammatory
			interleukin 10 (**IL-10**)	anti-inflammatory
			interleukin 1-beta (**IL1β**)	pro-inflammatory

Minor effects; mostly at 100 µg/mL!

Hazard, risk assessment – Human studies

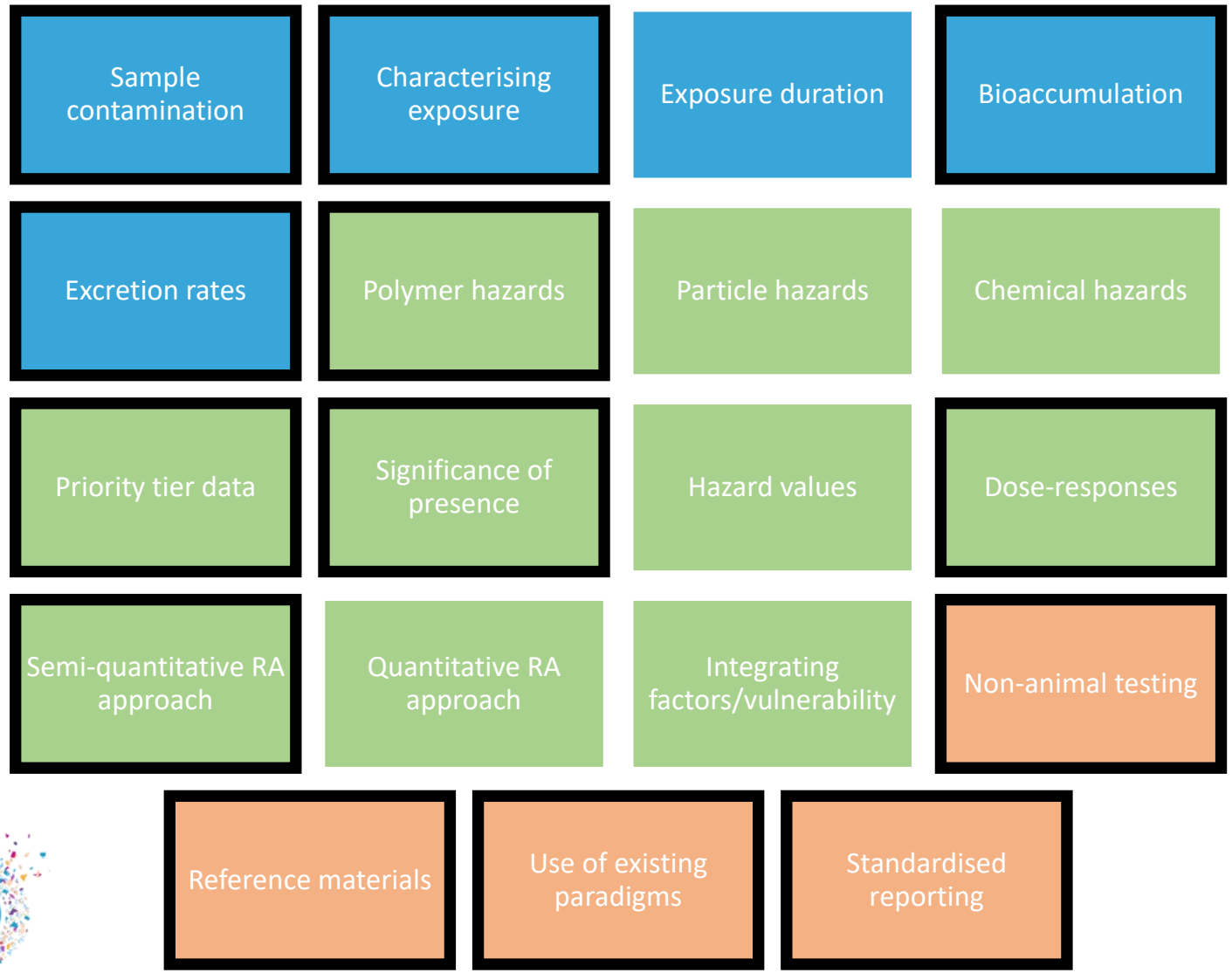
Study health effects of micro- and nanoplastics exposure (and associated chemicals) in birth cohorts (800 placenta, cord blood samples currently analysed)



Human studies - Contamination

- ***de novo* collection (n=25) – from mother to fetus (ongoing) - ENVIRONAGE birth cohort**
  - Glass collection tubes (**blood mother, cord blood**, urine mother)
  - Aluminum foil for tissue storage (**placenta tissue**)

Current gaps in Human Health Risk Assessment Framework MNPs on early-life health



Exposure assessment

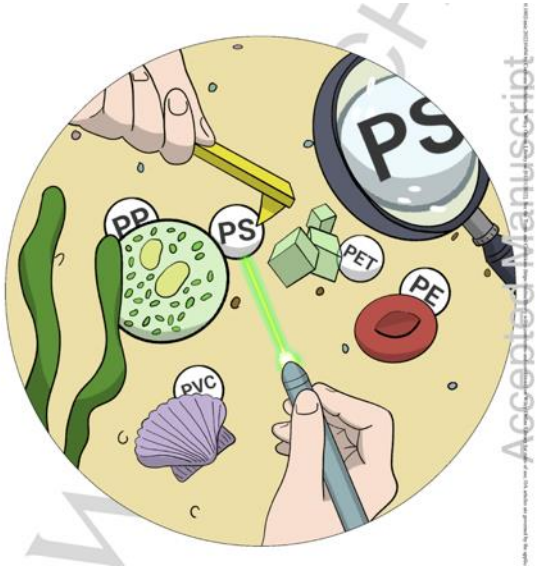
Hazard assessment

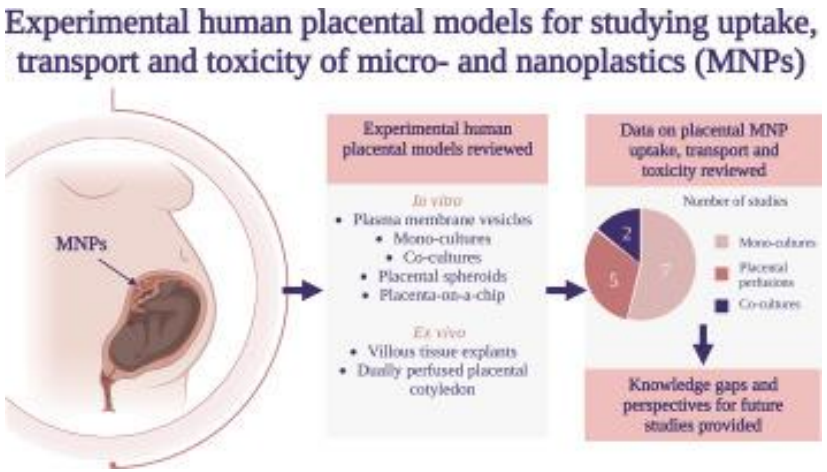
Paradigms / resources

Christopher et al. Microplastics and Nanoplastics https://doi.org/10.1186/s43591-024-00089-3

Publications

- Mandemaker LDB, Meirer F. Spectro-Microscopic Techniques for Studying Nanoplastics in the Environment and in Organisms. *Angew Chem Int Ed.* 2022; https://doi.org/10.1002/anie.202210494. (invited mini-review)
- Dusza HM, …, Vähäkangas KH. Experimental human placental models for studying uptake, transport and toxicity of micro-and nanoplastics. *Sci Total Environ.* 2022; https://doi.org/10.1016/j.scitotenv.2022.160403.
- Dusza HM, …, Legler J. Uptake, Transport, and Toxicity of Pristine and Weathered Micro-and Nanoplastics in Human Placenta Cells. Environ Health Perspectives, 2022; https://doi.org/10.1289/EHP10873.
- Shao K, …, Walker DI. Advancements in Assays for Micro- and Nanoplastic dectection: Paving the Way for Biomonitoring and Exposomics Studies. *Annu Rev Pharmacol Toxicol*. In press. (invited review)
- Christopher EA, …, Boyles MSP. Impacts of Micro- and Nanoplastics on Early-life Health: A Roadmap Towards Risk Assessment. *Microplastics and Nanoplastics*. In press.





Thanks

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Amanda Durkin, UMC Utrecht
Jane Muncke, Food Packaging Forum
Douglas Walker, Emory University
Petra Přibylová, Masaryk University
Nelly Saenen, Hasselt University
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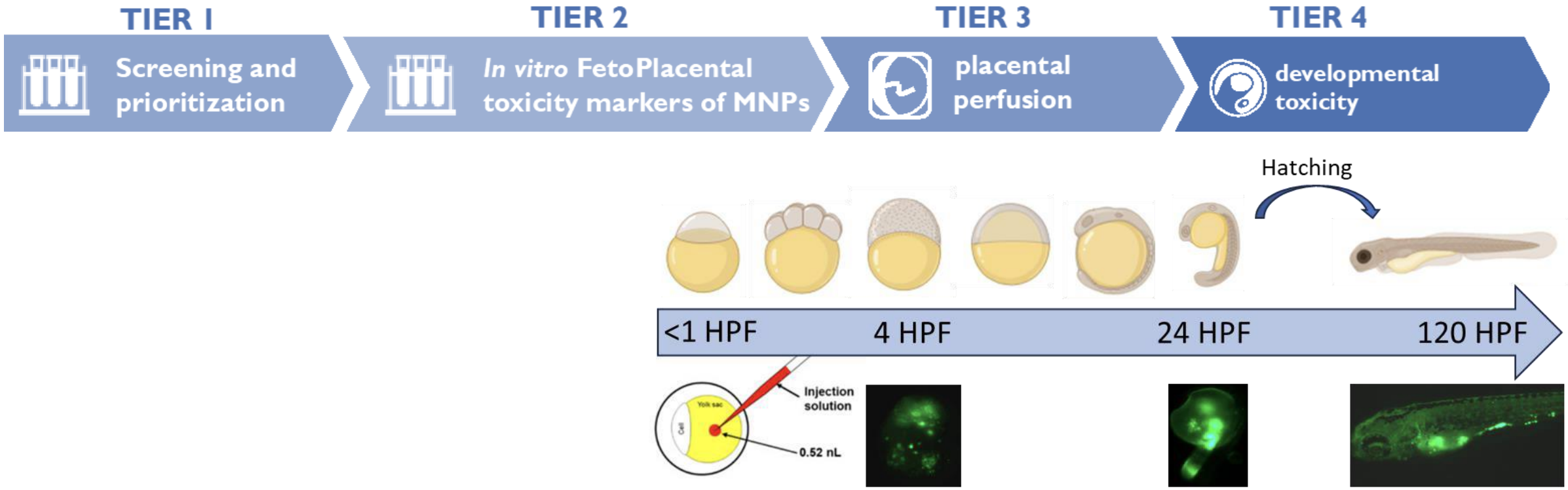
Hazard, in-vitro



Human studies – Exposure assessment

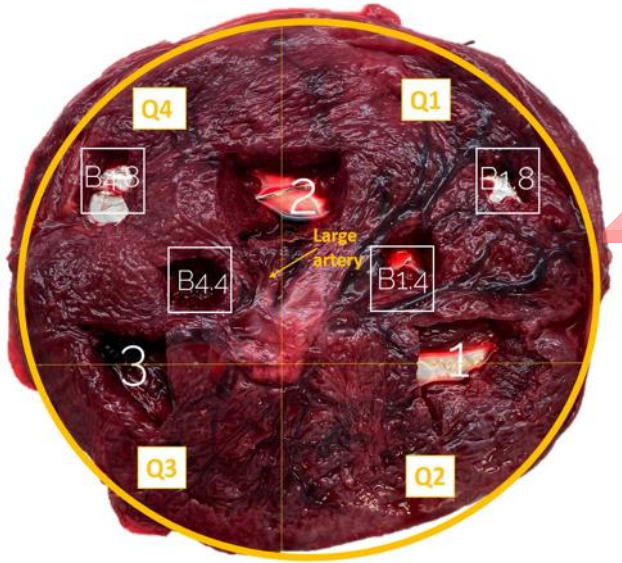
- 80 biopsies from 10 placenta's for MNP's inter- and intra-variability study transferred

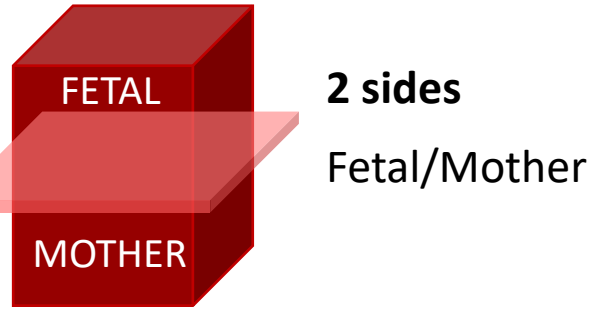
2 regions

Q1+Q4

2 distances

4 – 8 cm





2 sides

Fetal/Mother