Progress with Reference Materials in Europe

Wendel Wohlleben (BASF), Luke Parker (TNO)

Acknowledgments ECO59 and C10: Katherine Santizo, Hannah Mangold, George Sarau, Zeynab Mirzaei, Silke Christiansen, Tanja Hansen

Fraunhofer ITEM

BASF We create chemistry

Fraunhofer IKTS

LRI Long-Range Research Initiative

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Acknowledgements BRIGID: Elena Höppener, Andrea Brunner, Ingeborg Kooter, Evita van de Steeg, Dónal van Uunen, Maria Kloukinioti, Nanofract

PLASTICS EUROPE

Enabling a sustainable future

Selection of microplastics reference materials

- Describe Molecular and Particle Descriptors for Microplastic Particles
  - Polymer Backbone, Additives, Size Distribution, Degree of Crystallinity, etc.
- Final Descriptors will be chosen based on relevance for modeling approach and regulations
- Testing must be available for all test materials to demonstrate micronised test materials are representative of reference materials

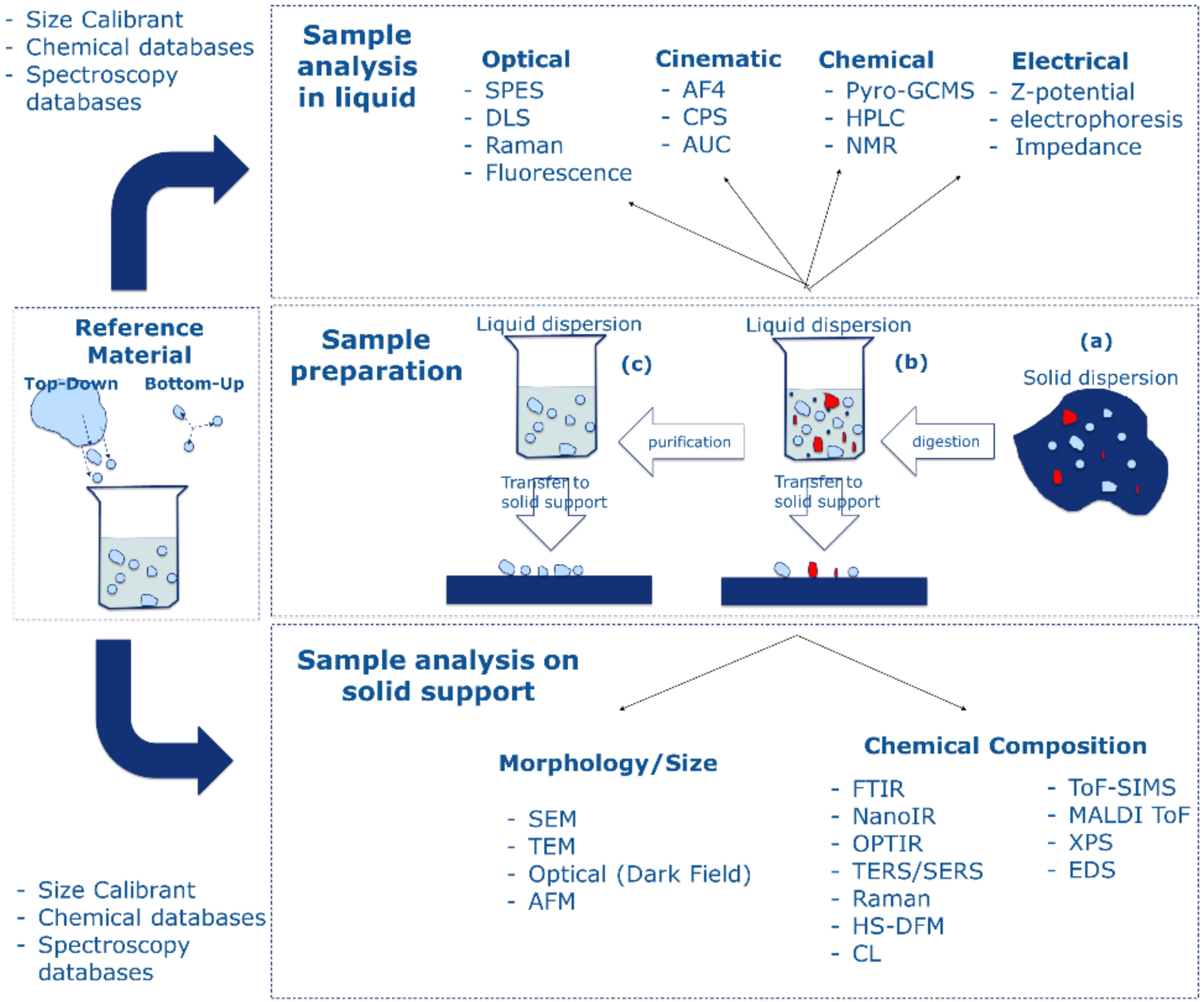

Figure courtesy of Andrea Valsesia, JRC-Ispra (SETAC panel with WW and Meredith Seeley, NIST)

Selection and physico-chemical characterisation

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- Final Descriptors will be chosen based on relevance for modeling approach and regulations
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Material Property	Analytical Method	Regulatory Criteria
Chemical Composition	Raman and FTIR	OECD_PLC, ECHA_restriction, polymer-REACH
Molecular Weight	GPC	OECD_PLC,
Molecular Mobility	TD-NMR	ECHA_restriction of „solids“
Crystallinity	DSC	Polymer-REACH
Particle Size Distribution	Mastersizer (micro) & AUC (nano)	Polymer-REACH, (ECHA_restriction)
Shape (morphology)	SEM	Polymer-REACH
Density	He-Pycnometry	-
Surface (re) activity	EPR	OECD_PLC, polymer-REACH
Surface Charge	Gel Electrophoresis	OECD_PLC, polymer-REACH
Impurities	HS-GC	-
Endotoxin	LAL Assay	-

Reference material progress at SETAC-EU (Dublin May 2023)

- Valsesia & team, JRC Ispra, IT (UV aging at NIST Gaithersburg)
  - Sequential stresses → nanoplastics
- Altmann & team, BAM Berlin, DE
  - Sequential & repeated stresses → respirable particles
  - Solvent precipitation → respirable particles / nanoplastics
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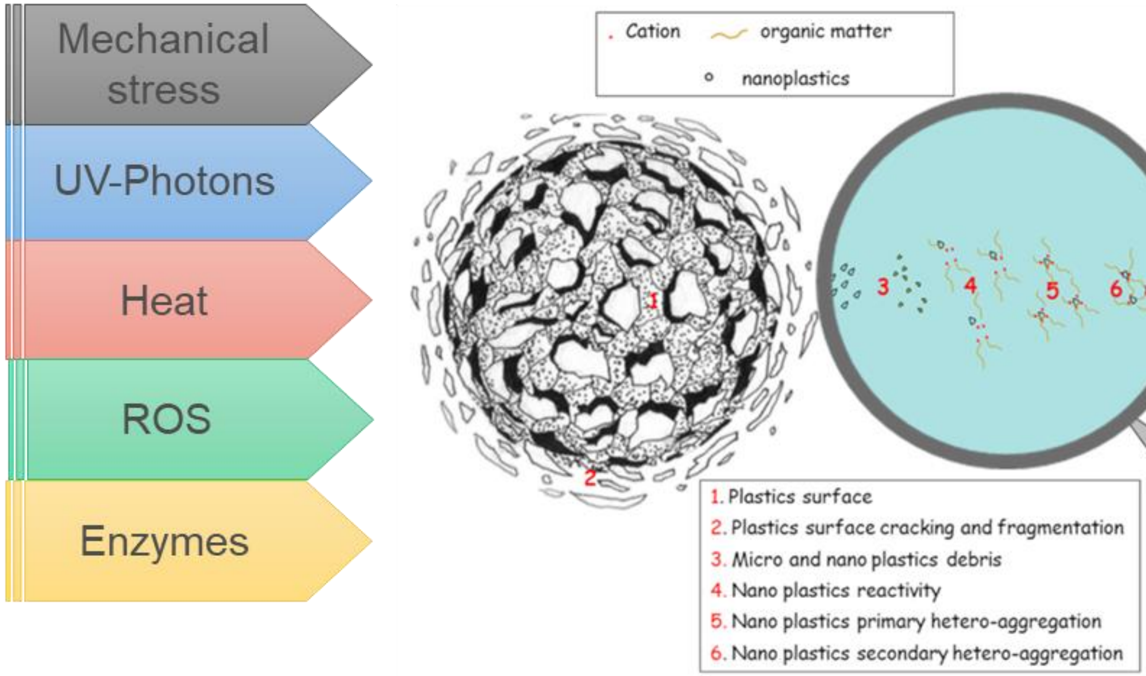

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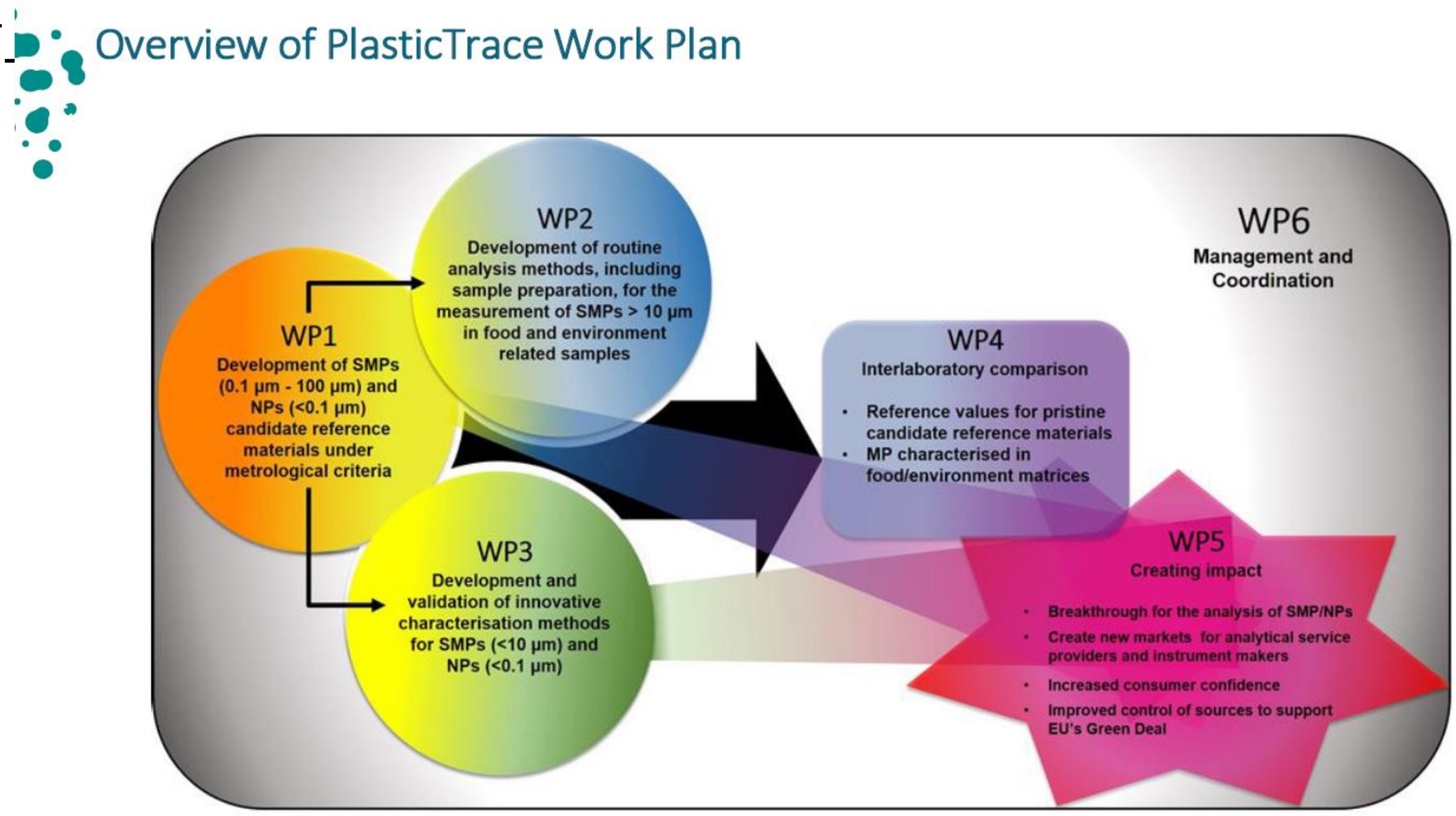

Slide courtesy of Korinna Altmann (BAM), shown at CUSP cluster & PlasticsEurope meeting

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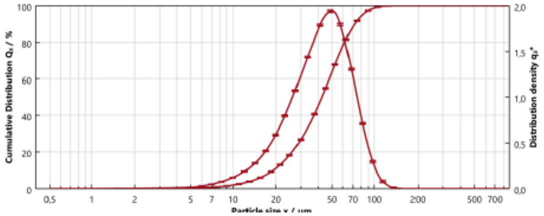
  - <u>Distribution as tablets + ILCs</u>

Harmonisation of samples

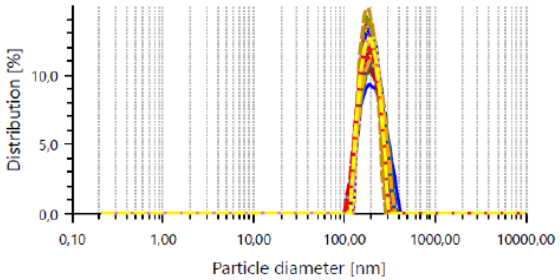
- Top down
- Starting material: granulate (kindly provided by PlasticEurope)

<u>Homogeneity control</u>

1. Microplastics (10 – 100 µm)
   - ➢ Polyethylene terephthalate
   - ➢ Used in WP2
   - ➢ D50: 42.4 µm



2. Nanoplastics (< 1 µm)
   - ➢ Polypropylene
   - ➢ Used in WP3
   - ➢ D50: 207.2 nm



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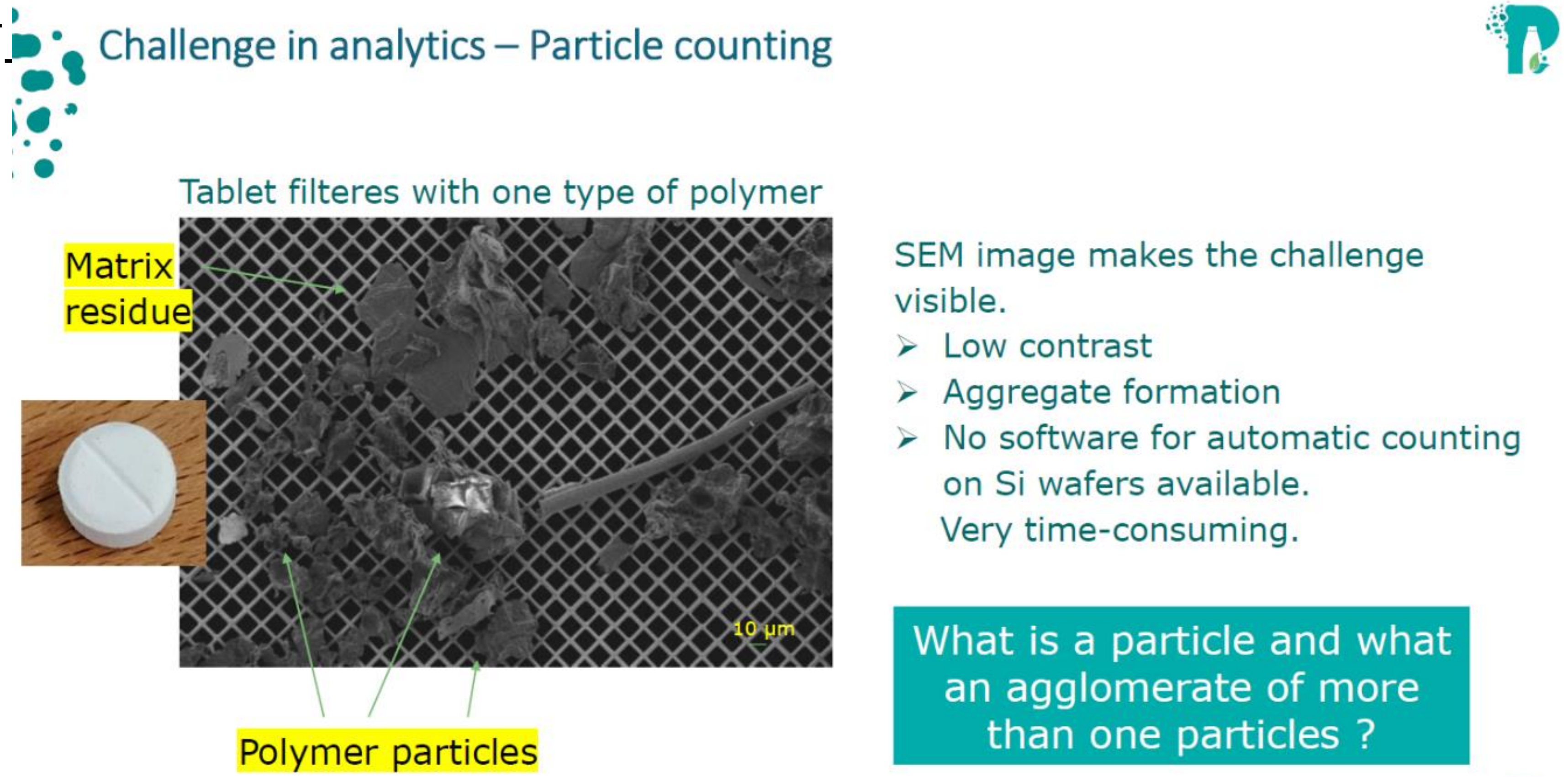

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Aqueous Dispersions of Polypropylene: Toward Reference Materials for Characterizing Nanoplastics

*Jana Hildebrandt and Andreas F. Thünemann**

DOI: 10.1002/marc.202200874

Figure 1. Bottle containing 10 mL of an aqueous dispersion of polypropylene nanoparticles. The dispersion is illuminated with a green laser from the left side to illustrate the light scattering properties.

Gravimetric concentration determination was performed three times. The concentration of polymer in the dispersion was 41 ± 4 mg L^{-1}.

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A new concept for the ecotoxicological assessment of plastics under consideration of ageing processes

Marcus Lukas*[1], Maria Kittner[2], Lisa Isernhinke[1], Korinna Altmann[2], Ulrike Braun[1]

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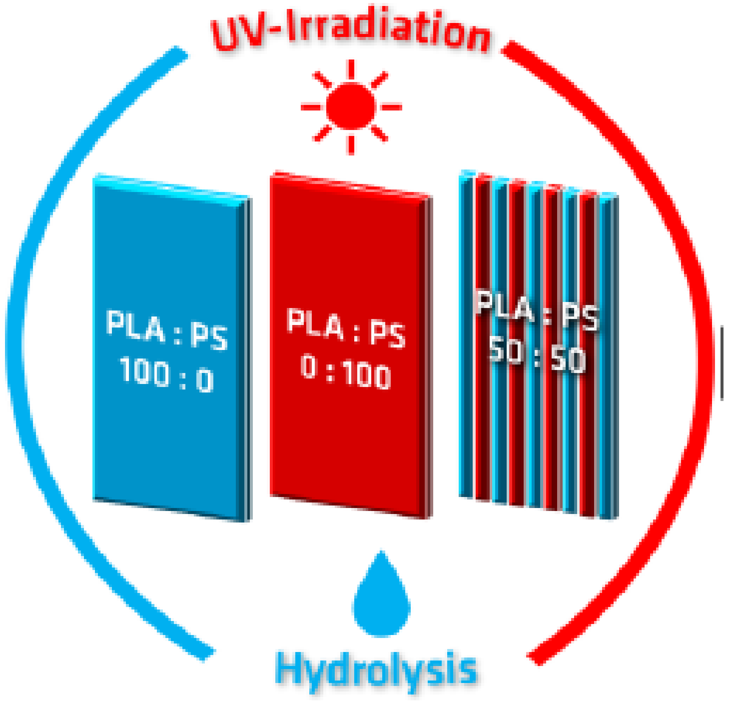
2 Federal Institute for Materials Research and Testing (BAM), Unter den Eichen 87, 12205 Berlin, Germany

* Corresponding author (marcus.lukas@uba.de)



representing a realistic scenario in the environment. Test specimens of PS, PLA or a PLA/PS layer (each 50 %) were alternately exposed to UV radiation for five days followed by hydrolysis for two days, for several weeks alternating. Ecotoxicological effects of the storage

doi: 10.1002/appl.202200124.

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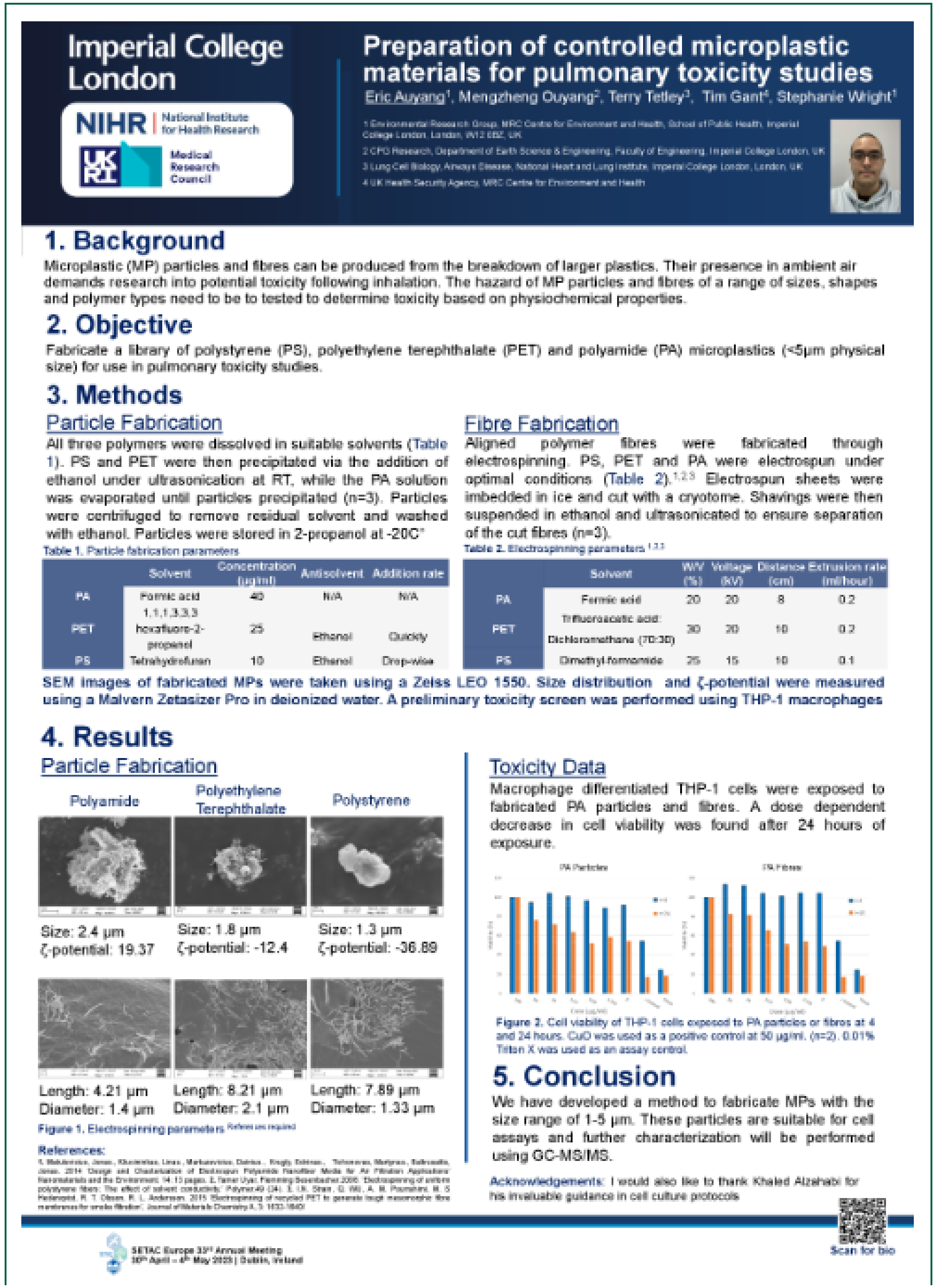
  - <u>Electrospray + microtome → fibers</u>



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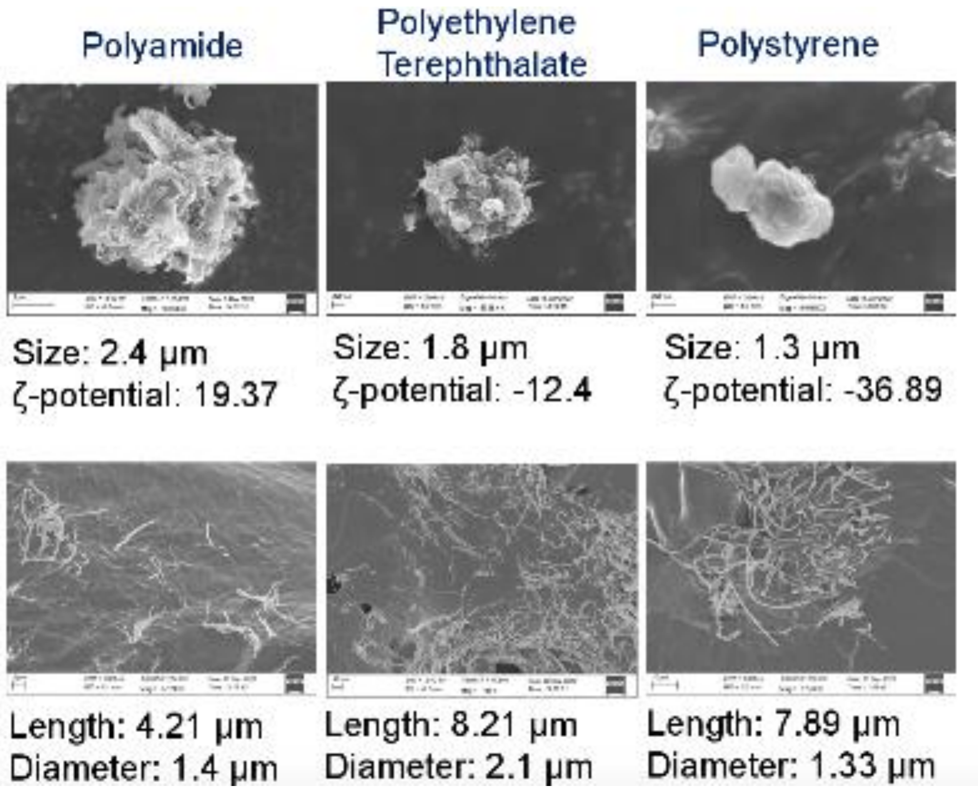

Particle Fabrication

All three polymers were dissolved in suitable solvents (Table 1). PS and PET were then precipitated via the addition of ethanol under ultrasonication at RT, while the PA solution was evaporated until particles precipitated (n=3). Particles were centrifuged to remove residual solvent and washed with ethanol. Particles were stored in 2-propanol at -20C°

Table 1. Particle fabrication parameters

	Solvent	Concentration (μg/ml)	Antisolvent	Addition rate
PA	Formic acid	40	N/A	N/A
PET	1,1,1,3,3,3 hexafluoro-2-propanol	25	Ethanol	Quickly
PS	Tetrahydrofuran	10	Ethanol	Drop-wise

Fibre Fabrication

Aligned polymer fibres were fabricated through electrospinning. PS, PET and PA were electrospun under optimal conditions (Table 2).[1,2,3] Electrospun sheets were imbedded in ice and cut with a cryotome. Shavings were then suspended in ethanol and ultrasonicated to ensure separation of the cut fibres (n=3).

Table 2. Electrospinning parameters [1,2,3]

	Solvent	W/V (%)	Voltage (kV)	Distance (cm)	Extrusion rate (ml/hour)
PA	Formic acid	20	20	8	0.2
PET	Trifluoroacetic acid: Dichloromethane (70:30)	30	20	10	0.2
PS	Dimethyl-formamide	25	15	10	0.1

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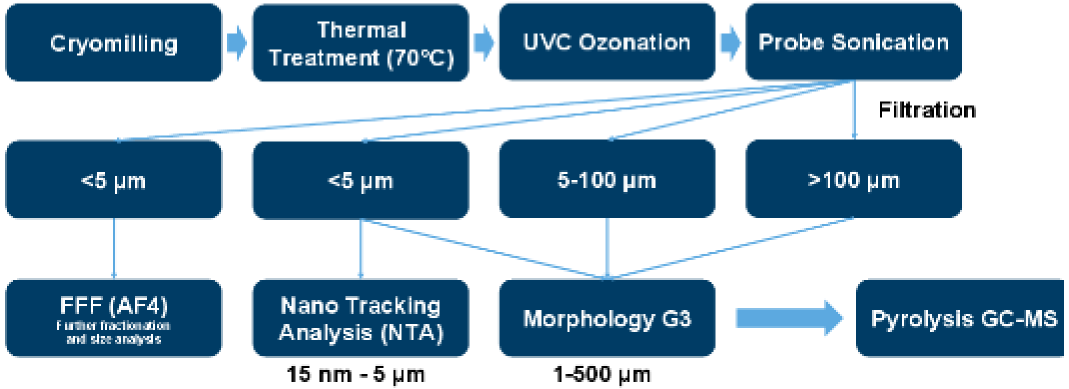
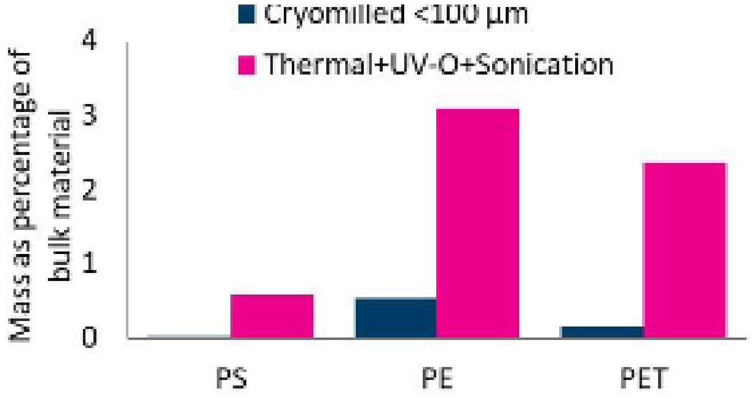


Fig 2: Overview of the degradation protocol and hyphenated fractionation and multi-instrument identification and quantification workflow for sMP and NP characterisation.

- UV-C ozonation increased the relative mass yield of NP (<5 µm) over small MP (5-100 µm) for all three polymers, with particular increase of particles between 1-7 µm (Figs. 3 & 4).

Future prospects

- UV treatment at some point in the top-down MP/NP production process is important for creating environmentally relevant reference materials.
- Need a focus on methods that increase the yield of particles <10 µm, especially those <1 µm.
- Assessment of UV-C vs UV-B as an accelerated aging method for producing environmentally degraded NP/MP test materials.



SETAC poster (abstract public at sintef.no)

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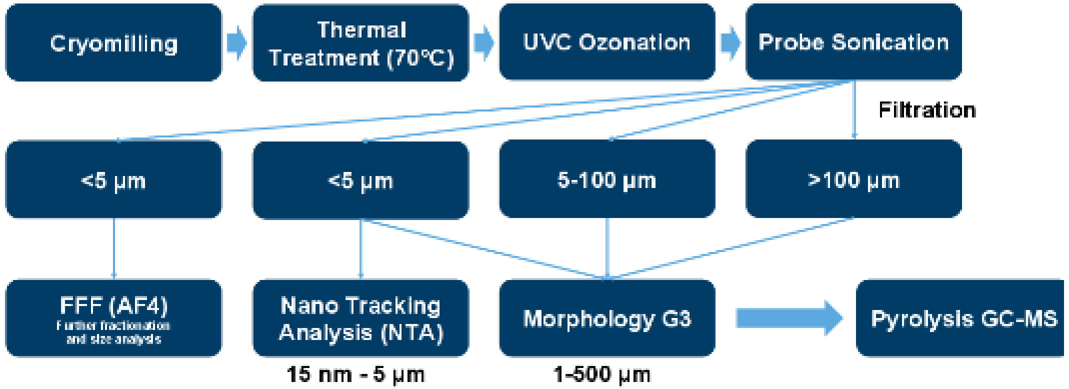

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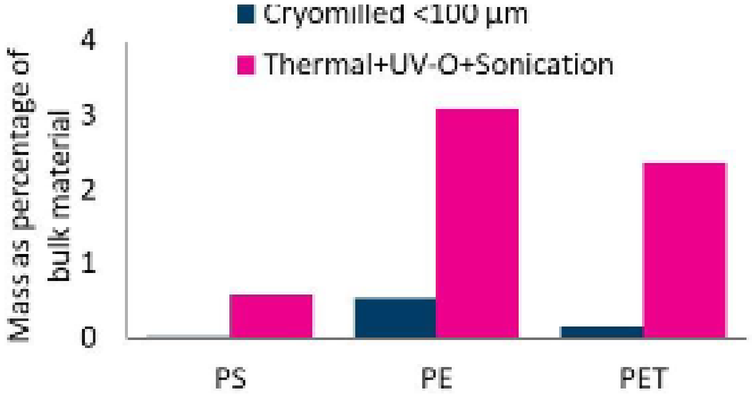
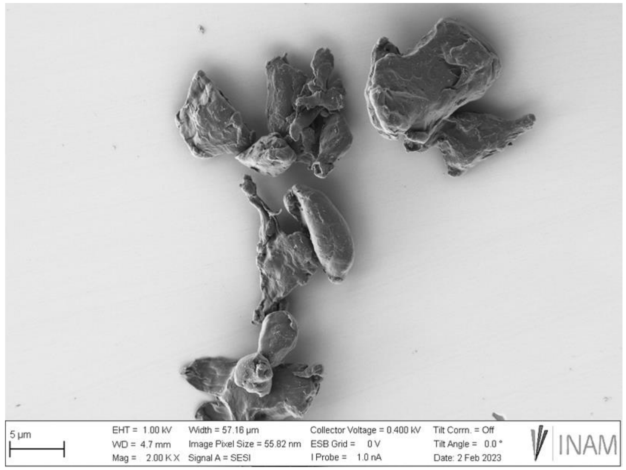


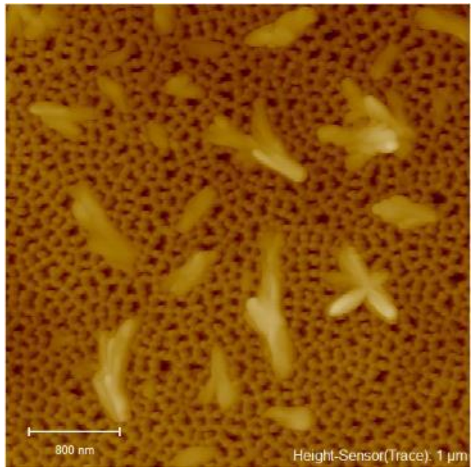
SETAC poster, abstract public at sintef.no

Polyamide in different sizes

PA_inhalable (7µm mass)



PA_precip (100nm mass)

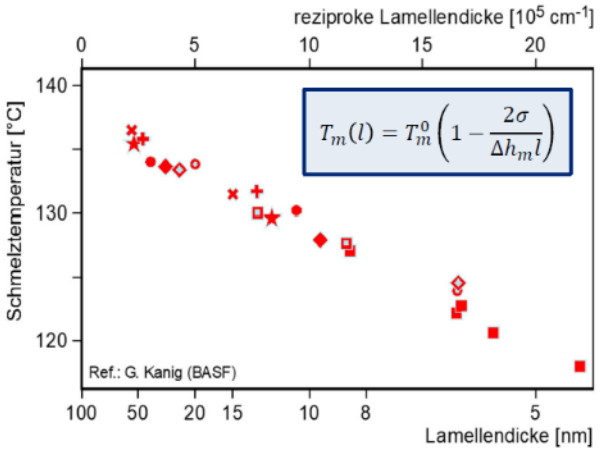


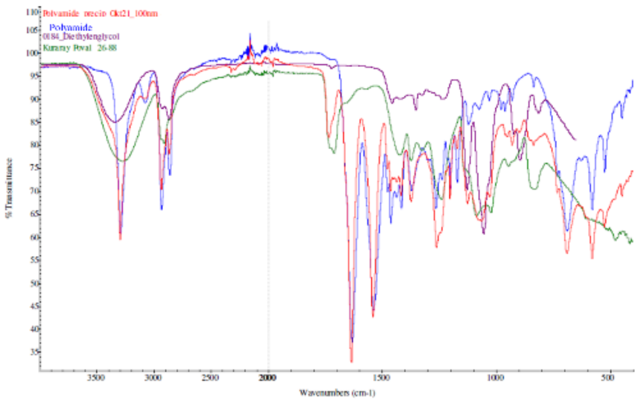
Sample Name (size in mass metrics)	Crystallization Peak (°C)	Melting Pt. (°C)	Density (g/cc)	Surface Area (m^2/g)	No. Ave. (M_n, g/mol)	Wt. Ave. (M_w, g/mol)	M_w/M_n	t_{50} (sec)
PA bulk (mm)	162.2	218.5	1.14	-	19600	61900	3.2	0.0110
PA cryomilled (47 µm)	186.4	219.9	1.15	0.36	16400	57900	3.5	0.0115
PA Inhalable (7 µm)	**176.0**	**217.2**		**1.90**				
PA_precip_100nm	**175.0**	**215.4**	**1.23**	**81.8**	**13100**	**68000**	**5.2**	**0.0130**

Thermal Analysis

Molar Mass

Representativeness – on the example of polyamide materials





TD-NMR:
Solid?
Mobile?

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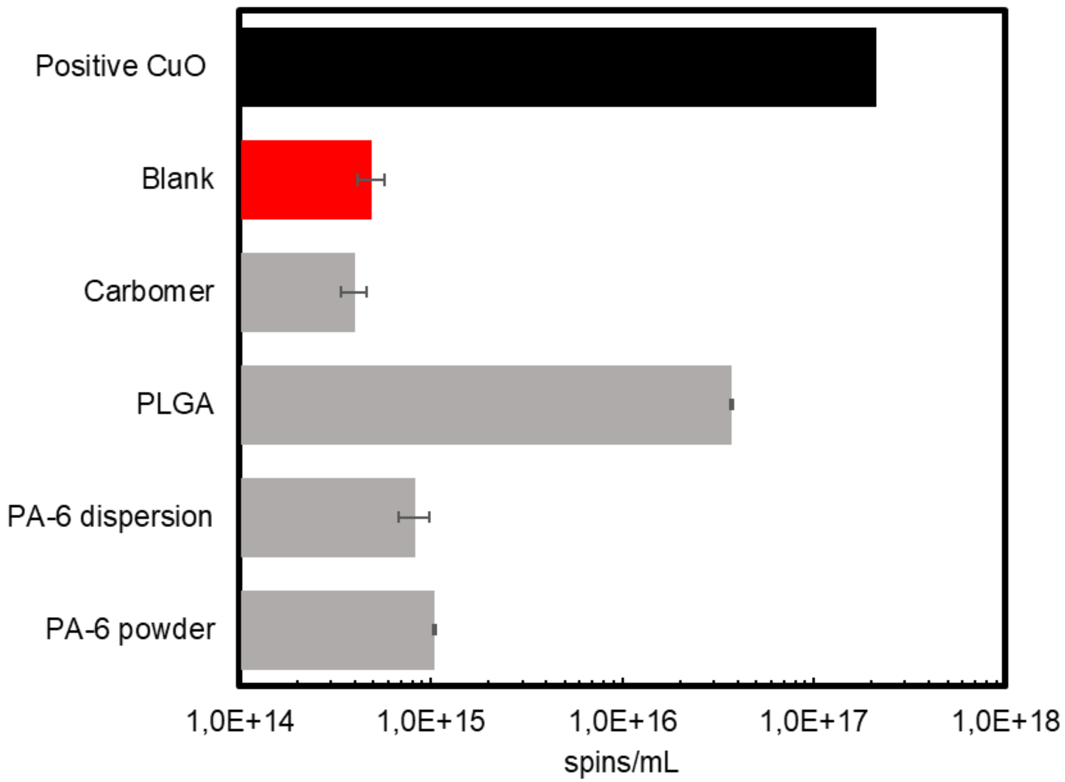
DSC: Thermal Analysis

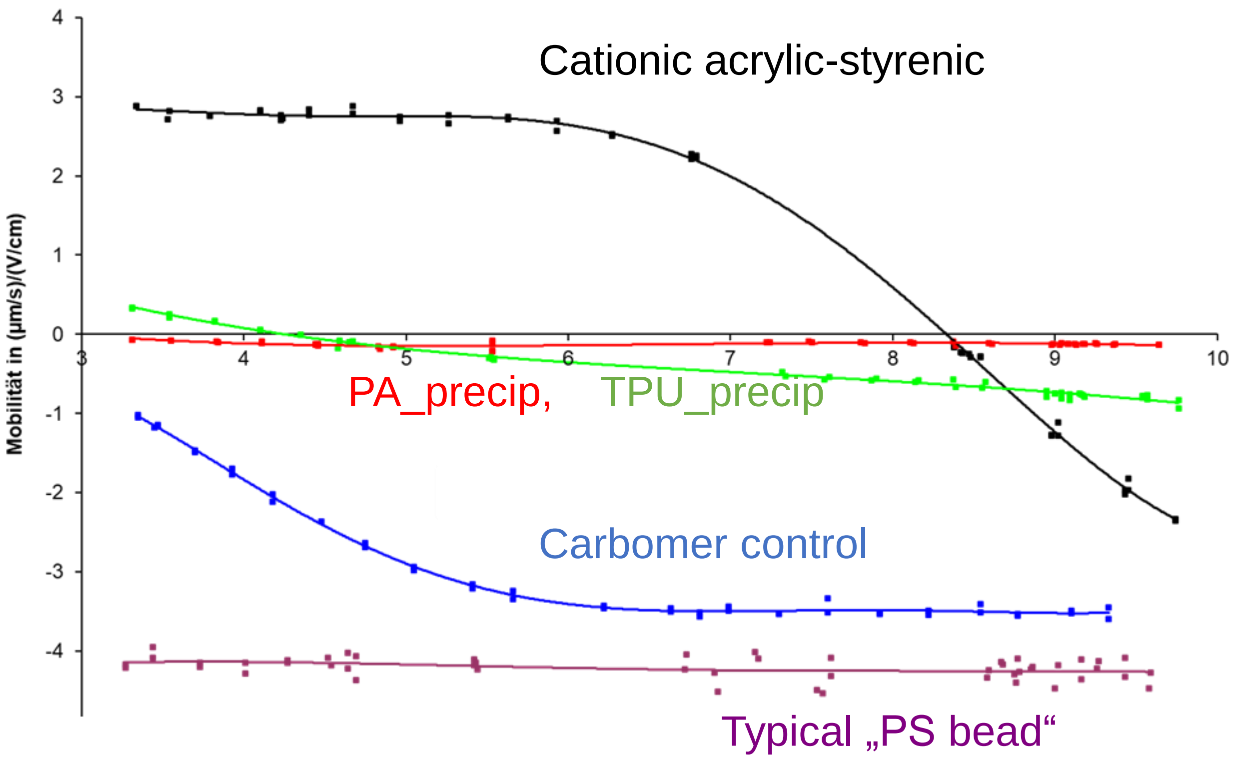
GPC: Molar Mass

Toxicity Relevant Characterization

Radical Generation: EPR_CPH



Charge: pH titration (in 1mmol KCl)



BASF We create chemistry
Fraunhofer IKTS
LRI Long-Range Research Initiative
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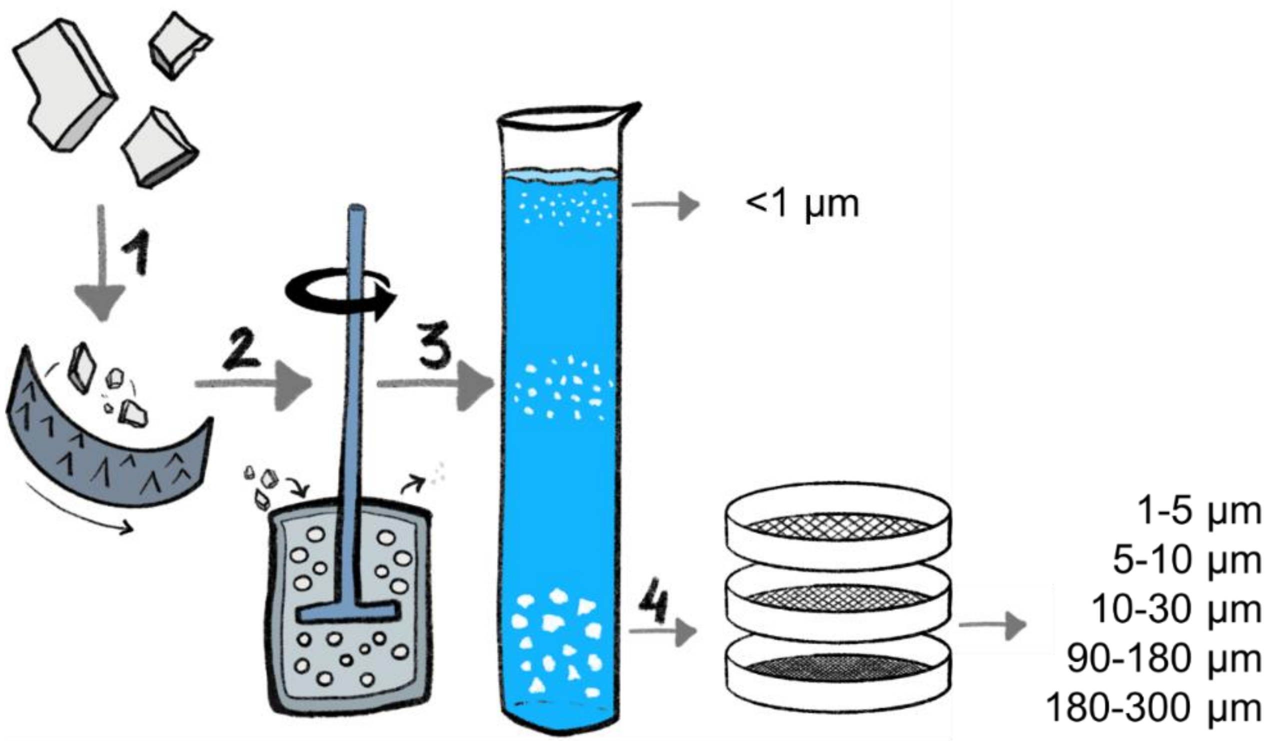
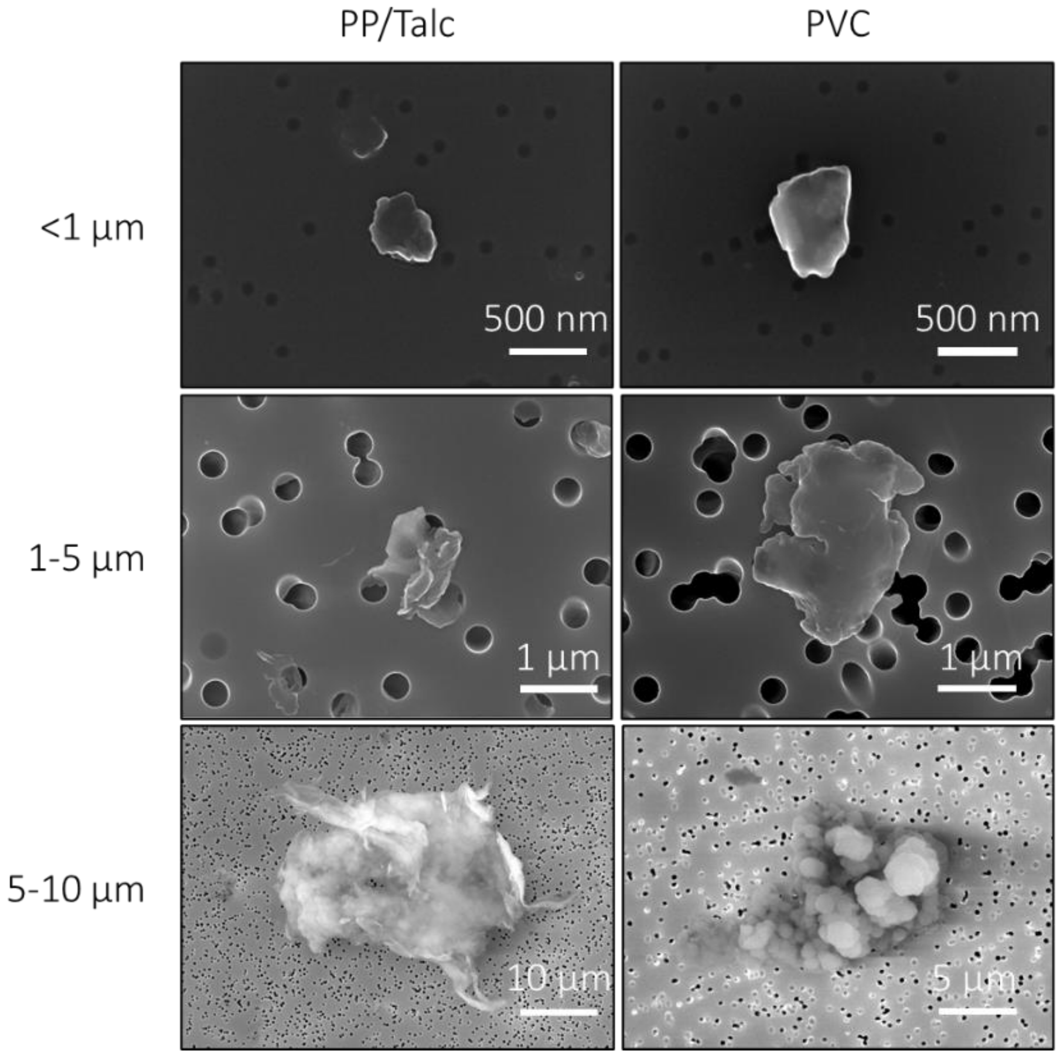
Milling and Fractionation

- Two size-reduction steps, grinding and ball milling
- Two fractionation steps, sedimentation and sieving
- Three plastics, three size classes

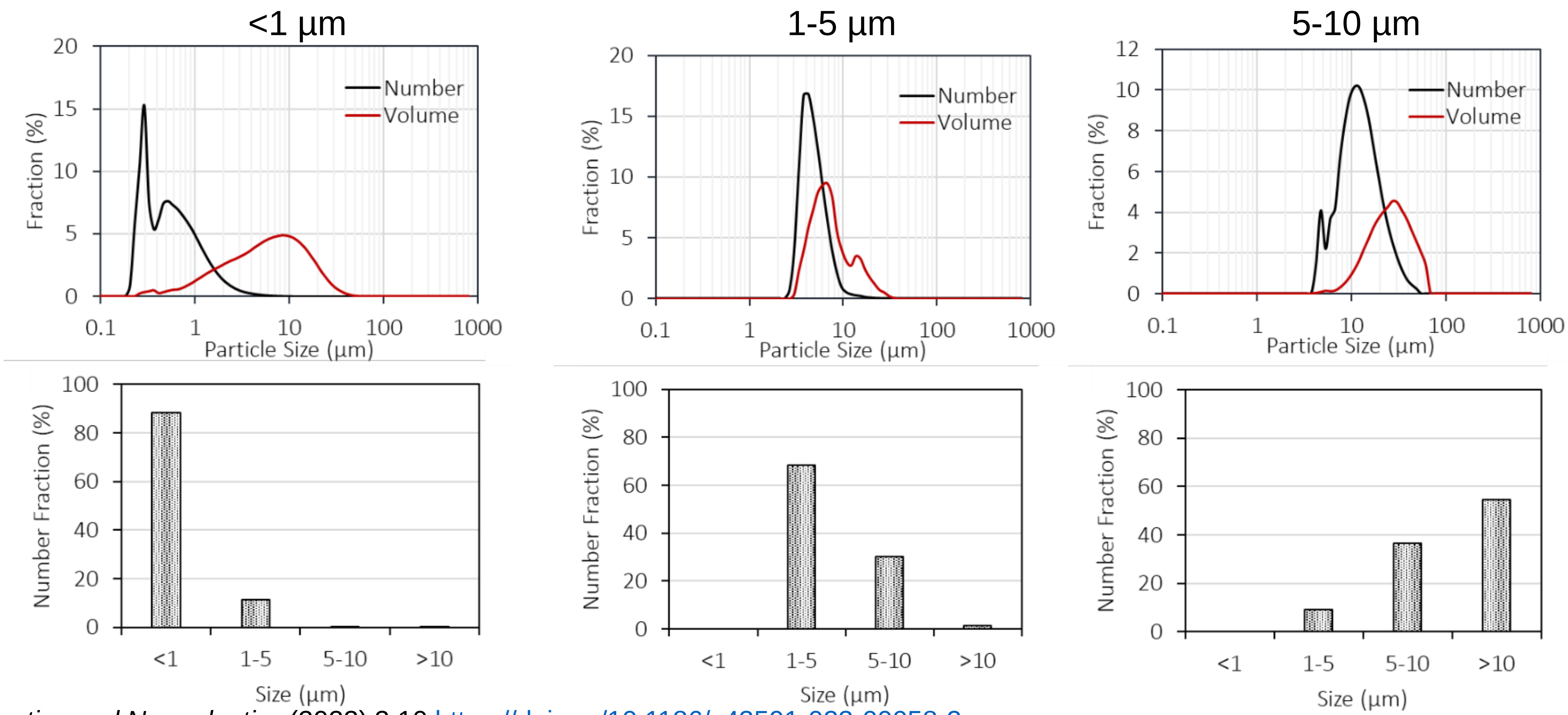




Parker et al. *Microplastics and Nanoplastics* (2023) 3:10 https://doi.org/10.1186/s43591-023-00058-2

Milling and Fractionation

- Narrow size distributions, >50% particles in desired size range

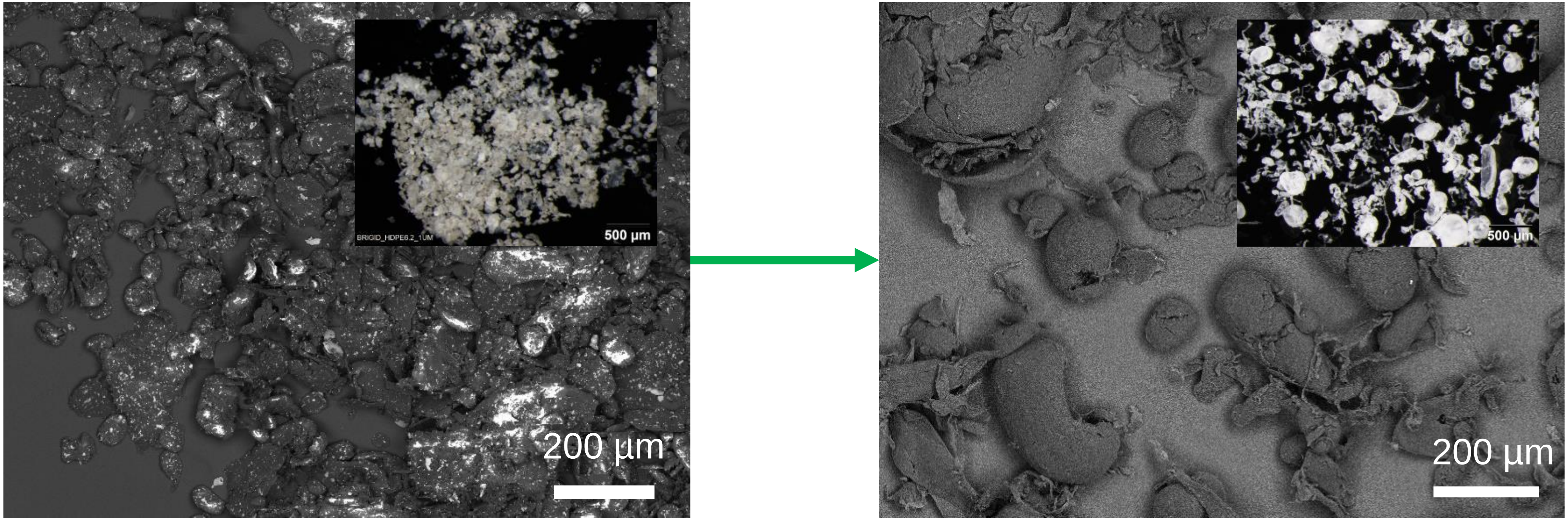


Parker et al. *Microplastics and Nanoplastics* (2023) 3:10 https://doi.org/10.1186/s43591-023-00058-2 brigid bridging the gap between microplastics and human health

Milling and Fractionation

PLASTICS EUROPE Enabling a sustainable future

- 8 different polymers: HDPE, LDPE, PP, PA, PC, PVC, PS and… PET
- Final batches still being prepared
- Optimising process with regards to:
- Contamination



Milling and Fractionation

- 8 different polymers: HDPE, LDPE, PP, PA, PC, PVC, PS and… PET
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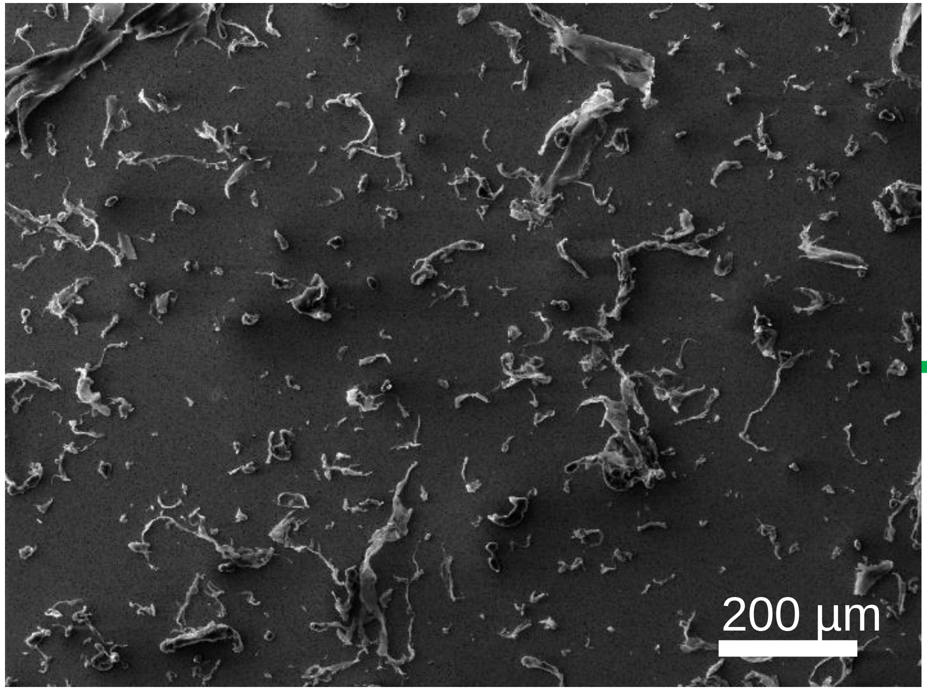
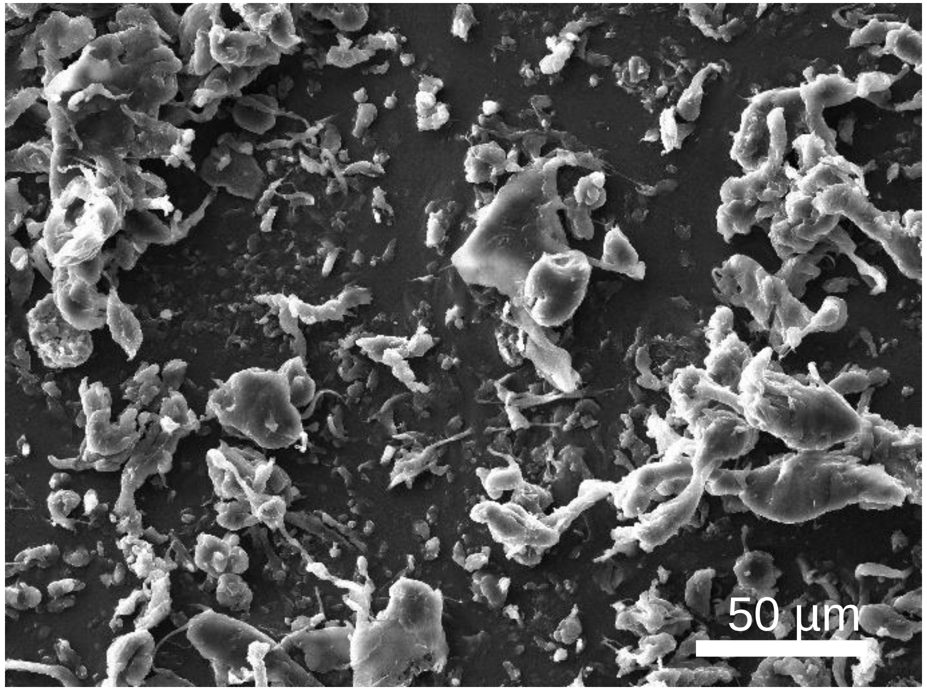
- Optimising process with regards to:
- Morphology





Suspending

- How can we best store and suspend reference materials?
- Hanson solubility parameters to predict solubility/swelling
- Glycerol promising option

Type	Solvent	HDPE	LDPE	PP	PC	PS	PVC	PA	PMMA
Aqueous	Water	21,8	13,2	7,4	6,8	7,8	7,8	6,3	4,0
	Urea	15,6	9,7	5,3	4,4	5,3	5,3	4,2	2,5
	Acetic Acid	7,8	4,1	2,7	1,8	2,6	1,4	3,8	1,2
Alcohols	Ethanol	10,0	5,8	3,5	2,5	3,4	3,4	1,7	1,6
	1-Butanol	7,7	4,3	2,7	1,8	2,6	2,6	3,3	1,3
	2-Butanol	7,2	4,0	2,6	1,6	2,4	2,4	3,6	1,2
	Iso-Butanol	8,1	4,4	2,8	2,0	2,8	2,8	3,3	1,4
	1-Propanol	8,7	4,9	3,0	2,1	2,9	2,9	2,6	1,4
	2-Propanol	8,1	4,6	2,9	1,9	2,8	2,8	3,0	1,4
Polyols	Glycerol	14,9	9,0	5,1	4,2	5,2	5,2	2,0	2,6
	Ethylene Glycol	13,2	7,9	4,6	3,6	4,6	4,6	0,9	2,2
	Propylene Glycol	8,3	4,8	2,9	1,7	2,6	2,6	3,0	1,1
	Diethylene Glycol	11,2	6,6	3,9	2,8	3,7	3,7	1,2	1,7
	Dipropylene Glycol	9,6	5,6	3,3	2,2	3,1	3,1	2,1	1,4
	Triethylene Glycol	10,6	6,1	3,7	2,6	3,5	3,5	1,9	1,5
Alkanes	Cyclohexane	1,5	1,0	0,4	1,7	1,2	1,2	8,8	1,3
	Hexane	3,3	1,1	1,0	2,0	1,7	1,7	8,8	1,4
Fatty Acids	Oleic Acid	3,0	1,1	1,1	0,9	1,0	1,0	6,7	0,9

Materials generated in BRIGID, C10, ECO59, NIST, BAM

sizes given as median mass $D_{50,M}$ and median number $D_{50,N}$

Size	PET	PA	LDPE	TPU	PVC	PP	PS
$D_{50,M}$ ≤ 1 µm		C10 (precip) $D_{50,M}$ = 0.1 µm = MOMENTUM	C10 (precip) to be char.	C10 (precip) $D_{50,M}$ = 1.0 µm			C10 $D_{50,N}$ = 0.08 µm $D_{50,M}$ = 0.09 µm
$D_{50,M}$ = 1-10 µm	C10 (precip) $D_{50,N}$ = 0.11 µm $D_{50,M}$ = 1.4 µm Brigid: n/a	C10 (cryo) $D_{50,M}$ = 6.7 µm (=InnoMat.Life) Momentum $D_{50,N}$ = 1.8, 2.3 µm $D_{50,M}$ = 5.3, 3.1 µm $D_{50,N}$ = 1.0 µm $D_{50,M}$ = 7.4 µm Brigid: n/a	NIST (cryo) $D_{50,M}$ = 4.6 µm Brigid: n/a		Momentum $D_{50,N}$ = 0.2 µm $D_{50,M}$ = 5.7 µm $D_{50,N}$ = 1.8 µm $D_{50,M}$ = 6.3 µm Brigid: n/a	Momentum $D_{50,N}$ = 0.6 µm $D_{50,M}$ = 5.8 µm $D_{50,N}$ = 5.0 µm $D_{50,M}$ = 6.4 µm Brigid: n/a	C10 $D_{50,N}$ = 2.0 µm Brigid: n/a
$D_{50,M}$ > 10 µm	ECO59 (cryo) $D_{50,M}$ = 41 µm Brigid: n/a	ECO59 (cryo) $D_{50,M}$ = 42 µm (=InnoMat.Life) Brigid: n/a	BAM (cryo) $D_{50,M}$ = 61 µm Brigid: n/a	ECO59 (cryo) $D_{50,M}$ = 236 µm (=InnoMat.Life)	Brigid: n/a Momentum $D_{50,N}$ = 4.9 µm $D_{50,M}$ = 10.6 µm	Momentum $D_{50,N}$ = 12.9 µm $D_{50,M}$ = 24.2 µm Brigid: n/a	BAM (cryo) $D_{50,M}$ = 206 µm Brigid: n/a

Reference Materials – an interim summary

- **PROs on alternatives**:
  - **Cryo/ball milling**: scalable production
  - **UV run-off**: most realistic (?): aged nanoplastics
  - **Precipitation**: nano in mass metrics, realistic charge (vanishing), realistic shape (non-spherical)
  - **Emulsion polymerisation** („beads“): ease of use, tagging options (dye, metal)

- **CONs of alternatives:**
  - **Cryo/ball milling**: inorg. contamination? Radicals? Size?
  - **UV run-off**: mg/m² quantity, demanding extraction
  - **Precipitation**: organic contamination?
  - **Emulsion polymerisation** („beads“): lack of representativeness, misleading properties (incl. ease of use), often surfactant content.

- **Representativeness issues**:
  - Define scenario to be represented: pristine, aged, contaminated, recycled, …
  - Measure & control phys-chem properties for representativeness:
    - Chemical Composition, Molecular Weight, Molecular mobility / solidity, Crystallinity, Particle Size Distribution, Shape (morphology), Density, Surface (re) activity, Surface Charge, Impurities, Endotoxin