Supplementary information

Addressing the relevance of polystyrene nano- and microplastic particles used to support exposure, toxicity and risk assessment: Implications and recommendations

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Microplastic Literature Search

The PRISMA workflow [1] was applied considering two complementary research strategies to retrieve potentially relevant studies, as follows: (i) comprehensive primary search using the PubMed-Medline electronic database, and (ii) secondary search through manual screening of the reference list of all relevant studies retrieved in the primary search.

Search filters and search strategy: To retrieve relevant research publications, a search strategy based on the use of relevant indexing keywords aimed at targeting studies that either generated and/or obtained nano- and microplastic particles (NMPs) from a supplier was adopted. Various descriptors and keywords were used to identify potentially relevant studies from a primary comprehensive literature database, which was created as an Endnote file from a search of the PubMed algorithm, used to obtain all studies aligned with the theme of “microplastic”. To increase the scope of the search strategy, relevant studies and literature reviews were identified from the primary reference list and were manually screened to identify additional relevant studies that were not included in the PubMed search results. All relevant studies published up to August 2023 were indexed and retrieved in full text to be included in the systematic review. No chronological or language limits were applied in the search strategy.

An initial keyword search of any Endnote listed field, including the main text of the publication of the primary 'microplastic' Endnote file, which contained a total of 10,217 references as of 27 July, 2023, was conducted using the keywords 'Cryogenic', 'Milling', 'Generation', 'Microtome', 'Grind' and 'Cryotome. Table S1 summarizes the results of studies identified that used and/or generated microplastic particles (MPs) as part of their study to assess an adverse effect from either an ecotoxicity or human health perspective, to evaluate the environmental fate or translocation of MPs across biological tissues, or to support the analytical method development regarding the analysis of MPs in various matrices. To widen the scope of the primary Endnote file to ensure a comprehensive search of all potentially relevant studies, a keyword search of the publication title field was included for studies not identified using the initial keywords described above and for which full text was not available. The keywords used were 'Extraction', 'Synthesis', 'Production', 'Reproductive', 'Metabolism', 'Growth', 'Inflammation', 'Oxidative', 'Polystyrene' (PS), 'Polyethylene' (PE), 'Polypropylene' (PP), 'Polyurethane', 'Nylon', 'Polyamide', 'Polycarbonate' (PC), 'polyvinyl', 'polylactic', 'effect', 'toxicity', and 'analysis'. All studies identified were manually screened for relevance, with full text articles being obtained for relevant studies.

Table S1: Studies identified that used and/or generated microplastic particles (MPs)

Keyword		Number of publications	
Listed in any field, including in the main text of the publication:			
Cryogenic		40	
Milling		68	
Generation		1712	
Microtome		26	
Grind		156	
Cryotome		3	
Used in the title of the publication:			

Extraction		92	
Synthesis		33	
Production		60	
Reproductive		47	
Metabolism		57	
Growth		108	
Inflammation		20	
Oxidative		94	
Polystyrene		551	
Polyethylene		436	
Polypropylene		39	
Polyurethane		21	
Nylon		18	
Polyamide		18	
Polycarbonate		2	
Polyvinyl		55	
Polylactic		16	
Effect		720	
Toxicity		255	
Analysis		261	
Total	>	4908	

All studies identified were manually checked for their relevance in the context of generating or using MPs purchased from a supplier with the polymer type categorized. Table S2 summarises the types of polymers that have been used across all studies.

Table S2: Types of polymers used across the studies

Polymer type	Number of publications	Percent of total (%)
High-density polyethylene (HDPE)	58	2.2
Low-density polyethylene (LDPE)	67	2.6
Polyethylene (PE)	308	11.8
Nylon / Polyamide	70	2.7
Polycarbonate	3	0.1
Polyethylene Terephthalate (PET)	82	3.1
Polylactic acid (PLA)	22	0.8
Polymethyl Methacrylate (PMMA)	13	0.5
Polyester	25	0.9
Polyurethane	8	0.3
Polypropylene (PP)	68	2.6
Polystyrene (PS)	1160	44.5
Expanded Polystyrene (EPS)	7	0.3
Polytetrafluoroethylene (PTFE)	3	0.1
Polyvinyl chloride (PVC)	91	3.5
Tire wear particles (TWPs)	24	0.9
Not reported	56	2.1
Studies using several types of polymers	542	20.8
Total	2607	

Observing that 44.5% of all studies use polystyrene, an assessment was performed to characterize the types of studies using polystyrene relative to other types of polymers. Table S3 summarizes studies categorized with respect to the type of study, the total number of publications for each category and the number of studies that report only using polystyrene.

Table S3: Studies using PS as part of their ecotoxicity or in mammalian in vivo or in vitro cell-based studies

Type of study	Number of publications	Number of publications using only polystyrene	Percent studies using polystyrene (%)
Analytical development	74	35	47
Particle aging	42	10	24
Biofilm formation	31	5	16
Ecotoxicity testing	1284	632	49
Environmental fate	373	131	35
Particle generation	39	2	5
Leaching	23	3	13
Sorption	164	40	24
Toxicity (human health)	445	265	60

It is important to note that studies reporting an adverse effect for MPs, from either an ecotoxicological or human health perspective, are dominated by the use of polystyrene. Indeed, approximately 60% of studies reporting an adverse effect based on results obtained from a mammalian in vivo study or an in vitro-based study with human health implications have relied entirely on the use of a monodispersed polystyrene microsphere (PSM). The PSMs are observed to have been obtained from a large number of different suppliers and/or distributors. The major suppliers/distributors identified across both ecotoxicity and human health toxicity studies are summarized in Table S4.

Table S4: Major suppliers/distributors of MPs and their occurrence in the studies

Supplier/distributor	Number of studies
Bangs Laboratories	27

Baseline Chromtech Research Centre	200
Microspheres-Nanospheres	15
Polysciences	76
Sigma Aldrich	78
Spherotech	21
Thermo Fisher Scientific	21
Generated by individual research group using emulsion polymerization method	41
Generated by cryomilling or mechanically grinding consumer products	6
Not reported	36

A general observation regarding the names of suppliers/distributors of PSMs listed by research groups is the dominance of companies that appear to have a Chinese origin. From Table S4, for instance, it can be seen that about one-third of research studies report obtaining their PSMs from BaseLine ChromTech Research Centre, which appears to be a distributor of the Unibead Series of crosslinked polystyrene polymers that are presumably produced by Tianjin Unibead Scientific Co., Ltd. (Tianjin, China). Both companies, BaseLine ChromTech Research Centre and Tianjin Unibead Scientific Co., Ltd., however, use the same website, suggesting that they are closely linked. The product data sheet for the Unibead PSMs refer to the product as being supplied in a water:ethanol (1:1 v/v) solution at varying inclusion levels, e.g. from 2.5-5% suspension (w/v), depending on the particle size obtained. The Unibead PSMs are understood to be used for a wide variety of purposes, such as standards for calibration purposes or as packing material for high-performance liquid chromatography columns, and in a variety of biomedical applications. In addition to the large number of studies using PSMs supplied by BaseLine Chromtech Research Centre we also note reference to several Chinese-based companies throughout the literature, where access to product data sheets and/or establishing whether a specific company listed is the distributor or the manufacturer, or the ability to assess what method of manufacturing was used to produce the PSMs is raised as a possible source of concern with respect to characterizing and quantifying the properties of PSMs used in a specific study. Table S5 summarizes other suppliers and/or distributors of PSMs, all of which appear to be of Chinese origin. The challenge identified, with respect to better understanding the properties of PSMs used in a specific study that results from the use of PSMs from such a large number of sources of particles, represents a significant limiting factor when it comes to supporting scientific experiments that can be reproducible. In this instance, tracking the sources and characterizing and quantifying the properties of PSMs used across all studies reported in the microplastic literature would require access to particles obtained from hundreds of different companies. Ideally, it would be preferable to have a small number of companies producing and distributing microplastic particles that are generated for the purposes of supporting and advancing the microplastic research community.

Table S5: Other suppliers and/or distributors of PSMs

	Supplier/distributor
1.	Aladdin Biochemical Technology
2.	Aladdin Industrial Corporation
3.	Aladdin Reagent Co., Ltd.
4.	Bei Si Le Chromatography Development Center
5.	Beijing Dekoshima Gold Technology Co.
6.	Beijing Dk Nano-Technology Co., Ltd.
7.	Beijing WeLab Science and Technology Corp.
8.	Beijing Zhongkeleiming Daojin Technology Co., Ltd.
9.	Beisler Chromatography Technology Development Center
10.	Big Goose Scientific Co., Ltd.
11.	Bio Nano New Materials Development Center
12.	Biotyscience
13.	Changchum Jingjie Technology Co., Ltd.
14.	Chengdu Huaxia Chemical Reagent Co., Ltd.
15.	China United Plastics Co., Ltd.
16.	Da’e Scientific Co.

17.	Daekj, Tianjin
18.	Diibio Biological Technology Co., Ltd.
19.	Dongguan Huachuang Plastic Co., Ltd.
20.	Dongguan Mingshuo Technology Co., Ltd.
21.	Dongguan Mingyuxing Plastic Materials Co., Ltd.
22.	Dongguan Tesulang Chemical Co., Ltd.
23.	Dongguan Yineng Plastics Co., Ltd.
24.	Dongguan Zhangmutou Plastic Co., Ltd.
25.	Dongguan Zhong Xin Raw Materials of Plastics Co., Ltd.
26.	Dongguan Zhongzheng Plastic
27.	Dushangzi Petrochemical Company of China National Petroleum Corporation
28.	Ganzhou Mxene Technology Co., Ltd.
29.	Goose Technology Co., Ltd.
30.	Guan Bu Tech.
31.	Guanbu Electromechanical Technology Ltd.
32.	Guangong Huachang Chemical Co., Ltd.
33.	Guangdong Yuancheng Plastic Chemical Materials Co., Ltd.
34.	Guangzhou Huayu Environmental Technology Inc.
35.	Guangzhou Pubo Biological Co., Ltd.
36.	Huachuang Chemical Co., Ltd.
37.	Huachuang Plastic Chemical Factory
38.	Huayu Co., Ltd.
39.	Huge Biotechnology
40.	Janus New-Materials
41.	Lianyungang Qihang Flame Retardant Material Co., Ltd.
42.	Luancheng District Guangsai Insulation Material Factory
43.	Macklin
44.	Mingshuo Chemical Co., Ltd.
45.	Mingshuo Chemical Plant
46.	Mingyuxing Plastic Raw Material Co., Ltd.

47.	Mxene Technology Co., Ltd.
48.	Nanjing Nanoeast Biotech Co., Ltd.
49.	Naking Janus Co.
50.	Regal Biotechnology Co., Ltd.
51.	Regal Nanoplastic Engineering Research Institute
52.	Rigor Biotechnology Co., Ltd.
53.	Rigor Science Co., Ltd.
54.	Rigor Technology Co., Ltd.
55.	Ruige Biotechnology Co., Ltd.
56.	Saierqun Technology Co., Ltd.
57.	Shanghai Aladdin Biochemical Technology Co., Ltd.
58.	Shanghai Guanbu Electromechanical Technology Co., Ltd.
59.	Shanghai Huge Biotechnology Co., Ltd.
60.	Shanghai HuiZhi Biotechnology Co., Ltd.
61.	Shanghai Macklin Biochemical Co., Ltd.
62.	Shanghai Macleans Biochemical Co., Ltd.
63.	Shanghai Yangli Electrical and Mechanical Technology Co., Ltd.
64.	Shanghai Youngling Electromechanical Technology
65.	Shanghai Zhenzhun Biotechnology Co., Ltd.
66.	Shanxi Heng Xue Material Technology Co., Ltd.
67.	Shengbang Co., Ltd.
68.	Shunjie Plastic Technology Co., Ltd.
69.	Suzhou NanoMicro Technology Co., Ltd.
70.	Suzhou Smartynano Technology Co., Ltd.
71.	Tesulang Chemical Company
72.	Tianjin Bestra Chromatography Technology Development Center
73.	Tianjin Big Goose Scientific Co., Ltd.
74.	Tianjin DaE Technology Co., Ltd.
75.	Tianjin Junyi Technology Co., Ltd.
76.	Tianjin Saierqun Technology Co., Ltd.

77.	Wuxi Regal Biotechnology Co., Ltd.
78.	Xi'an Ruixi Biological Technology Co., Ltd.
79.	Xiamen Huijia Biotechnology Co., Ltd.
80.	Xingwang Dongguan Plastic Co., Ltd.
81.	Yangling Electromechanical Technology Co., Ltd.
82.	Yineng Plastic Co., Ltd.
83.	Yiyuan Biotechnology Co., Ltd.
84.	Youngling Electromechanical Technology Co., Ltd.
85.	Yuan Biotech
86.	Zhengzhou Feynman Biotechnology Co., Ltd.
87.	Zhongcheng Plastics Co., Ltd.
88.	Zhongke Leiming Technology Co., Ltd.
89.	Zhongkeleiming Daojin Technology Co., Ltd.
90.	Zhonglian Petrochemical Materials

Based on the information from suppliers' literature, Table S6 provides an overview on the variety of sphere sizes available.

Table S6: PSMs sizes offered by major suppliers of PS microbeads

Supplier	Location	Sphere Size
Agilent [2]	Santa Clara, CA (USA)	50 nm – 1 µm
Bangs Laboratories (Ott Scientific Group) [3]	New York, NY (USA)	15 nm – 25 µm
Baseline ChromTech Research Centre [4]	Tianjin, China	50 nm – 10 µm
BS-Partikel [5]	Mainz, Germany	100 nm – 100 µm
CD Bioparticles [6]	Shirley, NY (USA)	50 nm – 750 µm
Cospheric LLC [7]	Santa Barbara, CA (USA)	1 – 1000 µm
J&K Scientific [8]	Beijing, China	unknown

JSR Life Sciences [9]	Sunnyvale, CA (USA)	50 nm – 500 nm
Magsphere [10]	Pasadena, CA (USA)	60 nm – 40 µm
Merck / Sigma Aldrich Co. [11]	Darmstadt, Germany	100 nm – 430 µm
Micromod [12]	Rostock, Germany	25 nm – 20 µm
Microparticles GmbH [13]	Berlin, Germany	100 nm – 300 µm
Microspheres-Nanospheres (Corpuscular Group) [14]	New York, NY (USA)	50 nm – 100 µm
NanoCS [15]	New York, NY (USA)	20 nm – 10 µm
Polysciences, Inc. (Ott Scientific Group) [16]	Warrington, PA (USA)	50 nm – 600 µm
Polysphere [17]	Chiba, Japan	80 – 520 nm
Shanghai Macklin [18]	Shanghai, China	50 nm – > 10 µm
Spherotech [19]	Chicago, IL (USA)	50 nm – 500 µm
Thermo Fisher Scientific (Invitrogen) [20]	Waltham, MA (USA)	20 nm – 16 µm

Alford et al. list common fluorophores used for PSMs [21]. We matched these fluorophores with their toxicity from the ECHA website [22], see Table S7.

Table S7: Fluorophores (information extracted from [21] and [22])

Fluorophore	Chemical name	Related CAS number	Toxicity / reference
Alexa Fluor 488	3-Amino-6-azaniumylidene-9-(2-carboxyphenyl)xanthene-4,5-disulfonate	247144-99-6	no toxicity-related information was available
Alexa Fluor 514	[6-(2-Carboxyphenyl)-2,2,4-trimethyl-10,12-	798557-07-0	no toxicity-related information was available

	disulfo-3,4-dihydro-1H-chromeno[3,2-g] quinolin-9-ylidene]ammonium		
BODIPY FL	4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid	146616-66-2	a BODIPY-verapamil conjugate is toxic to multidrug resistant human KB carcinoma cell lines in culture at concentrations greater than 10 µM (approximately 7.3 µg/mL) and is more toxic than verapamil alone. [21]
BODIPY R6G	4,4-Difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid	174881-57-3, 335193-70-9	no toxicity-related information was available
Carboxyrhodamine 6G	(Z)-ethyl-[2,6,7-trimethyl-9-(o-tolyl)xanthen- 3-ylidene]ammonium; methane	209112-21-0	no toxicity-related information was available
Cy5.5	[(2E,4E,6Z)-1-[1-(5,7-disulfo-1-naphthyl)-1-methyl-ethyl]-6-[3-(6-hydroxy-6-oxo-hexyl)-1,1-dimethyl-7,9-disulfo-4H-benzo[f]isoquinolin-2-ylidene]hexa-2,4-dienylidene]-diethyl-ammonium	172777-84-3, 442912-55-2	harmful if swallowed and may cause allergic respiratory and skin reactions [21]
Cy7	5-[(2E)-2-[(2E,4E,6E)-7-(1-ethyl-3,3- dimethyl-5-sulfo-indol-1-ium-2-yl)hepta-2,4,6-trienylidene]-3,3-	169799-14-8	no toxicity-related information was available

	dimethyl-5-sulfo-indolin-1-yl]pentanoic acid		
Cypate	4-[Ethyl-[(1Z,3E,5E,7E)-8-[3-(4-hydroxy-4-oxo-butyl)-1,1-dimethyl-4H-benzo[f]isoquinolin-3-ium-2-yl]-1-[1-methyl-1-(1-naphthyl)ethyl]octa-1,3,5,7-tetraenyl]amino] butanoic acid	317809-26-0, 95736-60-0	In general, cypate is considered a “biocompatible” dye [21]
Fluorescein	2-(3,6-Dihydroxy-9H-xanthen-9-yl)benzoic acid	2321-07-5	widely used, FDA-approved fluorescent dye, that can have toxic effects in cell culture and on patients at high doses [21]
Indocyanine green	4-[(2Z)-2-[(2E,4E,6E)-7-[1,1-dimethyl-3-(4-sulfobutyl)benzo[e]indol-3-ium-2-yl]hepta-2,4,6-trienylidene]-1,1-dimethyl-benzo[e]indol-3-yl]butane-1-sulfonic acid	3599-32-4	cytotoxic effects in vitro at a concentration of 2.5 mg/mL widely used, FDA-approved fluorescent dye that at much higher doses than used clinically causes in vitro and in vivo toxicity [21]
Oregon green 488	2-(2,7-Difluoro-3-hydroxy-6-oxo-xanthen- 9-yl)benzoic acid	198139-51-4	Information on the cytotoxicity, toxicity to tissues, in vivo toxicity, and mutagenicity of Oregon green is limited [21]
8-phenyl BODIPY	4,4-Difluoro-1,3,5,7-tetramethyl-8-phenyl- 4H-4a-aza-3a-azonia-4-bora-s-indacene	180156-50-7	no toxicity-related information was available

Rhodamine 110	[6-Amino-9-(2-carboxyphenyl)xanthen- 3-ylidene]ammonium	13558-31-1	Limited toxicity information is available for rhodamine 110 cytotoxic at concentrations greater than 100 µM (approxi-mately 51 µg/mL) and accumulates in Friend leukemia cells, causing cell death [21]
Rhodamine 6G	(Z)-ethyl-[2,6,7-trimethyl-9-(o-tolyl)xanthen-3-ylidene]ammonium; methane	989-38-8	non–FDA-approved fluorophore that has demonstrated mutagenicity and toxicity in cells, tissues, and organisms [21]
Rhodamine X	9-(2,5-Dicarboxyphenyl)-2,3,6,7,12,13,16,17-octahydro-1H,5H,11H,15H-xantheno[2,3,4-ij:5,6,7-i’j’]diquinolizin-18-ium	216699-36-4, 194785-18-7	substance causes serious eye irritation, causes skin irritation and may cause respiratory irritation [22]
Rhodol	2-(3-Amino-6-hydroxy-3H-xanthen-9-yl) benzoic acid	3086-44-0	substance is harmful if swallowed [22]
6-TAMRA	2-(3-Dimethlyamino-6-methylene-xanthen-9-yl)benzoic acid	150810-69-8	substance causes serious eye irritation, causes skin irritation and may cause respiratory irritation [22]
Texas red	9-(2,4-Disulfophenyl)-2,3,6,7,12,13,16,17-octahydro-, hydroxide-1H,5H,11H,15H-xantheno(2,3,4-ij:5,6,7-i’j’)-diquinoliz- in-18-ium	82354-19-6	Only information on Texas red conjugates was available: Conjugates of Texas red have shown phototoxic effects in rat erythrocytes exposed to epi-illumination [21]

Tokyo green	6-Hydroxy-9-(o-tolyl)xanthen-3-one	n.a.	Information on the tissue toxicity, in vivo toxicity, and mutagenicity of Tokyo green is limited. [21]

Table S8 provides a selection of products and functional groups that are formed in the photooxidation of polystyrene along with their characteristic IR absorption bands.

Table S8: Selected photodegradation products of polystyrene (data compiled from [23 - 30])

Substance or functional group	**Chemical formula**	**Characteristic IR absorption bands [cm^{-1}]**
Peroxy radicals, peroxides and hydroperoxides	O-O• | $-CH_2-C(Ph)-CH_2-$	800-1050, 1200-1300 (ν (O-O))
	R-O-O-R’	3400-3600 (ν (O-H))
	R-O-OH	
Hydroxyl groups	R-OH	3000-3800 (ν (O-H))
• Free hydroxyl groups	$-CH_2-C(OH)(Ph)-CH_2-$	3540 (ν (O-H))
• Intermolecularly bonded hydroxyl groups	-CH2-C(O)-Ph(OH)	3450 (ν (O-H))
Carbonyl groups	>C=O	1500-1800 (ν (C=O))
• Aliphatic ketones (chain ketone)	$-(Ph)(CH_3)C-C(O)-C(Ph)(CH_3)-$	1708 (ν (C=O))
• Aromatic ketones (ketonic end groups)	$-CH_2-C(O)-Ph$	1690 (ν (C=O))

• Acetophenone	Ph-C(O)-CH_3	1690 (ν (C=O))
• Benzaldehyde	H-C(O)-Ph	1708 (ν (C=O))
• Benzoic anhydride	Ph-C(O)-O-C(O)-Ph	1725, 1785 (ν (C=O))
• Dibenzoyl methane	Ph-C(O)-CH_2-C(O)-Ph	1515, 1603 (ν (C=O))
Carboxylic acids	R-C(O)-OH	1690-1750 (ν (C=O))
• Benzoic acid	Ph-C(O)-OH	1698-1732 (ν (C=O))
• Formic acid	H-C(O)-OH	1710 (ν (C=O))
• Acetic acid	H_3C-C(O)-OH	1710 (ν (C=O))
Esters	R-C(O)-O-R’	1725-1735 (ν (C=O))
• Methyl benzoate	Ph-C(O)-O-CH_3	1728 (ν (C=O))
Styrene	Ph-CH-CH_2	
Benzene	C_6H_6	

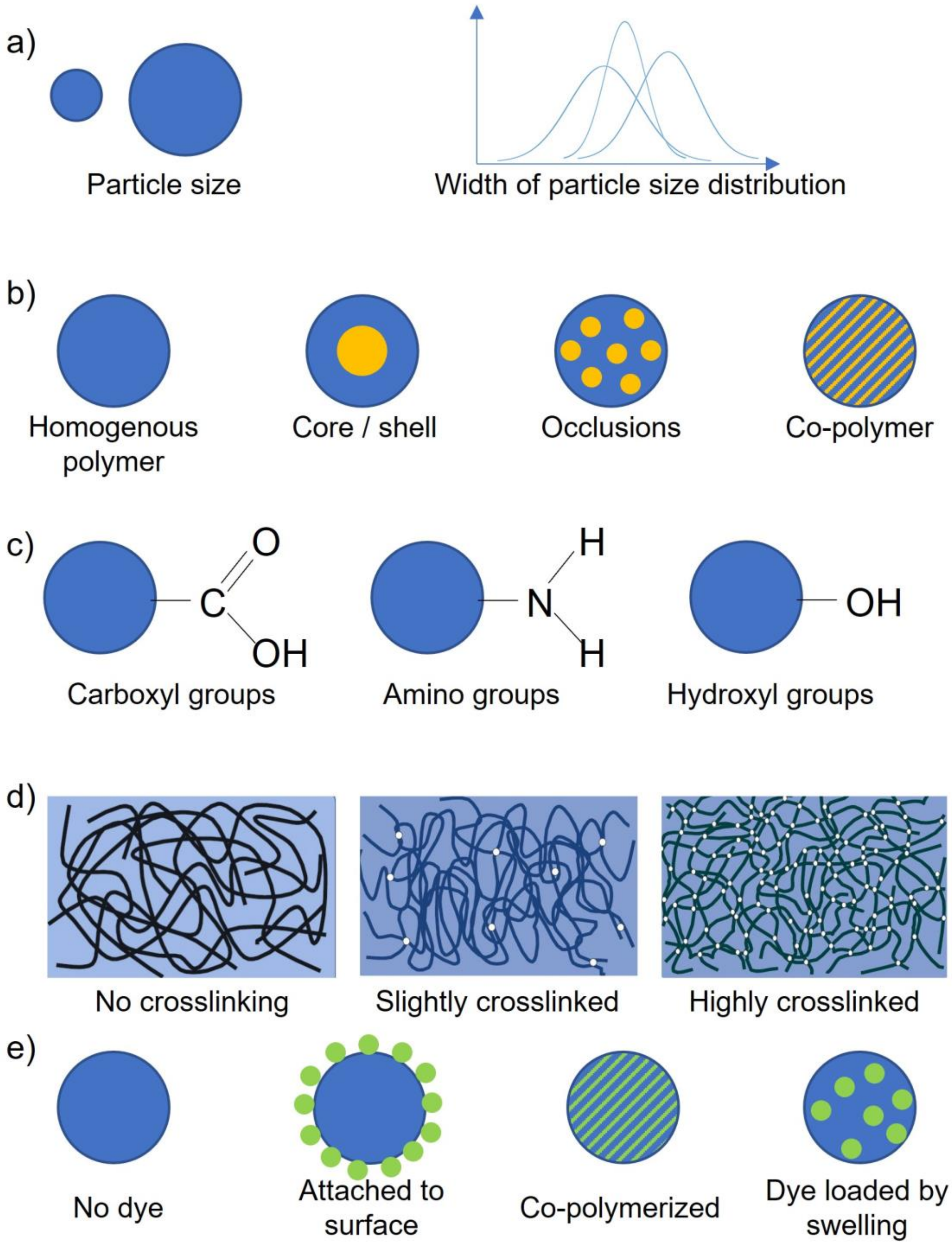

Figure S1: Schematic representation on selected properties of PSMs: a) Particle size and particle size distribution, b) Polymer composition, c) Surface modification, d) Degree of crosslinking, e) Incorporation of dyes.

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