Supplementary Information

Review of ecotoxicological studies of widely used polystyrene nanoparticles

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List of abbreviations

SDS – Sodium dodecyl sulfate

EC_{50} - half maximal effective concentration

PTA/NTA – Particle tracking analysis or nanoparticle tracking analysis

DLS – Dynamic light scattering

SLS – Static light scattering

ELS – Electrophoretic light scattering

TEM – Transmission electron microscopy

SEM - Scanning electron microscope

DCS – Differential centrifugal sedimentation

UV-vis – Ultraviolet visible spectroscopy

SAXS – Small-angle x-ray scattering

Table S1. The information extracted from the reviewed published papers. "hpf" stands for hours post fertilization.

Study subject	Polystyrene nanoparticles size (nm)		Concentration (mg/L; unless stated differently)		Exposure duration	*If* and *how* polystyrene nanoparticle suspension were cleaned?	Other measured parameters of the test media and/or polystyrene nanoparticles	The main outcome	Polystyrene nanoparticles supplier	Ref.
	Before adding to the test media, detection method(s) performed in the pure water, unless stated differently, particle surface modification	After adding to the test media, detection method(s)	Before and after adding to the test media; (nominal, unless stated differently)	After adding to the test media, detection method(s)						
Daphnia magna	70 TEM, surface modification not specified	Not mentioned	0.22 – 150	Not mentioned	21 d	No, because sodium dodecyl sulfate concentration was below toxicity threshold	pH; Temperature; Light-dark period; Oxygen concentration; Conductivity	Reduction in body size and neonates at 0.22 mg/L; Malformations at 30 mg/L	Synthesized at AVT-PCC, Wageningen UR	[1]
D. magna	100 DLS, $-NH_2$	150-250, DLS	0.1 – 1000	Not mentioned	24 h	No comments	pH; Temperature	Increased mortality at 1 mg/L	Obtained from a previous FP7 project (QualityNano)	[2]
D. magna	26 DLS, ELS, SEM, -COOH	347 and 436, DLS, ELS	0.0001 – 100	Yes (SLS)	48 h	Dialysis (a comparison between dialysed and non-dialysed nanoparticles)	pH; ζ-potential; Conductivity;	Increased mortality at >100 mg/L; Increased mortality at EC_{50} = 22.0±0.7 mg/L	Bangs Laboratories, Inc., US	[3]

D. magna	100 DLS, ELS, SEM, -COOH	95, DLS, ELS	0.01 – 100	Yes (SLS)	48 h	Dialysis (a comparison between dialysed and non-dialysed nanoparticles)	pH; ζ-potential; Conductivity	Increased mortality at >100 mg/L; Increased mortality at EC_{50} = 13.0±1.4 mg/L	Bangs Laboratories, Inc., US	[3]
D. magna	50-100, 110 and 300 DLS, $-NH_2$	110, 370 and 360, DLS	1, 40, 70, 75, 100	Not mentioned	48 h	No comments	pH; ζ-potential; Temperature; Light-dark period; Dissolved oxygen; Ammonia	Increased mortality at LC_{50} = 5.24 mg/L; Behavioural changes and elevated rates at ROS at 1 mg/L	Aladdin; Thermo Scientific, China	[4]
D. magna	50 DLS, NTA, DCS, $-NH_2$	120-150, NTA	1.4 and 2.7	Not mentioned	24 and 48 h	Dialysis	pH; Temperature; Light-dark period; Turbidity	Increased mortality at 1.4 mg/L	Bangs Laboratories, Inc., US	[5]
D. magna	26, 53, 62 DLS, $-NH_2$, -COOH	Not mentioned	0.0032, 0.032, 0.32, 0.76, 3.2, 7.6	Not mentioned	103 d	Dialysis	pH; Temperature; Light-dark period; Sedimentation	Increased mortality at 0.32 mg/L	Bangs Laboratories, Inc., US	[6]
D. magna	52 DLS, $-NH_2$	Not mentioned	25 – 150	Not mentioned	24 h	Dialysis	Not mentioned	Increased mortality at >75 mg/L	Bangs Laboratories, Inc., US	[7]
D. magna	130-150, surface modification not specified	130-150, DLS	0.30, 0.51 0.60, 1.20, 2.40, 4.80 mmol	Not mentioned	48 h	No comments	pH; ζ-potential	A 2-fold reduction in the acute toxicity after nanoplastics sonication process	Synthesized by authors	[8]
D. pulex	75 DLS, TEM, surface modification not specified	71, DLS, TEM	0.1, 1, 10, 50, 100, 150, 200, 400	Not mentioned	48 and 96 h	No comments	ζ-potential; Temperature; Light-dark period; Dissolved oxygen	Increased mortality at LC_{50} = 80.02 mg/L; Changes in gene expression at 0.1 mg/L	BaseLine Chromtech Research Centre, China	[9]

D. pulex	75 DLS, TEM, surface modification not specified	71, DLS, TEM	0.1, 0.5, 1, 2	Not mentioned	21 d	Centrifugation followed by washing with ultrapure water	Temperature; Light-dark period; Dissolved oxygen	Changes in genes expression at 0.5 mg/L	BaseLine Chromtech Research Centre, China	[10]
D. pulex	75 DLS, surface modification not specified	71, DLS	0.1, 0.5, 1, 2, 10, 50, 100, 150, 200, 400	Not mentioned	48 h, 21 d	No comments	nPS structure; Temperature; Light-dark period; Dissolved oxygen	Increased mortality at LC_{50} = 76.69 mg/L; Growth inhibition at 0.5 mg/L; Reproduction impairment at 0.1 mg/L	BaseLine Chromtech Research Centre, China	[11]
D. pulex	75 DLS, TEM, surface modification not specified	71, DLS	0.1, 0.5, 1, 2	Not mentioned	21 d	No comments	Temperature; Light-dark period	Changes in genes expression at 2 mg/L	BaseLine Chromtech Research Centre, China	[12]
D. pulex	75 DLS, TEM, surface modification not specified	71, DLS	0.1	Not mentioned	21 d	No comments	Temperature; Light-dark period	Reproduction impairment and growth inhibition	BaseLine Chromtech Research Centre, China	[13]
D. pulex	75 DLS, TEM, surface modification not specified	71, DLS	1	Not mentioned	21 d	No comments	ζ-potential; Temperature; Light-dark period; Dissolved oxygen	Changes in genes expression	BaseLine Chromtech Research Centre, China	[14]
D. pulex	75 DLS, TEM, surface modification not specified	71, DLS	1	Not mentioned	96 h	No comments	Temperature; Light-dark period	Changes in genes expression	BaseLine Chromtech Research Centre, China	[15]
D. pulex	75 DLS, TEM, surface	71, DLS	0.001, 0.1, 0.5, 1, 2	Not mentioned	21 d	No comments	Temperature; Light-dark period	Changes in genes expression	BaseLine Chromtech Research	[16]

	modification not specified								Centre, China	
D. pulex	71.18 DLS, TEM, surface modification not specified	71, 18 DLS	0.1, 0.5, 1, 2	Not mentioned	21 d	No, because sodium azide concentration was below toxicity threshold	Temperature; Light-dark period	Inhibition of fecundity and population growth; Changes in proteins expression	BaseLine Chromtech Research Centre, China	[17]
Carassius carassius (size is not provided)	24 DLS, surface modification not specified	Not mentioned	100	Not mentioned	42 d	Dialysis	Temperature; Light-dark period	Changes in metabolism and behaviour	Bangs Laboratories, Inc., US	[18]
C. carassius (8.2−9.6 cm, 7.5−13.7 g)	24 and 27 DLS, NTA, -SO_3H	Not mentioned	71.35	Not mentioned	61 d	Dialysis	Temperature; Light-dark period	Changes in metabolism and behaviour	Bangs Laboratories, Inc., US	[19]
C. carassius (size is not provided)	52 and 180 DLS, -NH_2	56 and 174, DLS	29 – 100	Not mentioned	67 d	Dialysis	Not mentioned	Changes in behaviour	Bangs Laboratories, Inc., US	[7]
Danio rerio (3–4 months old)	45 DLS, surface modification not specified	30.67±8.97, DLS	0.05	Not mentioned	7 d	Centrifugation followed by washing with ultrapure water	ζ-potential; pH; Temperature; Light-dark period	Changes in oxygen consumption and elevated heart rate	Bangs Laboratories, Inc., US	[20]
D. rerio (embryo; 0, 24, 48 and 72 hpf)	100 SEM, surface modification not specified	Not mentioned	2.2×10^{-7}	Not mentioned	72 h	Centrifugation followed by washing with ultrapure water	Temperature; Light-dark period	Elevated heart rate and blood flow, changes in development	Bangs Laboratories, Inc., US	[21]
D. rerio (adults, ~6 months old)	70 TEM, surface modification not specified	Not mentioned	0.5, 1.5, 5	Not mentioned	7 d, 7 weeks	No comments	pH; Temperature; Light-dark period; Dissolved oxygen; Electrical conductivity; Water hardness	Changes in the activity of the nervous system at 1.5 and 5 mg/L	BaseLine ChromTech Research Centre, China	[22]

D. rerio (larvae, 3 hpf)	50 TEM, surface modification not specified	Not mentioned	1	Not mentioned	48 h, 72 h	No comments	ζ-potential; Temperature	Inhibition of the larval locomotion; Reduced body length of larvae; Changes in genes expression	Polysciences Co., US	[23]
D. rerio (larvae, 72, 96, or 120 hpf)	50 DLS, SEM, TEM, surface modification not specified	50, DLS, SEM, TEM	10	Not mentioned	24 h	No comments	ζ-potential; pH; nPS structure; Light-dark period; Temperature	Accumulation in the cytoplasm; Changes in genes expression	Polyscience, Inc., US	[24]
D. rerio (adult, 6 months old)	42 Methodology is not mentioned, surface modification not specified	Not mentioned	1×10^{-4}, 0.001, 0.01	Not mentioned	5 d	No comments	Not mentioned	Decreased heart rate and locomotor activity	Bangs Laboratories, Inc., US	[25]

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