

Supplementary Materials

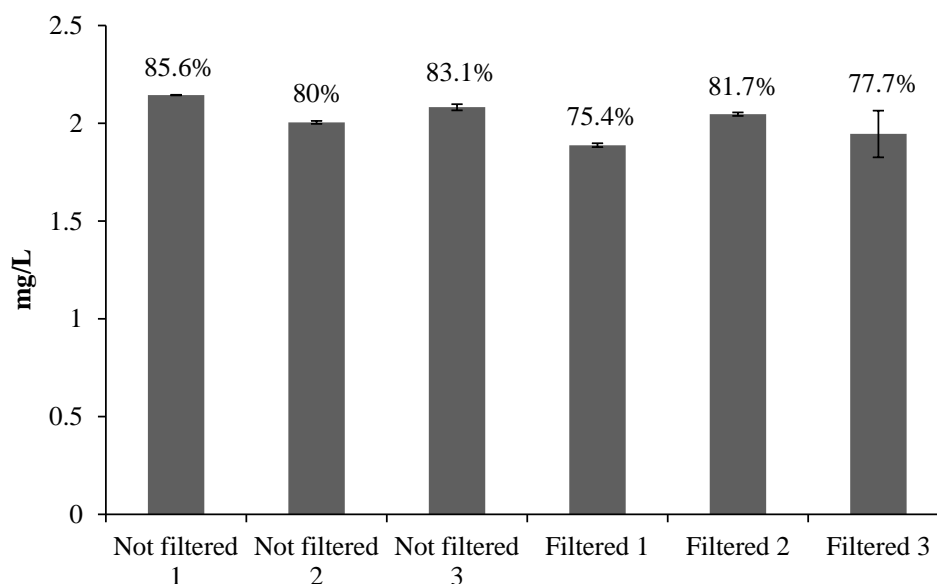
# Screening of the Toxicity of Polystyrene Nano- and Microplastics Alone and in Combination with Benzo(a)pyrene in Brine Shrimp Larvae and Zebrafish Embryos

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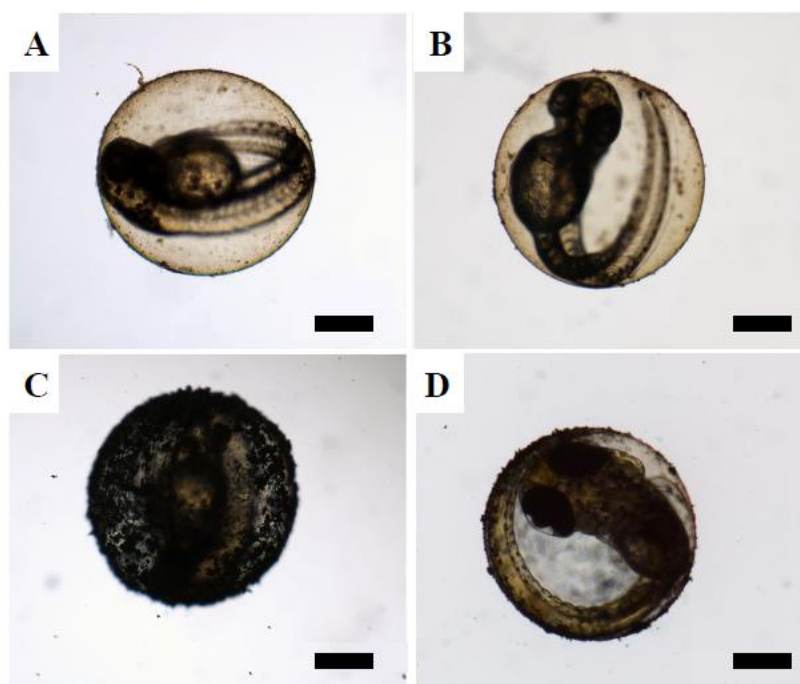
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**Figure S1-** Measured concentration from a nominal concentration of 2.51 mg/L or  $5.10^4$  particles/mL of 4.5 µm MPs using a cell counter before and after filtration using a polyethersulfone filter (0.45 µm filter pore). Bars represent the mean of 3 instrumental replicates with their corresponding standard deviation. The percentage of MP measured from the nominal concentration is given above each bar.



**Figure S2.** Micrographs of zebrafish embryos exposed to similar masses of plastics of different sizes. A) 48 hpf embryo exposed to 6.87 mg/L of 50 nm NPs; B) 48 hpf embryo exposed to 6.87 mg/L of 500 nm NPs; C) 48 hpf embryo exposed to 5.01 mg/L of 4.5  $\mu$ m MPs; D) non-hatched 120 hpf alive embryo exposed to 5.01 mg/L of 4.5  $\mu$ m MPs-B(a)P. Scale bars: 100  $\mu$ m.

**Table S1-** Effect on survival (%) of the exposure of 24 hph and 48 hph brine shrimp larvae to DMSO for 24 h and 48 h.

Concentration (v/v)	24 hph		48 hph	
	24 h	48 h	24 h	48 h
0	100	97	100	97
0.01%	97	93	97	93
0.10%	100	93	100	100

**Table S2-** Odd ratio values indicating the risk of death (immobilisation) for brine shrimp larvae exposed to MPs alone or in combination with B(a)P.

Treatment test		Treatment for comparison	Conc. (mg/L)	Odd ratio	Confidence interval (5%, 95%)	<i>p</i> value
500 nm NPs-B(a)P	24 hph 48 h	Control	0.00034	$1.94 \cdot 10^{54}$	$7.83 \cdot 10^{50} - 4.78 \cdot 10^{57}$	0
			0.00069	$1.94 \cdot 10^{54}$	$7.83 \cdot 10^{50} - 2.71 \cdot 10^{271}$	0
			6.87	1.422	1.179 - 1.715	0.000
	48 hph 48 h	Control	0.00034	$1.94 \cdot 10^{54}$	$7.83 \cdot 10^{50} - 2.72 \cdot 10^{271}$	0
B(a)P	24 hph 48 h	Control	0.1	$2.82 \cdot 10^8$	$25.257 - 3.16 \cdot 10^{15}$	0.008
			0.5	196	7.939 - 4838.811	0.000
			1	10.706	2.148 - 53.348	0.001
			5	1.741	1.263 - 2.399	0.000
			10	1.250	1.064 - 1.469	0.002
500 nm NPs-B(a)P	24 hph 48 h	500 nm NPs	0.00069	9.333	1.866 - 46.683	0.001
			6.87	24.182	4.808 - 121.625	0.000
	48 hph 48 h	500 nm NPs	0.00034	25.375	3.050-211.104	0.000
			0.687	22.176	2.661-184.798	0.000
			6.87	19.333	2.313-161.565	0.000
4.5 $\mu$ m MPs-B(a)P	24 hph 48 h	4.5 $\mu$ m MPs	0.025	19.333	2.313 - 161.565	0.000
			0.501	10.706	2.148 - 53.348	0.001
			50.1	16.789	2.001 - 140.898	0.001
4.5 $\mu$ m MPs-B(a)P	24 hph 48 h	500 nm NPs-B(a)P	5.01, 6.87	0.706	0.539 - 0.924	0.009
	48 hph 48 h	500 nm NPs-B(a)P	0.501, 0.687	0.617	0.436 - 0.875	0.003
			5.01, 6.87	0.639	0.450 - 0.906	0.006

**Table S3-** Odd ratio values indicating the risk of malformation in 120 hpf zebrafish embryos exposed to 4.5  $\mu\text{m}$  MPs alone or in combination with B(a)P or to B(a)P alone.

Treatment test	Treatment for comparison	Conc. (mg/L)	Odd ratio	Confidence interval (5%, 95%)	<i>p</i> value
4.5 $\mu\text{m}$ MPs-B(a)P	Control	50.1	1.045	1.019 - 1.072	0.0001
B(a)P	Control	5	1.373	1.081 - 1.744	0.0063
		10	1.239	1.098 - 1.397	0.0002
4.5 $\mu\text{m}$ MPs-B(a)P	4.5 $\mu\text{m}$ MPs	50.1	5.71	1.82 - 20.66	0.0000

**Table S4.** Effects of 120 h DMSO exposure on developmental parameters of zebrafish embryos.

Concentration (% v/v)	% survival	hatching time (h)	% malformed embryos
0.0	100	70.0	8.3
0.01	100	68.7	10.0
0.1	100	69.3	13.3