

Supplementary Information

Comparative Toxicity of Polystyrene, Polypropylene, and Polyethylene Nanoplastics on *Artemia franciscana* Nauplii: A Multidimensional Assessment

Marriya Sultan ^{a,b}, Xing-Yi Wei ^{a,b}, Jin-Jing Duan ^c, Bao-Fu Zhang ^c, Ming-Fei Wu ^c, Zi-Xin Cai ^c,
De-Sheng Pei ^{c*}

^a Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences,
Chongqing School, University of Chinese Academy of Sciences, Chongqing 400714, China.

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c School of Public Health, Chongqing Medical University, Chongqing 400016, China

*Corresponding author.

E-mails: peids@cqmu.edu.cn (D.S.P) and deshengpei@gmail.com (D.S.P)

Table S1. Size of *Artemia franciscana* Nauplii after acute exposure to PS-NP, PP-NP (n=10) and PENP. Results are shown as mean $\pm$ standard deviation and *p*-value (T-test).

Conc (mg/L)	PS-NP		PP-NP		PE-NP	
	Mean $\pm$ Std	<i>p</i> - value	Mean $\pm$ Std	<i>p</i> - value	Mean $\pm$ Std	<i>p</i> - value
0	0.80 $\pm$ 0.005	0	0.81 $\pm$ 0.005	0	0.80 $\pm$ 0.005	0
0.05	0.80 $\pm$ 0.005	0.68	0.80 $\pm$ 0.006	0.28	0.80 $\pm$ 0.006	0.82
0.5	0.80 $\pm$ 0.008	0.10	0.80 $\pm$ 0.011	0.06	0.80 $\pm$ 0.011	0.28
5	0.80 $\pm$ 0.008	0.20	0.80 $\pm$ 0.010	0.08	0.80 $\pm$ 0.010	0.35
50	0.80 $\pm$ 0.006	0.10	0.80 $\pm$ 0.006	0.09	0.80 $\pm$ 0.006	0.50
100	0.80 $\pm$ 0.009	0.09	0.79 $\pm$ 0.013	0.07	0.78 $\pm$ 0.013	0.06

Table S2. The mean fluorescence intensity acquired using Image. J software for bioaccumulation of PS-NP, PP-NP and PE-NP in *Artemia franciscana* nauplii (n=3). Results are shown as mean $\pm$ standard deviation and *p*-value.

Conc (mg/L)	PS-NP		PP-NP		PE-NP	
	Mean $\pm$ Std	<i>p</i> - value	Mean $\pm$ Std	<i>p</i> - value	Mean $\pm$ Std	<i>p</i> - value
0.05	1080 $\pm$ 15	0.003	445 $\pm$ 55	0.03	745 $\pm$ 42	0.0002
0.5	1925 $\pm$ 40	0.005	693 $\pm$ 148	0.2	892 $\pm$ 24	0.0002
5	1999 $\pm$ 44	0.05	1683 $\pm$ 76	0.0003	1171 $\pm$ 60	0.005
50	3557 $\pm$ 61	0.001	1970 $\pm$ 115	0.002	1777 $\pm$ 72	0.009

Table S3. The mean fluorescence intensity acquired using Image.J software for cell apoptosis in *Artemia franciscana* nauplii exposed to PS-NP, PP-NP and PE-NP (n=3). Results are shown as mean $\pm$ standard deviation and *p*-value

Conc (mg/L)	PS-NP		PP-NP		PE-NP	
	Mean $\pm$ SD	<i>p</i> - value	Mean $\pm$ SD	<i>p</i> - value	Mean $\pm$ SD	<i>p</i> - value
0.05	1202 $\pm$ 52	0.08	1018 $\pm$ 54	0.02	960 $\pm$ 45	0.002
0.5	1525 $\pm$ 88	0.02	1110 $\pm$ 34	0.2	1443 $\pm$ 34	0.0002
5	2002 $\pm$ 89	0.01	1511 $\pm$ 17	0.0008	1704 $\pm$ 26	0.02
50	2666 $\pm$ 108	0.007	2029 $\pm$ 77	0.3	2162 $\pm$ 101	0.04

Table S4. Primer sets of growth and development-related genes of *Artemia franciscana* used for real-time PCR analysis

Gene ID	Forward primer	Reverse Primer	Accession No.
<i>Clap</i>	<i>GCAGACAATTCTTGCCGGTG</i>	<i>TCACTGTTCCATGTCGTGCT</i>	AY757920
<i>Cstb</i>	<i>GCGAGAAGTCTTATCAAGT</i>	<i>TCTTTTACAGGAGTGATGG</i>	XM 029224569.1
<i>Had- like</i>	<i>AAGTCGATTCCCTGACAC</i>	<i>TCAGGTCGGAACCTCTAAC</i>	XM029224570.1
<i>Ucp2</i>	<i>TGACCATTGCTAGAGAAGA</i>	<i>GCACCAGTAGACATACCAG</i>	XM029224571.1
<i>Cdc48</i>	<i>AATGGCAGGTGAATCCG</i>	<i>CTTCAAACCGTCCATCA</i>	XM029224572.1
<i>AP15</i>	<i>TGTTGAGACGGAGGTACCCA</i>	<i>CCATTAAACCGGCCTCGGAA</i>	EU142258
<i>Tcp1</i>	<i>CTCTTGCTCAACTGCCCACT</i>	<i>TGAGCATAAAGAGCCCGCAA</i>	EF062491
<i>Gapdh</i>	<i>GTTGATGGCAAACCTCGTCATA</i>	<i>CCACCTTCCAAGTGAGCATT</i>	XM029224575.1

Table S5. Data of % fold change in gene expression for PSNP, PENP, and PPNP. Results are shown as mean $\pm$ standard deviation and *p*-value (T-test).

Gene	PS-NP			PP-NP			PE-NP		
	Exposed	Control	<i>p</i> -value	Exposed	Control	<i>p</i> -value	Exposed	Control	<i>p</i> -value
<i>Cstb</i>	4.29 $\pm$ 0.3	0.23 $\pm$ 0.01	0.05	4.77 $\pm$ 0.007	0.21 $\pm$ 0.003	0.00	1.84 $\pm$ 0.008	0.54 $\pm$ 0.002	0.01
<i>clap</i>	0.41 $\pm$ 0.04	2.46 $\pm$ 0.02	0.01	0.21 $\pm$ 0.12	2.44 $\pm$ 0.50	0.12	0.22 $\pm$ 0.01	4.57 $\pm$ 0.23	0.04
<i>Cdc48</i>	5.22 $\pm$ 0.1	0.19 $\pm$ 0.004	0.02	3.00 $\pm$ 0.03	0.33 $\pm$ 0.003	0.01	0.88 $\pm$ 0.11	1.14 $\pm$ 0.14	0.51
<i>Had-like</i>	4.47 $\pm$ 0.06	0.23 $\pm$ 0.02	0.06	1.18 $\pm$ 0.43	0.97 $\pm$ 0.3	0.83	1.43 $\pm$ 0.17	0.71 $\pm$ 0.08	0.22
<i>Tcp1</i>	0.21 $\pm$ 0.17	5.05 $\pm$ 1.0	0.18	1.194 $\pm$ 0.32	1.46 $\pm$ 0.3	0.03	0.41 $\pm$ 0.15	2.95 $\pm$ 1.17	0.31
<i>API5</i>	0.43 $\pm$ 0.23	2.47 $\pm$ 0.6	0.23	1.82 $\pm$ 0.36	1.87 $\pm$ 1.4	0.98	0.35 $\pm$ 0.04	2.89 $\pm$ 0.39	0.11
<i>Ucp2</i>	0.24 $\pm$ 0.1	4.25 $\pm$ 0.6	0.10	0.26 $\pm$ 0.009	3.84 $\pm$ 0.1	0.03	3.45 $\pm$ 0.42	0.29 $\pm$ 0.03	0.09