Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting materials *for*

Estrogenic activity of plastic nanoparticles mixture under in vitro settings

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CONTENT:

Figures S7. Dose-response curve for the estrogen receptor activity of PS-NPs generated using free online tool PROAST (https://proastweb.rivm.nl/).

Figures S8. Dose-response curve for the estrogen receptor activity of PE-NPs, PP-NPs, PS-NPs mixtures generated using free online tool PROAST (https://proastweb.rivm.nl/).

Tables S1. Literature search results based on relevant keyword combinations using the Web of Science database.

Table S2. Literature research results based on relevant keyword combinations for micro- and nanoplastics (PMPs and PNPs). Literature research was conducted using the Web of Science database.

Table S3. Literature research results based on relevant keyword combinations for combined effects of EDCs and PMPs/PNPs. Literature research was conducted using the Web of Science database.

Table S4. Literature research results based on relevant keyword combinations for polystyrene nanoparticles (PSNPs). Literature research was conducted using the Web of Science database.

Table S5. Literature research results based on relevant keyword combinations for polyethylene nanoparticles (PENPs). Literature research was conducted using the Web of Science database.

Table S6. Literature research results based on relevant keyword combinations for polypropylene nanoparticles (PPNPs). Literature research was conducted using the Web of Science database.

Table S7. Literature research results based on relevant keyword combinations for combined effects of PSNPs, PENPs and PPNPs. Literature research was conducted using the Web of Science database.

Table S8. Physico-chemical characteristics of mixture of PS-NPs, PP-NPs and PE-NPs determined by DLS.

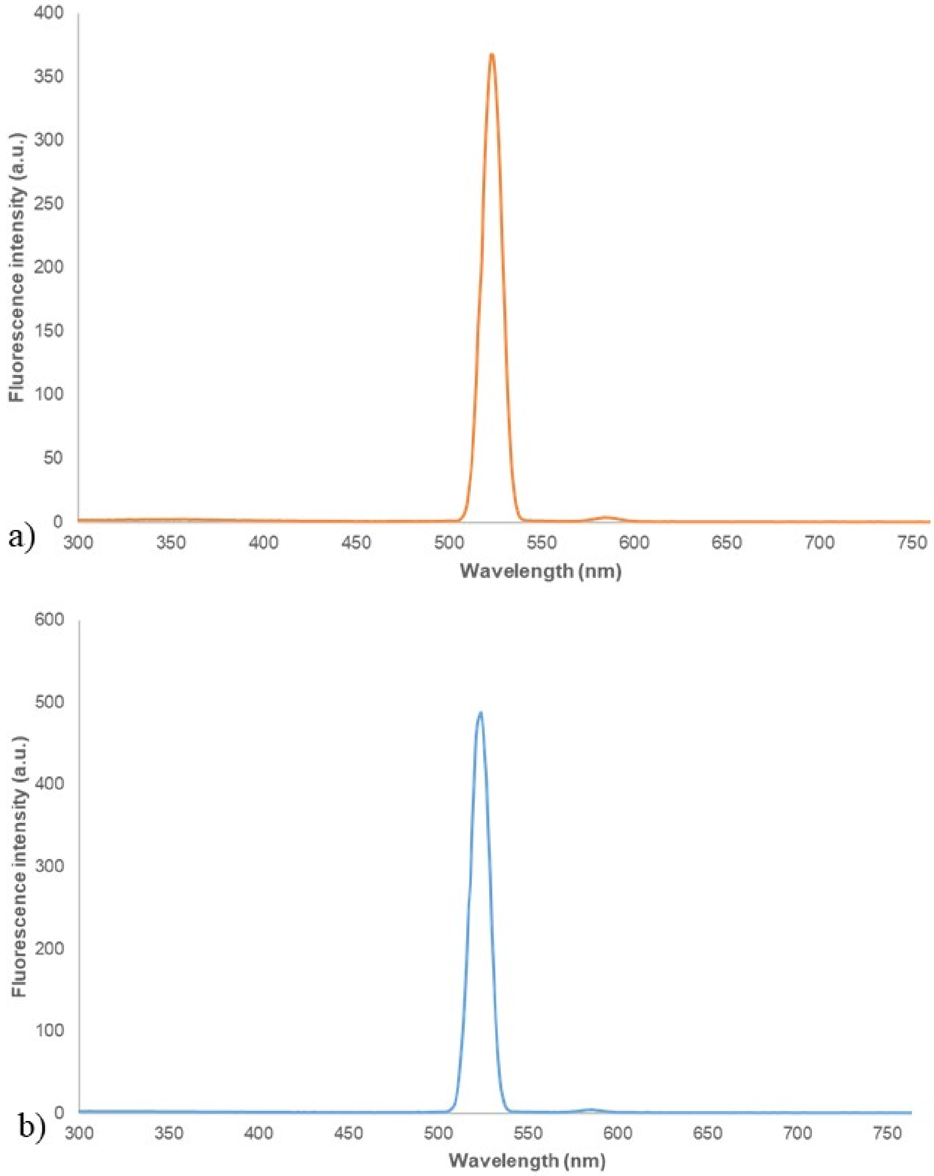
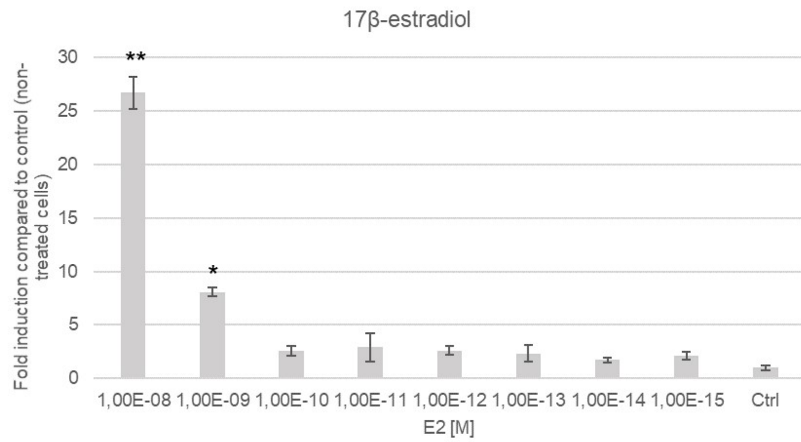
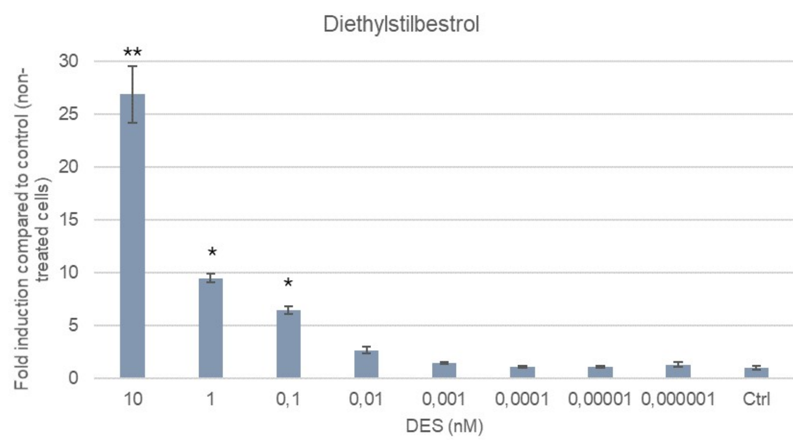


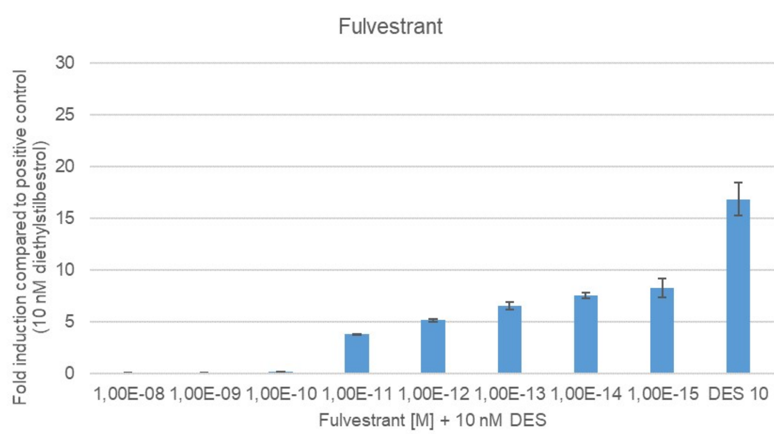
Figure S1. Fluorescence emission spectra of polyethylene nanoparticles (PE-NPs) (a) and polypropylene nanoparticles (PP-NPs) (b). PE-NPs show emission maximum at 523 nm and PP-NPs emission maximum is at 524 nm.



a)



b)



c)

Figure S2. Responsiveness of the system used for analysis of in vitro modulation of ER tested by two agonists - 17β-estradiol (a), diethylstilbestrol (b) and an antagonist - fulvestrant (c).

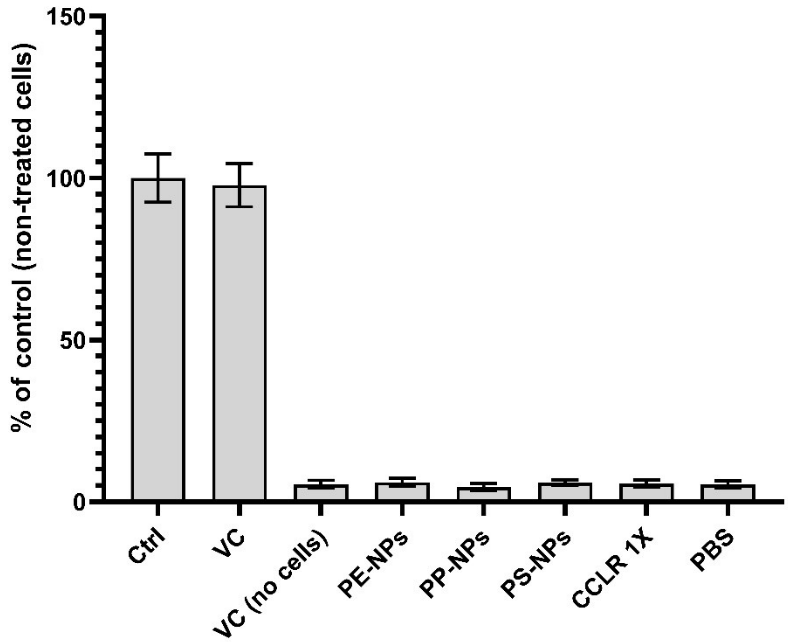


Figure S3. Comparison of non-treated cells (Ctrl), vehicle control (0.0001& Tween and 2 μM sodium azide) with (VC) and without cells (VC (no cells)), PE-NPs, PP-NPs, PS-NPs, diluted Cell Culture Lysis Buffer (CCLR 1X) and phosphate buffered saline (PBS). PE-NPs, PP-NPs and PS-NPs were tested at concentration of 10 mg/L (highest concentration used) and without cells. Results are shown as % of Ctrl.

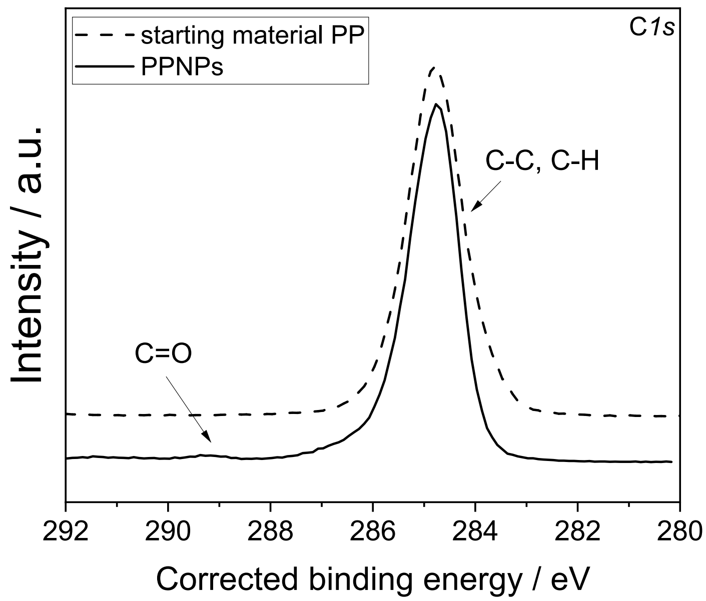


Figure S4. X-ray photoelectron spectroscopy (XPS) spectra of C 1s peaks of PP starting material and PP-NPs.

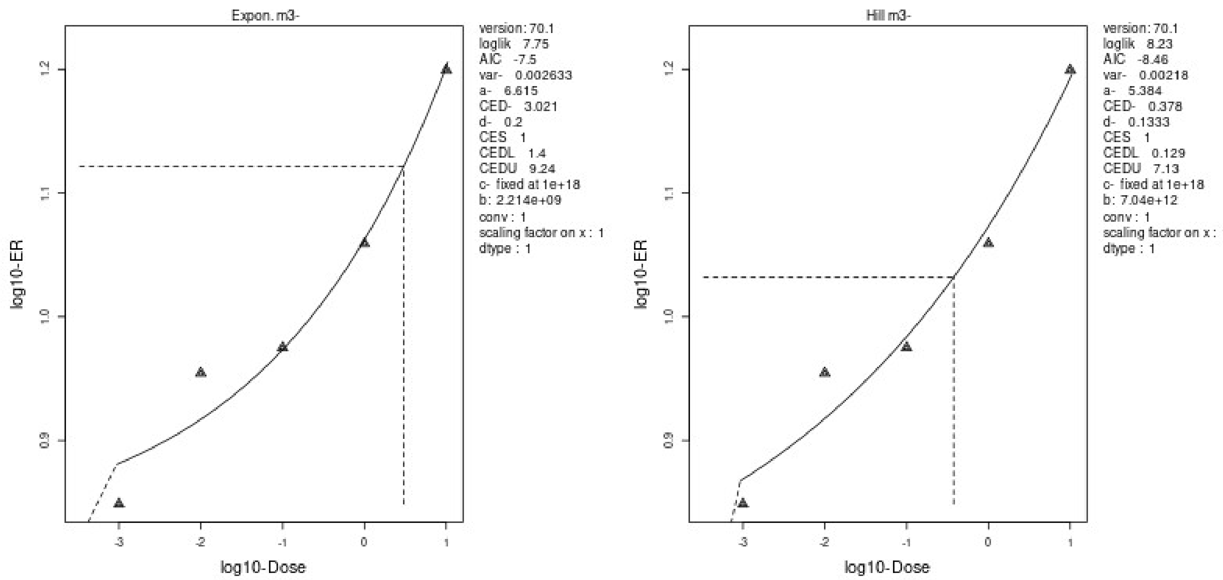


Figure S5. Dose-response curve for the estrogen receptor activity of PENPs generated using free online tool PROAST (https://proastweb.rivm.nl/). Dose-response curve enables determination of benchmark dose (BMD) needed to calculate relative potency factor (RPF).

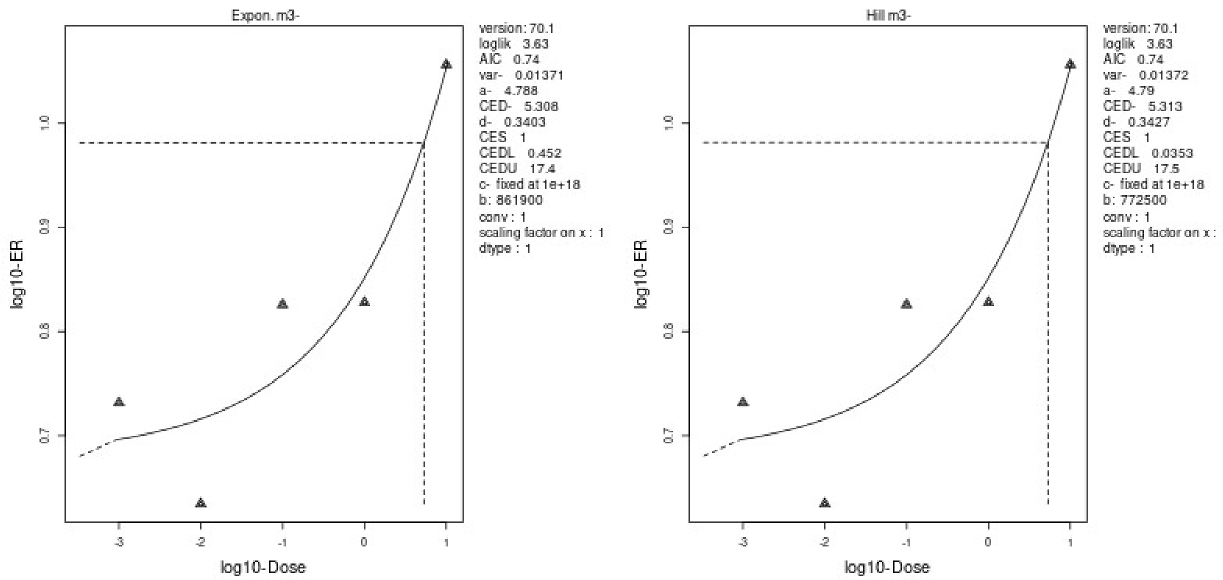


Figure S6. Dose-response curve for the estrogen receptor activity of PPNPs generated using free online tool PROAST (https://proastweb.rivm.nl/). Dose-response curve enables determination of benchmark dose (BMD) needed to calculate relative potency factor (RPF).

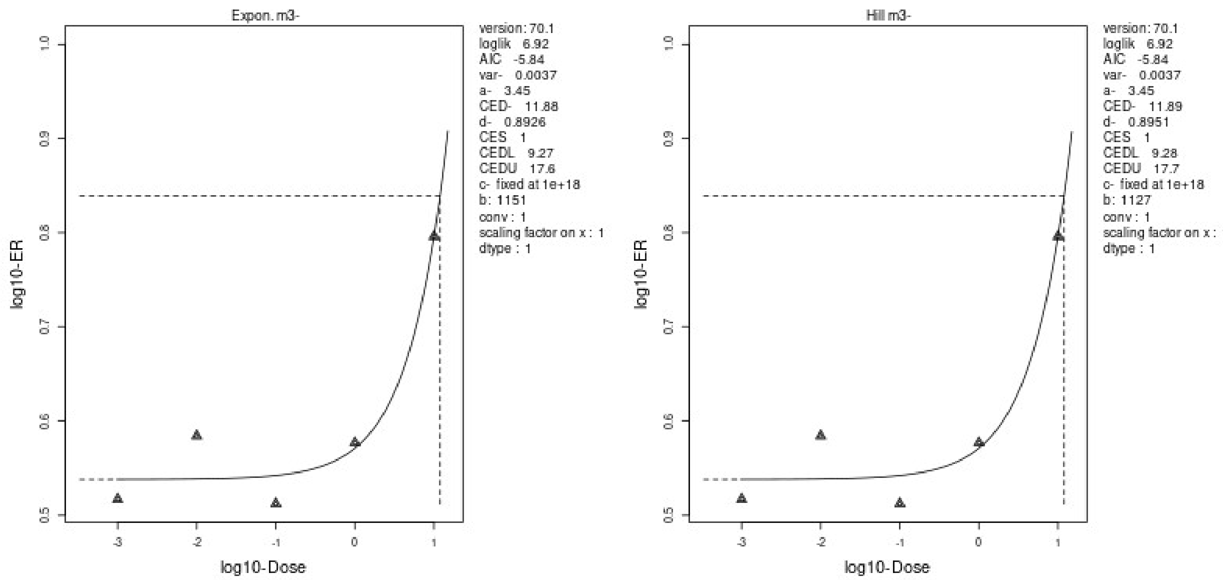


Figure S7. Dose-response curve for the estrogen receptor activity of PSNPs generated using free online tool PROAST (https://proastweb.rivm.nl/). Dose-response curve enables determination of benchmark dose (BMD) needed to calculate relative potency factor (RPF).

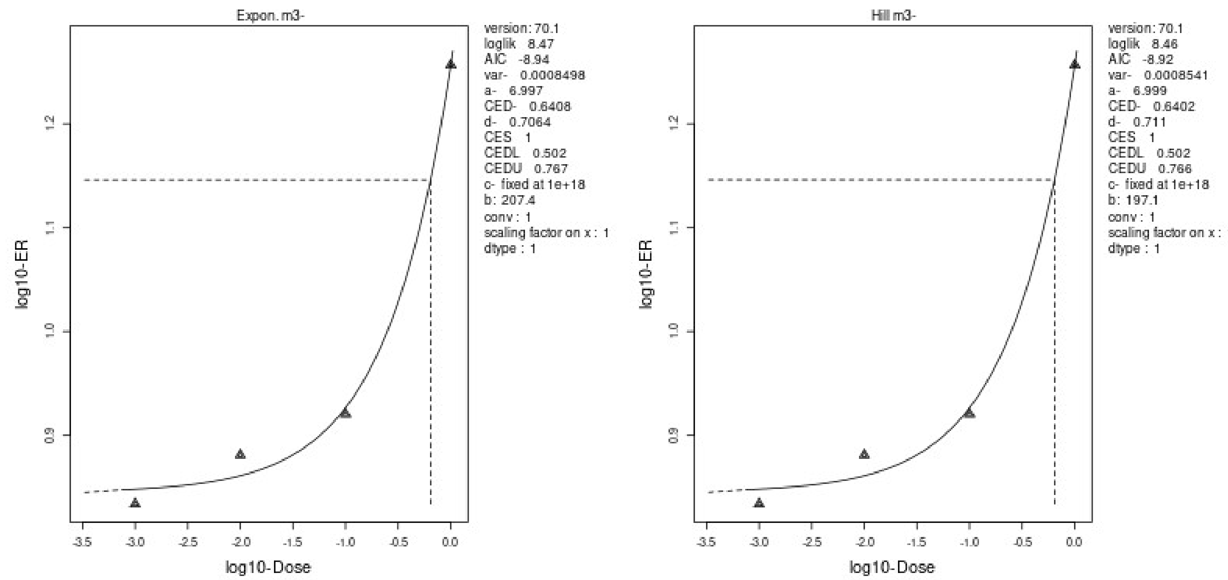


Figure S8. Dose-response curve for the estrogen receptor activity of PENPs/PPNPs/PSNPs mixture generated using free online tool PROAST (https://proastweb.rivm.nl/). Dose-response curve enables determination of benchmark dose (BMD) needed to calculate relative potency factor (RPF).

Table S1. Literature research results based on relevant keyword combinations for endocrine disrupting chemicals (EDC). Literature research was conducted using the Web of Science database.

Keyword combination	**Number of results**
(ALL=(endocr*)) AND ALL=(disrupt*)	45114
((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	3472
(((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(human)	1575
(((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(in vitro)	763
(((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(animal)	581
((((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(human)) AND ALL=(estrog*)	469
((((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(in vitro)) AND ALL=(estrog*)	405
((((ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(animal)) AND ALL=(estrog*)	198

Table S2. Literature research results based on relevant keyword combinations for micro- and nanoplastics (PMPs and PNPs). Literature research was conducted using the Web of Science database.

Keyword combination	Number of results
((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)	41708
(ALL=(microplast*)) AND ALL=(nanoplast*)	2143
(ALL=(microplast*)) OR ALL=(nanoplast*)	16109
(((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)	3494
((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)	144
((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)	921
(((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*) AND ALL (estrog*)	41
((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(estrog*)	4
((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(estrog*)	59
((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(human)	182
(((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(human)	27
(((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(human)	144
((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(in vitro)	95
(((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(in vitro)	11
(((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(in vitro)	22
((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(animal)	43
(((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(animal)	12

(((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(animal)	78
(((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(human)) AND ALL=(estrog*)	3
((((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(human)) AND ALL=(estrog*)	1
((((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(human)) AND ALL=(estrog*)	1
(((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(in vitro)) AND ALL=(estrog*)	2
((((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(in vitro)) AND ALL=(estrog*)	1
((((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(in vitro)) AND ALL=(estrog*)	2
(((((ALL=(nano*)) AND ALL=(micro*)) AND ALL=(plast*)) AND ALL=(mix*)) AND ALL=(animal)) AND ALL=(estrog*)	1
((((ALL=(microplast*)) AND ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(animal)) AND ALL=(estrog*)	0
(((ALL=(microplast*)) OR ALL=(nanoplast*)) AND ALL=(mix*)) AND ALL=(animal)	0

Table S3. Literature research results based on relevant keyword combinations for combined effects of EDCs and PMPs/PNPs. Literature research was conducted using the Web of Science database.

Keyword combination	Number of results
((((ALL=(micro*)) AND ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	110
((((ALL=(micro*)) OR ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	834
(((((ALL=(micro*)) AND ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	12
(((((ALL=(micro*)) OR ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	71
((((((ALL=(micro*)) OR ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*) AND ALL=(estrog*))	24
(((((((ALL=(micro*)) OR ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*) AND ALL=(estrog*))) AND ALL=(in vitro)	8
(((((((ALL=(micro*)) OR ALL=(nano*)) AND ALL=(plast*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*) AND ALL=(estrog*))) AND ALL=(human)	11

Table S4. Literature research results based on relevant keyword combinations for polystyrene nanoparticles (PSNPs). Literature research was conducted using the Web of Science database.

Keyword combination	Number of results
(((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	92
((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)	13
(((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(human)	5
(((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(in vitro)	6
(((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(animal)	3
((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	9
(((((ALL=(polystyrene nano*)) OR ALL=(polystyrene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(estrog*)	0

Table S5. Literature research results based on relevant keyword combinations for polyethylene nanoparticles (PENPs). Literature research was conducted using the Web of Science database.

Keyword combination	Number of results
(((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	105
((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)	21
(((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(human)	13
(((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(in vitro)	9
(((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(animal)	3
((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	12
(((((ALL=(polyethylene nano*)) OR ALL=(polyethylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(estrog*)	5

Table S6. Literature research results based on relevant keyword combinations for polypropylene nanoparticles (PPNPs). Literature research was conducted using the Web of Science database.

Keyword combination	**Number of results**
(((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	53
((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)	11
(((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(human)	1
(((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(in vitro)	2
(((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(estrog*)) AND ALL=(animal)	1
((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	5
(((((ALL=(polypropylene nano*)) OR ALL=(polypropylene micro*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)) AND ALL=(estrog*)	1

Table S7. Literature research results based on relevant keyword combinations for combined effects of PSNPs, PENPs and PPNPs. Literature research was conducted using the Web of Science database.

Keyword combination	Number of results
(((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)	2705
((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(mix*)	566
(((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(mix*)) AND ALL=(human)	11
(((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(mix*)) AND ALL=(in vitro)	3
(((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(mix*)) AND ALL=(animal)	2
(((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(mix*)) AND ALL=(estrog*)	1
(((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(endocr*)) AND ALL=(disrupt*)	8
((((((ALL=(polyethyl*)) NOT ALL=(glycol)) AND ALL=(polystyr*)) AND ALL=(polypropy*)) AND ALL=(endocr*)) AND ALL=(disrupt*)) AND ALL=(mix*)	1

Table S8. Physico-chemical characteristics of mixture of polystyrene (PS-NPs), polypropylene (PP-NPs) and polyethylene (PE-NPs) nanoparticles. Hydrodynamic diameters (d_H, nm) and polydispersity index (PDI) were determined by DLS in cell culture media (CCM) after 48 h. All measurements were done at 25°C and the concentration of mixture was varied from 0.001 to 10 mg/L.

NPs mixture	d_H (nm)	%	PDI
PP_PE_PS 10 ppm	261.2 ± 9.4	100	0.2 ± 0.0
PP_PE_PS 1 ppm	240.0 ± 41.0	73.2	1.0 ± 0.0
	29.6 ± 8.2	15.5	
	8.8 ± 0.8	11.3	
PP_PE_PS 0.1 ppm	252.0 ± 23.5	15.7	0.6 ± 0.0
	48.9 ± 4.6	44.7	
	10.9 ± 0.9	39.6	
PP_PE_PS 0.01 ppm	12.0 ± 1.5	55.6	0.4 ± 0.0
	55.9 ± 13.2	44.4	
PP_PE_PS 0.001 ppm	12.4 ± 0.6	55.7	0.4 ± 0.0
	52.9 ± 12.3	44.3	