

Supplementary Information

Table S1. Levels of polystyrene (PS), polyethylene (PE), polypropylene (PP), and other microplastics with a wide range of sizes that produce an effect or no effect on biomarkers of interest

MP Type	MP Characteristics	Experimental Subject	Exposure Duration	Level with no effect	Level with adverse effect	Adverse Effect Observed	Reference
PS	5 μm and 20 μm	Mice	28 days	-	0.01 mg/day	Decreased adenosine triphosphate level	67
				-	0.01 mg/day	Increased lactate dehydrogenase	
				-	0.01 mg/day	Decreased total cholesterol (lipid metabolism)	
				-	0.1 mg/day	Decreased triacylglycerol (lipid metabolism)	
				-	0.1 mg/day	Decreased catalase (oxidative stress)	
				-	0.1 mg/day	Increased glutathione peroxidase (oxidative stress)	
				-	0.01 mg/day (20 μm); 0.1 mg/day (5 μm)	Increased superoxide dismutase (oxidative stress)	
				-	0.01 mg/day	Increased acetylcholinesterase	
				-	0.5 mg/day	Histological – inflammation and formation of lipid droplets in liver	
PS	0.5 μm and 50 μm	Mice	5 weeks	-	1000 $\mu\text{g/L}$ (0.5 μm)	Decreased body weight	68
				-	1000 $\mu\text{g/L}$	Decreased relative liver and fat weights	
				-	100 $\mu\text{g/L}$	Decreased mucin secretion	
COOH-modified PS	46.3 \pm 6 nm (nano), 465.8 \pm 10.2 nm (micro)	Caco-2/HT29-MTX-E12-culture (intestinal barrier)	24 hours	-	100 $\mu\text{g/mL}$ nano-PS	Increased cell viability	45
				100 $\mu\text{g/mL}$ nano-PS; 100 $\mu\text{g/mL}$ micro-PS	-	Trans-epithelial electrical resistance	

		BeWo b30 (placental barrier)		-	5 µg/mL nano-PS; 0.01 µg/mL micro- PS (dose-dependent up to 10 µg/mL)	Increased cell viability	
				100 µg/mL nano- PS; 100 µg/mL micro-PS	-	Trans-epithelial electrical resistance	
		NIH/3T3 fibroblasts (embryotoxicity)		100 µg/mL nano- PS;	12.6 µg/mL micro- PS	Cytotoxicity (IC ₅₀ – 50% inhibition of cell growth)	
		Mouse ES-D3 (embryotoxicity)		100 µg/mL nano- PS	12.6 µg/mL micro- PS	Cytotoxicity (IC ₅₀)	
		Cardiomyocytes		-	89.9 µg/mL nano-PS; 0.1 µg/mL micro-PS	Cytotoxicity (ID ₅₀ – 50% inhibition of cell differentiation)	
		HepG2CDKN1A -DsRed biosensor cells	5 days	50 µg/mL nano- PS; 10 µg/mL micro-PS	-	Genotoxicity – p53 expression	
		CHO-K1 cells	24 hours	100 µg/mL nano- PS; 100 µg/mL micro-PS	-	Genotoxicity – micronuclei formation	
PS	1, 4, 10 µm	Human intestinal epithelial cell line Caco-2	48 hours		1 x 10 ⁷ particles/mL or 1 x 10 ⁷ pg/mL (1 µm) 1.5 x 10 ⁷ particles/mL or 1 x 10 ⁹ pg/mL (4 µm)	Loss of cell viability	57
		Mice	28 days	Mixture of 4.55 x 10 ⁷ particles (1 µm & 4 µm respectively) and 1.49 x 10 ⁶ particles (10 µm) at a volume of 10 mL/kg-bw	-	No significant changes in the weights of body and organ (heart, kidney, liver, lungs, spleen, testes)	
				As above	-	No significant change of β- galactosidase reporter activity	
				As above	-	heme oxygenase 1- dependent reporter was not	

PS	0.1 μm , 5 μm	Caco-2 cells	12 hours	-	200 $\mu\text{g}/\text{mL}$	observed to be induced Increased intracellular ROS	43
				-	≥ 20 $\mu\text{g}/\text{mL}$ (0.1 μm) and ≥ 1 $\mu\text{g}/\text{ml}$ (5 μm)	Mitochondrial depolarization	
				80 $\mu\text{g}/\text{ml}$	-	No significant change in plasma membrane integrity and fluidity	
PE	10-150 μm	C57BL/6 mice model	5 weeks	-	600 μg	Intestinal inflammation	53
PS	500 nm, 1 μm , 3 μm , 6 μm	Caco-2 cells	24 hours	-	120 $\mu\text{g}/\text{mL}$ (mixture of different particle sizes)	Increased intracellular ROS	72
				-	120 $\mu\text{g}/\text{mL}$ (300 nm, 500 nm, 1 μm , 3 μm and 6 μm respectively)	Mitochondrial depolarization (more pronounced for larger MPs)	
MPs (PP_Sun, polyamide6 and thermoplastic polyurethane ester) resembling those released from tires and polyolefins	50-500 μm	3-dimensional in-vitro intestinal model comprising Caco-2, HT29-MTX-E12, macrophages and dendritic cells	6, 24 and 48 h	Hardened polyurethane (1263.25 $\mu\text{g}/\text{cm}^2$); tire rubber (1066.00 $\mu\text{g}/\text{cm}^2$); PP_Sun (1305.50 $\mu\text{g}/\text{cm}^2$); Polyamide6 (1028.58 $\mu\text{g}/\text{cm}^2$); Thermoplastic polyurethane ester (1098.02 $\mu\text{g}/\text{cm}^2$)	-	Release of lactate dehydrogenase due to cell membrane rupture	39
				-	-	Release of cytokines IL-8, TNF α and IL-1 β due to inflammatory response	
PS	0.05-0.1 μm	Caco-2/HT29 intestinal cells and Caco-2/HT29 + Raji-B cells	24 hours	200 $\mu\text{g}/\text{mL}$	-	Cytotoxicity	50
				100 $\mu\text{g}/\text{mL}$	-	Barrier's integrity	
				100 $\mu\text{g}/\text{mL}$	-	Barrier's permeability	
				100 $\mu\text{g}/\text{mL}$	-	Production of intracellular ROS	
				100 $\mu\text{g}/\text{mL}$	-	Genotoxic and oxidative DNA damage	
PS	0.05-0.1 μm	Caco-2 cells	24 and 48 hours	150 $\mu\text{g}/\text{mL}$ (48	200 $\mu\text{g}/\text{mL}$ (24 and	Mild cytotoxicity	36

				hours)	48 hours)		
				100 µg/mL (24 hours)	-	Intracellular ROS levels	
				100 µg/mL (24 hours)	-	Genotoxic and oxidative DNA damage	
				100 µg/mL (48 hours)	-	Chromosome damage (micronucleus induction)	
				-	50 µg/mL (24 hours); 25 µg/mL (48 hours)	Increased expression of ROS-related genes, HO1	
PS (original and transformed by an in-vitro digestive process)	100 nm, 5 µm	Caco-2 cells	96 hours	20 µg/mL (100 nm and 5 µm t-PS-MPs)	20 µg/mL (100 nm PS-MPs)	Increased lactate dehydrogenase	37
				20 µg/mL (100 nm and 5 µm t-PS-MPs)	20 µg/mL (100 nm PS-MPs)	Decreased intestinal transport indicated by increased permeability of paracellular marker Lucifer Yellow	
				-	20 µg/mL (100 nm PS-MPs); 1 µg/mL (5 µm PS-MPs); 20 µg/mL (100 nm t-PS-MPs); 1 µg/mL (5 µm t-PS-MPs)	Down-regulated adenosine triphosphate binding cassette transporters, ABCC2	
				-	20 µg/mL (100 nm PS-MPs); 1 µg/mL (5 µm PS-MPs); 20 µg/mL (100 nm t-PS-MPs); 20 µg/mL (5 µm t-PS-MPs)	Down-regulated adenosine triphosphate binding cassette transporters, ABCG2	
PS	20 nm	Rats	24 hours	-	Intratracheal instillation of 2.64 x 10 ¹⁴ particles	Lower fetal and placenta weights	54
PE	16.9 ± 1.9 µm	Mice	90 days	-	0.125 mg/day	Lesions in lung tissues	
				-	0.125 mg/day (male)	Lesions in kidney tissues	
				-	0.5 mg/day (female)	Lesions in spleen tissues	
				-	0.5 mg/day	Lesions in testicular tubules	
				-	0.125 mg/day (male)	Germ cell degeneration	
				-	0.125 mg/day	Lesions in stomach tissues	

				-	0.125 mg/day (female)	Adverse changes in the ovary	
				2 mg/day	-	No remarkable changes of epididymitis, heart, duodenum, ileum, colon, thymus, uterus, and brain tissues	
				-	0.125 mg/day	Increased helper T cells	
				-	0.125 mg/day	Decreased number of live births per dam and	
				-	2 mg/day	Decreased body weight of pups (within 6 hours after birth)	
PE	40-48 µm	Mice	90 days	-	0.5 mg/day (15 mg/kg-bw/day) (P ≤ 0.5)	Increased ratio of helper T cells to cytotoxic T cells (CD4 ⁺ /CD8 ⁺)	64
				-	2 mg/day (60 mg/kg-bw/day) (P ≤ 0.05)	Decreased ratio of mature dendritic cells (CD11b ⁻ /CD11c ⁺)	
				-	2 mg/day (60 mg/kg-bw/day)	Increased IgA concentration	
				-	2 mg/day (60 mg/kg-bw/day) (P ≤ 0.5)	Reduced number of live births per dam	
				-	2 mg/day (60 mg/kg-bw/day) (P ≤ 0.5)	Reduced body weight of pups (within 6 hours after birth)	
				-	0.125 mg/day (3.75 mg/kg-bw/day)	Pathological – degenerated testicular germ cell	
				-	0.125 mg/day (3.75 mg/kg-bw/day)	Pathological – stomach mucosal hypertrophy/hyperplasia	
				-	0.125 mg/day (3.75 mg/kg-bw/day)	Pathological – ovary cysts	
				0.125 mg/day (3.75 mg/kg-bw/day)	-	Pathological – heart, intestinal, uterine and brain tissues	
				-	0.5 mg/day (15 mg/kg-bw/day)	Pathological – spleen extramedullary hemopoiesis	

				-	0.125 mg/day (3.75 mg/kg-bw/day)	Pathological – lung and kidney tissues	
PS	5.0-5.9 μm	Mice	42 days	-	0.01 mg/day	Decreased spermatogenic cells	69
				-	0.01 mg/day	Decreased sperm count	
				-	0.01 mg/day	Increased rate of teratosperm	
				-	0.1 mg/day	Reduced succinate dehydrogenase activities	
				-	0.01 mg/day (not dose-dependent; no significant effect at 0.1 mg/day)	Reduced lactate dehydrogenase activities	
				-	0.01 mg/day	Decreased testosterone level	
				-	0.01 mg/day	Increased ROS	
				-	0.01 mg/day	Increased malondialdehyde	
				-	1 mg/day	Decreased glutathione	
				-	0.01 mg/day	Increased apoptotic factor, Casp-3	
-	0.01 mg/day	Increased inflammatory factors TNF- α , IL-1 β and IL-6					
PE	35.46 $\mu\text{m} \pm 18.17 \mu\text{m}$	Mice	7 days	-	60 mg/L or 4.24×10^6 particles/ m^3	Reduced locomotion activity, i.e. shorter distance traveled, lower speed and higher anxiety index	74
PS	0.5 μm	Rats	90 days	-	1.5 mg/day	Decreased volume of growing follicle	38
				-	0.15 mg/day	Reduced secretion of anti-Mullerian hormone	
				-	0.15 mg/day	Increased malondialdehyde level; decreased catalase, superoxide dismutase and glutathione peroxidase	
				-	1.5 mg/day	Decreased integrated optical density value of collagen fibers; increased	

						fibronectin expression	
				-	0.15 mg/day	Increased expressions of Wnt and TGF- β in ovarian tissues	
				-	1.5 mg/day	Increased expressions of β -catenin, p- β -catenin, α -SMA, collagen I, collagen II and fibronectin	
		Rat granulosa cells	24 hours	-	5 μ g/mL	Increased reactive oxygen species	
				-	5 μ g/mL	Higher GC apoptotic rate	
				-	5 μ g/mL	Higher protein expression wnt	
				-	1 μ g/mL	Higher expression of p- β -catenin	
PS	0.5 μ m, 4 μ m, 10 μ m	Mice	28 days	-	100 μ L (10 mg/L)	Declined sperm quality and testosterone level	58
				100 μ L (10 mg/L) (0.5 μ m)	100 μ L (10 mg/L) (4 μ m, 10 μ m)	Decreased weight	
				-	100 μ L (10 mg/L)	Increased testicular inflammatory factor	
				-	100 μ L (10 mg/L)	Disrupted integrity of the blood-testis barrier	
PE	200-9900 nm	Inverted triple culture transwell model of healthy and inflamed intestine (Caco-2/HT29-MTX-E12/THP-1)	24 hours	-	50 μ g/cm ² (healthy and inflamed cultures)	Increased lactate dehydrogenase level	65
				50 μ g/cm ² for inflamed culture	50 μ g/cm ² for stable healthy culture	Increased inflammatory cytokine, IL-8	
				50 μ g/cm ² for healthy and inflamed cultures	-	Pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α	
				50 μ g/cm ² for healthy and inflamed cultures	-	DNA damage	
Fresh (f-) and weathered (w-) PP and PS	100 μ m	Caco-2 cells	24 hours	20000 particles/mL	-	Lactate dehydrogenase level	63
				20000 particles/mL	-	Levels of IL-6, IL-8 and TNF- α	
		HepG2 cells		20000	-	Lactate dehydrogenase	

				particles/mL		level	
				20000 particles/mL	-	Levels of IL-8 and TNF- α	
				-	5000 f-PS particles/mL (lack dose-dependence)	Increased level of IL-6	
		THP-1 macrophage		20000 w-PP particles/mL	20000 f-PP particles/mL; 5000 f-PS particles/mL; 10000 w-PS particles/mL	Increased lactate dehydrogenase level	
				20000 w-PP particles/mL; 20000 w-PS particles/mL	5000 f-PP particles/mL; 5000 f-PS particles/mL	Increased IL-1 β	
				-	5000 particles/mL	Increased MIP-1 β	
PS	5 μ m	Mice	35 days	-	100 μ g/L (0.6-0.7 μ g/day)	Decreased ratio of live sperm in epididymis to total sperm number	70
				-	10 mg/L (60-70 μ g/day)	Increased sperm malformation	
				-	100 μ g/L (0.6-0.7 μ g/day)	Increased expression of NF- κ Bp65 and p-NF- κ Bp65 nuclear factor	
				1000 μ g/L (6-7 μ g/day)	-	Expression of p-I κ B α	
				-	1000 μ g/L (6-7 μ g/day)	Reduced expression of p-I κ B α nuclear factor	
				-	1000 μ g/L (6-7 μ g/day)	Increased IL-1 β	
				-	100 μ g/L (0.6-0.7 μ g/day)	Increased IL-6	
				-	10 mg/L (60-70 μ g/day)	Increased TNF α	
				-	1000 μ g/L (6-7 μ g/day)	Decreased levels of Nrf2 and HO-1	
				-	100 μ g/L (0.6-0.7 μ g/day)	Increased pro-apoptotic protein to anti-apoptotic protein ratio (Bax/Bcl2)	

PE	30-140 μm	Caco-2 cells	48 hours	-	1000 mg/L	Decreased cell viability	66
				-	100 mg/L	Increased intracellular ROS	
				-	100 mg/L	Reduced mitochondrial membrane potential	
				1000 mg/L	-	Lactate dehydrogenase level	
PS	20 nm, 40 nm, 1 μm carbonylated PS; sulfate- and amine-modified 100 nm PS; sulfate-modified 10 μm PS	HepaRG cells	24 hours	-	2.5×10^{11} (100 nm sulfate (-) and 100 nm neutral PS); 5×10^{11} (100 nm amino (+) PS); 2.5×10^{12} (40 nm carboxy (--) PS surface/mL; 1.5×10^{11} (20 nm carboxy (--) PS (dose-dependent)	Slightly increased caspase-3 activity	73
				-	2×10^{11} (20 nm carboxy (--) PS (dose-dependent); 5×10^{11} (100 nm amino (+) PS) Unit = μm^2 particle surface/mL	Increased caspase-9 activity indicating apoptosis induction	
UV-treated PS	1.0-1.9 μm	Caco-2 cells	24 hours	-	500 $\mu\text{g/mL}$ (6-hour and 12-hour UV irradiation)	Decreased cell viability	44
				-	500 $\mu\text{g/mL}$ (6-hour and 12-hour UV irradiation)	Increased lactate dehydrogenase level, which implies plasma membrane damage	
PS	50 nm	Mice	30 days	10 mg/kg	-	Body weight	60
				10 mg/kg	-	Anxiety-like behavior	
				10 mg/kg	-	Locomotor function	
				10 mg/kg	-	Relative mRNA expression of IL-1 β , IL-6, Tnf- α (intestinal inflammation)	
				10 mg/kg (Muc2)	10 mg/kg (Tff3 and	Relative mRNA	

					Klf3)	expression of Muc2, Tff3 and Klf3 (intestinal mucus secretion)	
				10 mg/kg	-	Relative mRNA expression of Nfe212, Hmox-1, Txn-1, Txn-2 (oxidative stress)	
				10 mg/kg	-	Relative mRNA expression of Zo-1, Cldn3, Cldn7, Occludin (intestinal epithelial cell tight junction proteins)	
				10 mg/kg	-	Inflammation and oxidative stress of cortex	
				10 mg/kg	-	Inflammation and oxidative stress of lung	
				10 mg/kg	-	Inflammation and oxidative stress of liver	
				10 mg/kg	-	IL-1 β , IL-6 and TNF- α in serum	
PS	1 μ m	Mice	33 days	-	80 μ g/kg/day	Transcriptional change in colon	71
				-	80 μ g/kg/day	Mild inflammatory signature in colon	
			33 and 41 days	-	80 μ g/kg/day	Increased inflammation and increased leukocytes	