Supplementary Information (SI) for Environmental Science: Nano.

Interactive toxicity of non-/biodegradable NPs and butyl methoxydibenzoyl methane on intestinal health and metabolism of zebrafish

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Chemicals and reagents

BD-DBM (purity > 98%, CAS-no. 118-60-5), PLA-NPs and PS-NPs (1000 nm) were purchased from AccuStandard Inc (Newhaven, USA) and Guangxi Chenchen Plasticizing Co., Ltd (Guangxi, China) respectively. BD-DBM stock solutions and serial dilutions were pre-configured in dimethyl sulfoxide (DMSO; purity > 99.9%; Amresco, Solon, USA). PLA-NPs and PS-NPs stock and dilutions were pre-configured in a deionized water solution. Phosphate buffer saline (PBS) was purchased from J&K Scientific (Shanghai, China). 4 % paraformaldehyde solution was purchased from Labgic Technology Co., Ltd (Beijing, China).

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Two microlitres of original cDNA (diluted 10-fold) were taken out and added into a reaction tube containing 0.1 μM primer and 0.25×FastStart Universal SYBR GREEN Master (Roche, Germany), making a total volume of 20 μL. Expression of target genes was quantified by an Eppendorf main ring EP real-time PCR detection system (Eppendorf, Germany). The quantitative RT-PCR amplification procedures were as follows: the first was a pre-denaturation at 95℃ for 3 min, then 40 cycles of denaturation at 95℃ for 10 s and annealing and extension at 60℃ for 1 min. All reactions were repeated 3 times.

Intestinal microbiome analysis

The highly variable V3-V4 region of bacterial 16S rRNA gene was amplified by PCR thermal cycler, and the primers were upstream primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and downstream primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The original sequencing sequence was quality-controlled by fastp (0.20.0, https://github.com/OpenGene/fastp) software and spliced by FLASH (1.2.7, http://www.cbcb.umd.edu/software/flash) software. Then, the sequences were subjected to OTU clustering based on 97% similarity by UPARSE software (7.1, http://drive5.com/uparse/). Finally, each OUT representative sequence

was annotated and analyzed for species classification using RDP classifier (2.2, http://rdp.cme.msu.edu/) and a confidence threshold (70%) combined with the Silva 16S rRNA database (v138).

Metabolomic analysis

The mass spectrometry data were searched, compared and analyzed against three major mass spectrometry databases: mzCloud, mzVault and MassList. After detection and identification, metabolomics data obtained in positive (833 species) and negative (404 species) ion modes were combined, and orthogonal partial least squares discriminant analysis (OPLS-DA) was performed on the entire sample set. Statistical significance (p-value) was calculated for each metabolite using a t-test to compare the two groups, and the fold change (FC value) of the differences between metabolites in the different groups was calculated. metabolites with VIP > 1, $p < 0.05$, and $|\log_2 FC| \geq 1$ were identified as significantly changed metabolites.

Table 1S. Primers used for quantitative real-time PCR analysis and their sources. Gene-specific primers of all the genes were designed based on zebrafish sequences available at NCBI (http://www.ncbi.nlm.nih.gov/).

Primer	Gene ID	Primer (5′-3′)
β-actin	57934	F: 5′-CTGTCTTCCCATCCATCGTGGGTC-3′ R: 5′- CTCCATATCATCCCAGTTGGTGACA -3′
TNF-α	405785	F: 5′-GCTGGATCTTCAAAGTCGGGTGTA -3′ R: 5′-TGTGAGTCTCAGCACACTTCCATC-3′
IL-1β	405770	F: 5′-CATTTGCAGGCCGTCACA-3′ R: 5′-GGACATGCTGAAGCGCACTT-3′
IL-10	553957	F: 5′- CCCTATGGATGTCACGTCATG -3′ R: 5′- CATATCCCGCTTGAGTTCCTG -3′
PPAR-αa	563298	F: 5′-CATCTTGCCTTGCAGACATT-3′ R: 5′-CACGCTCACTTTTCATTTCAC-3′
GCK	751668	F: 5′-GCTGTGAAGTCGGCATGATA-3′ R: 5′-CTTCAACCAGCTCCACCTTAC-3′
UCP2	555812	F: 5′-TGGCTAACCCACTGATGTA-3′ R: 5′-CAATGGTCCGATATGCGTC-3′
SOD	30553	F: 5′-GTCGTCTGGCTTGTGGAGTG-3′ R: 5′-TGTCAGCGGGCTAGTGCTT-3′
CAT	30068	F: 5′-CAGGAGCGTTTGGCTACTTC-3′ R: 5′-ATCGGTGTCGTCTTTCCAAC-3′

Table 2S. Alpha diversity in zebrafish intestines after exposure to PLA, PS, B, PLA+B and PS+B.

Group of exposure	Chao1	ACE	Shannon	Simpson	Pielou
CK	1302.93±844.06	1410±997.30	2.28±0.39	0.66±0.03	0.36±0.02
PLA	935.43±134.44	1048.74±205.57	2.53±0.81	0.68±0.19	0.38±0.10
PS	1352.38±190.42	1460.76±186.78	3.82±0.35*	0.90±0.06	0.54±0.05*
B	899.79±614.96	983.82±704.59	2.71±0.71	0.76±0.14	0.42±0.09
PLA+B	1487.61±508.90	1660.83±494.68	3.21±0.31	0.84±0.07	0.48±0.04
PS+B	870.32±281.69	961.88±306.11	3.45±0.28*	0.90±0.04	0.52±0.02*

Table 3S. OPLS-DA analysis of different metabolites in zebrafish intestines.

Group of exposure	Upregulated the number of metabolites	Down-regulating the number of metabolites	Total
PLA	30	33	63
PS	27	41	68
B	48	35	83
PLA+B	81	34	115
PS+B	97	61	158

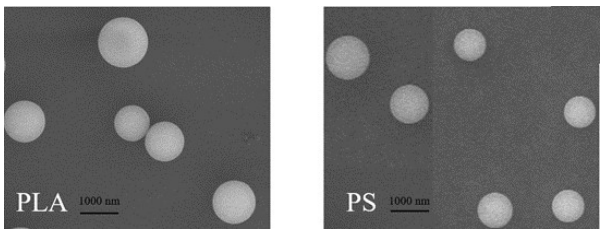

Fig. 1S. Scanning electron microscopy of PLA-NPs and PS-NPs.

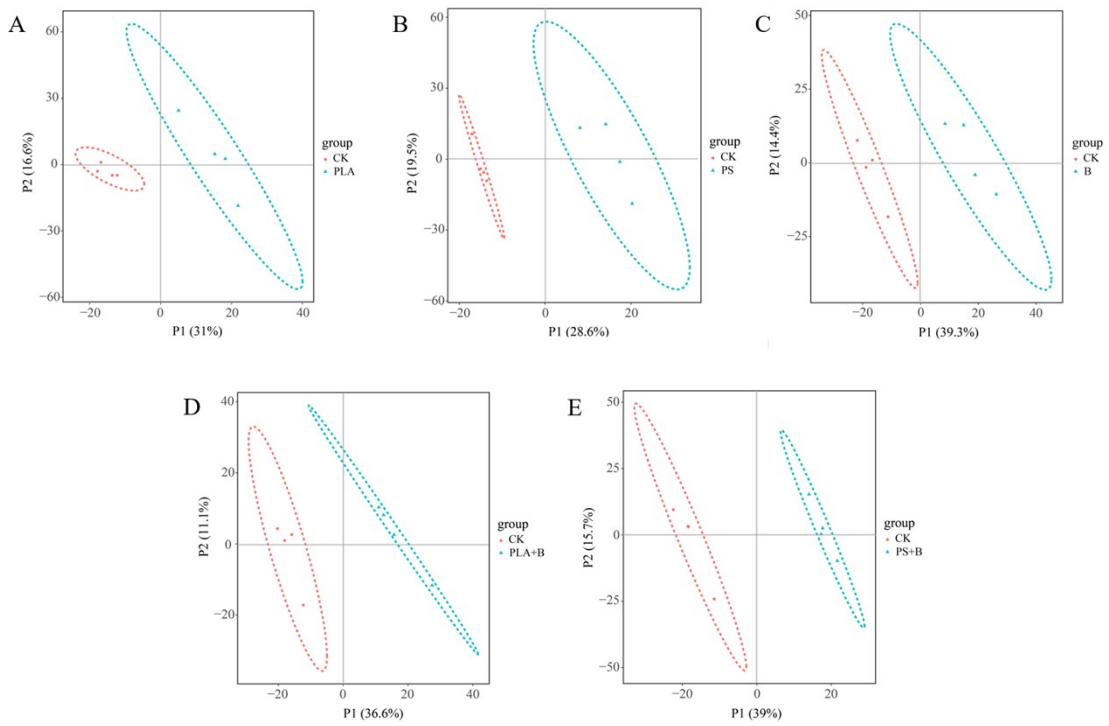

Fig. 2S. Sequencing verification of PLS-DA of the metabolome in zebrafish intestines with different concentrations of PLA, PS, B, PLA+B and PS+B treatments.