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

Supporting Information

Interactive transgenerational effects of polystyrene nanoplastics and ethylhexyl salicylate on zebrafish

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Chemicals and reagents

EHS stock solutions and serial dilutions were prepared in dimethyl sulfoxide (DMSO; purity > 99.9%; Amresco, Solon, OH, USA). Dichloromethane, ethyl acetate, methanol, acetone, hexane and caustic potash (HPLC grade) were supplied by Merk Serono Co., Ltd. (Darmstadt, Germany). Tricaine methane sulfonate (MS-222) was purchased from J & K Scientific (Shanghai, China). Kits for the analysis of glutathione (GSH), reactive oxygen species (ROS), MDA, triiodothyronine (T3) and thyroxine (T4) contents, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) activities and protein level were supplied by Nanjing Sen Bei Jia Biological Technology Co., Ltd. (Nanjing, China). DNA assay kits were obtained from Qiyi Biotechnology (Shanghai) Co., Ltd. (ShangHai, China).

Quantification of EHS and PS-NPs in F0 and F1 eggs

EHS was extracted by ultrasound method. Specifically, a 0.5 g ww sample was mixed with 1 g of active alumina (removing interference of fat), 10 mL of extractant ethyl acetate/dichloromethane (v/v = 1:3) was added and ultrasound was performed for 2 h. Then, the mixture was centrifuged at 120 r/h for 3 min, and the extracts were evaporated and reconstituted in acetone/hexane (v/v = 1:9) to a final volume of 1 mL in a 2 mL amber glass vial. EHS identification and quantification were performed using a Thermo Scientific TSuantum XLS gas chromatography triple quadrupole mass spectrometry (GC-QqQ-MS/MS) instrument equipped with column DB-5MS (0.25 mm × 30 m × 0.25 μm). Oven temperature program started at 50 ℃, held for 1 min, then up

to 290 ℃ at 30 ℃/min and finally to 330 ℃ at 5 ℃/min and held for 2 min. Helium was the carrier gas at a constant flow of 1.2 mL/min. The interface line and ion source temperature were maintained at 290 and 260 ℃, respectively. The limits of detection (LODs) and quantification (LOQs) were defined as 3 and 10 times the signal-to-noise (S/N) ratio. For tissue samples, the LODs and LOQs were 0.023 − 0.044 ng/g ww and 0.077 – 0.147 ng/g ww for EHS, respectively. The mean recoveries of EHS ranged from 83% to 94%.

PS-NPs was extracted by KOH digestive method. Another 0.5 g muscle tissue sample was digested using 10 mL of KOH (10% w/v, 60 °C) for 24 h which has been shown to have no effect on the fluorescence intensity, morphology and composition of fluorescent polystyrene beads. The quantification of PS-NPs in the digested solution was performed using a Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan), with excitation at 488 nm and emission at 518 nm using external standard method. The mean recoveries of PS-NPs ranged from 80% to 89%.

GSH, ROS, MDA, T3 and T4 contents and SOD, CAT, GR activities assay

The competition method was used for all indicators. Specifically, Samples and antigens labelled by horse radish peroxidase (HRP) were successively added to an enzyme well that had been pre-coated with antibodies, competing with both solid phase antigen and formed immune complex after incubation for 1 hour at 37°C. Then, the combined HRP catalyseds tetramethylbenzidine (TMB) turned into blue after washing with phosphate buffer-saline with tween-20. Finally, the solution was converted to

yellow with the addition of sulfuric acid and determined at a wavelength of 450 nm with a microplate reader (Molecular Device VersaMax, USA). All indicators were quantified by comparing the absorbance of samples with the standard curve.

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

Total RNA extraction was performed using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) based on the manufacturer's instructions. Two microlitres of original cDNA (diluted 10-fold) was 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°C for 3 min, then 40 cycles of denaturation at 95°C for 10 s and annealing and extension at 60°C for 1min. All reactions were repeated 3 times.

DNA damage qualitative

3, 3′-diaminobenzidine colorimetric method were used for DNA qualitative. Under the action of deoxyribonucleotide transferase, the deoxyribonucleotide and fluorescein, biotin derivatives, peroxidase and alkaline phosphatase can be labelled to the 3′-OH terminals of DNA which are cause by DNA double strand or single bond breaks on chromosomes, and then apoptotic cells can be detected by the labelled 3′-OH terminals. Specifically, liver tissues fixed with 4% paraformaldehyde solution were embedded in paraffin wax and sectioned. The slices were treated with 3% H_2O_2 for 10

minutes and TdT, digoxygenin-d-UTP and labelling buffer complex reagents were dripped into slices at 37°C for 2 h. Then 50 μL of seal solution was added to the slices and dumped (not washed) after 30 min at room temperature. The 1: 100 diluted biotinylated anti-digoxin antibody was added and incubated at 37°C for 30 min and then 1:100 diluted streptavidin-biotin complex reagents were added. The samples were further processed with DAB coloration, slightly restained with haematoxylin, washed, dehydrated to transparency and observed.

DNA damage quantitative

Avidin-biotin method were used for DNA quantitative. The aldehyde reactive probe (ARP; -aminomethylcarbonyl hydrazine-D-biotin) can specifically react with the aldehyde groups on the ring opening of the single base-free sites (AP sites) which are the main type of damage produced by oxidative damage. After the reaction with excessive ARP, all AP sites on the DNA were labeled with biotin, and these biotin-labeled AP sites can be counted by colorimetric method using peroxidase or alkaline phosphatase that linked to the avidin. Specifically, DNA was extracted using a centrifugal adsorption column (RNase-Free Spin Columns CR2) that specifically binds nucleic acids and a unique buffer elution system based on the manufacturer's instructions. After extraction, 10 μL purified genomic DNA solution was mixed with 10 μL of aldehyde reactive probe (ARP) solution in a 0.5 ml centrifuge tube of and incubated at 37℃ for 1 h. The reaction mixture was transferred to the centrifuge tube and centrifuged at 5000 g for 15 min and the filtrate was poured out. Next, 200 μL TE buffer were added to dissolve the DNA obtained by centrifuge and transferred to the

1.5 mL tube, and the above operation was repeated 5 times. Then the ARP labeled standard or sample solution and DNA binding solution were mixed into the culture plate and placed overnight at room temperature. Next day, after pouring out the solution and washing with TE buffer, HRP- streptavidin solution was added and incubated at 37℃ for 1 h. Finally, the solution was poured out and washed with TE buffer, and the substrate solution was added and also incubated at 37℃ for 1 h, then the absorbance was determined at 630-670 nm.

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	Gen Bank number	Sense primer (5′-3′)	Antisense primer（5′-3′）
β-actin	NC_007112.7	F: TGCTGTTTTCCCCTCCATTG	R: TCCCATGCCAACCATCACT
Dio1	NC_007119.7	F: GGTGGTGGATGAGATGAACAAC	R: TCCGATGCCTCCCTGATAGA
Dio2	NC_007128.7	F: CGCGAAATGGGCTTGCT	R: CCAGGCAAAATCTGCAAAGTTA
TRA	NC_007114.7	F: CAATGTACCATTTCGCGTTG	R: GCTCCTGCTCTGTGTTTTCC
TRB	NC_007130.7	F: TGGCATGGCTAAGACTTGGT	R: TCAGCTTCCGCTTGGCTAA