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

Effects of polystyrene nanoplastics on the bioaccumulation, distribution and parental transfer of ethylhexyl salicylate

Ranran Zhou[1,2], Guanghua Lu[1,*], Zhenhua Yan[1], Runren Jiang, [1] Yu Sun[1], and Peng Zhang[1]

[1]*Key Laboratory for Integrated Regulation and Resources Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China*

[2]*School of Environmental Science & Engineering, Nanjing Tech University, 30 Puzhu Southern Road, Nanjing, 211816, China*

***Corresponding author:** Guanghua Lu; E-mail address: ghlu@hhu.edu.cn; Tel.: +86-025-8378-7306

Chemicals and reagents

EHS (purity > 98%) was obtained from AccuStandard Inc (NewHaven, USA). EHS stock solutions were prepared in dimethyl sulfoxide (DMSO; purity > 99.9%; Amresco, Solon, USA). Fluorescent PS-NPs (100 nm) were purchased from Tianjin Saierqun Technology Co., Ltd (Tianjin, China). Dichloromethane, ethyl acetate, methanol, acetone, hexane (HPLC grade) and glutaraldehyde were supplied by Merk Serono Co., Ltd. (Darmstadt, Germany). Tricaine methane sulfonate (MS-222) was purchased from J&K Scientific (Shanghai, China).

Yolk extraction

Specific operation of yolk extraction are as follows: the fertilized normal embryos were placed under the microscope, the chorion was removed mechanically with micro tweezers to obtain the yolk sac, and then the material in the yolk sac was sucked out and transferred to 1.5 mL centrifuge tube with very fine capillary tube. Finally, the yolk sac and pinhole were washed repeatedly with quantitative methanol and transferred to the same centrifuge tube.

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

Ultrasonic method was used to extract EHS from biological tissues. Specifically, a 0.5 g (wet weight, ww) sample was taken out and mixed with 1 g of activated alumina in a 15 mL glass tube to remove the interference of fat. Then 10 mL of extractant (ethyl acetate/dichloromethane, v/v = 1:3) was added and ultrasound for 2 h. After that, the supernatant was transferred to a 10 mL centrifuge tube and centrifuged at 120 r/h for 3 minutes. Then the supernatants were evaporated by nitrogen blowing 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 by 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). Specific parameters are as follows：Oven temperature program started at 50 °C, held for 1 min, then up to 290 °C at 30 °C /min and finally to 330 °C at 5 °C /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 °C, 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.

PS-NPs in parental biological tissues were observed using tissue sections combined with fluorescence microscopy (Eclipse 80i, Nikon, Tokyo, Japan). Specific steps are as follows: fresh tissue samples were dehydrated with alcohol and transparent with 100% alcohol and xylene. Then dewaxed, embedded and sliced were implemented with xylene and paraffin and finally placed under a fluorescence microscope for PS-NPs fluorescence observation. PS-NPs distribution in offspring was observed by fluorescence microscope directly.

The quantitative determination of PS-NPs was carried out by KOH digestion and fluorescence spectrophotometer. In detail, 0.5 g of muscle tissue samples were digested with 10 mL of KOH (10% w/v, 60 °C) for 24 h and the KOH used had been proven to have no effects on the fluorescence intensity, morphology and composition of fluorescent polystyrene particles. Then, the PS-NPs in the digestion solution were quantified by comparing the absorbance of samples with the standard curve using a Hitachi F-7000 spectrofluorometer (Hitachi, Ltd., Tokyo, Japan) with an excitation

wavelength of 488 nm and an emission wavelength of 518 nm. Before the application of this method, we carried out the spiked recovery experiment of biological samples with the spiked concentration of 1, 10, and 100 μg/g ww, and the recovery experiment was repeated three times with the relative standard deviation less than 20%. The mean recoveries of PS-NPs ranged from 80% to 89%.

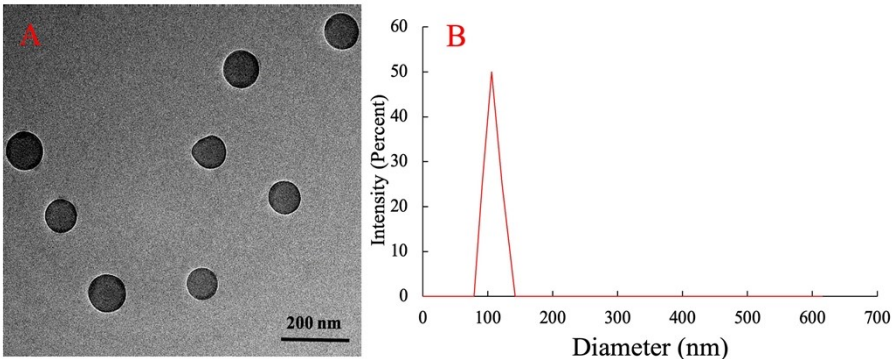

Fig. 1S. Transmission electron microscope analysis (A) and Hydrodynamic diameter distribution (B) of PS-NPs