Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2018, 52, 11411−11418

pubs.acs.org/est

Nanoplastic Ingestion Enhances Toxicity of Persistent Organic Pollutants (POPs) in the Monogonont Rotifer *Brachionus koreanus* via Multixenobiotic Resistance (MXR) Disruption

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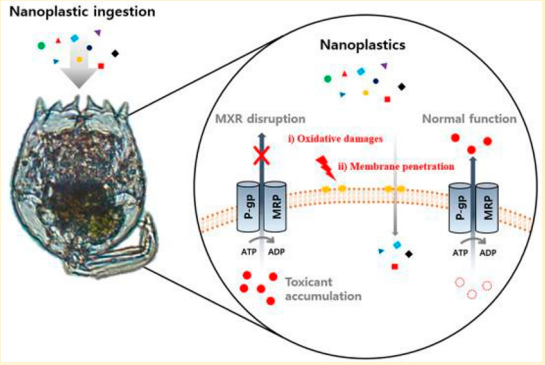
Supporting Information

ABSTRACT: Among the various materials found inside microplastic pollution, nanosized microplastics are of particular concern due to difficulties in quantification and detection; moreover, they are predicted to be abundant in aquatic environments with stronger toxicity than microsized microplastics. Here, we demonstrated a stronger accumulation of nanosized microbeads in the marine rotifer *Brachionus koreanus* compared to microsized ones, which was associated with oxidative stress-induced damages on lipid membranes. In addition, multixenobiotic resistance conferred by *P*-glycoproteins and multidrug resistance proteins, as a first line of membrane defense, was inhibited by nanoplastic pre-exposure, leading to enhanced toxicity of 2,2′,4,4′-tetrabromodiphenyl ether and triclosan in *B. koreanus*. Our study provides a molecular mechanistic insight into the toxicity of nanosized microplastics toward aquatic invertebrates and further implies the significance of synergetic effects of microplastics with other environmental persistent organic pollutants.



INTRODUCTION

Plastic pollution is considered one of the most concerning environmental problems due to its abundance and persistence in the aquatic environment.[1,2] The biggest issue regarding plastic pollution is "microplastic", which generally refers to plastic particles less than 5 mm. These particles are associated with larger plastic debris in aquatic environments, accounting for up to 80% of marine debris as they shatter into smaller plastic particles via natural weathering and aging processes.[3] These plastic particles are more bioavailable when their sizes decrease with an increased surface area to the volume ratio, and therefore are considered more toxic to aquatic organisms.[4] Furthermore, as the size of these particles can vary and become similar to that of food sources for aquatic organisms, organisms with low feeding selectivity ingest these plastic particles, which is likely accompanied by negative effects.[5−8] For example, among aquatic invertebrates, various zooplanktons have shown the ability to ingest polystyrene microbeads ranging from 1.7 to 30.6 μm with decreased algal feeding in the copepod *Centropages typicus*.[6] The rotifer *Brachionus manjavacas* was also shown to be capable of ingesting the polystyrene microbeads within the range of 0.05 to 3 μm.[9] In another study, size-dependent negative effects of 0.05, 0.5, and 6 μm microplastic ingestion were shown on reproduction and growth rates in the rotifer *Brachionus koreanus* and copepod *Paracyclopina nana* due to microplastic-induced oxidative stress.[4,10]

One particular concern of microplastic pollution is related to the issues regarding nanoplastics. While the abundance of nanoplastics in nature via nanofragmentation of larger plastic particles and their release from plastic products has been suggested by previous studies,[11,12] there is no available information on nanoplastic concentration in nature due to difficulties in detection and quantification. Assuming the worst-case scenario, the number of plastic particles increases inversely with the radius of the particle. While in vivo adverse effects of microplastics on aquatic organisms have been relatively well documented, molecular responses and subsequent consequences in aquatic organisms have not been studied, particularly for nanoplastics. Therefore, in the present study, we focused on multixenobiotic resistance (MXR) to investigate the molecular effects of microplastic and/or nanoplastic on marine zooplanktons with further assessment

Received: June 12, 2018
Revised: September 5, 2018
Accepted: September 7, 2018
Published: September 7, 2018

DOI: 10.1021/acs.est.8b03211
Environ. Sci. Technol. 2018, 52, 11411−11418

of synergetic effects of nanoplastic in the rotifer *B. koreanus* in response to 2,2′,4,4′-tetrabromodiphenyl ether (BDE- 47) and triclosan (TCS) as model persistent organic pollutants (POPs). MXR is a phenomenon conferred by ATP-binding cassette (ABC) transporters, namely, *P*-glycoprotein (P-gp), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP), which play a first line of defense in aquatic invertebrates in response to xenobiotics including environmental pollutants.[10]

Rotifers occupy an important ecological niche in the aquatic ecosystem, linking the energy flow from primary producers to organisms at higher levels. Rotifers are planktonic, floating along water currents and ingesting ambient particles (e.g., algae) with low feeding selectivity as a filter feeder,[13,14] which possibly increases the opportunities for rotifers to ingest microplastics. As a laboratory experimental species, they have a small body size (~170 μm for adults), short generation cycle (~24 h), high fecundity, and are easy to culture.[13,14] On the basis of these advantages, a genome database has been recently constructed that facilitates rotifers as a model species for ecotoxicological studies.[15−17]

In the present study, we demonstrate the inhibition of MXR by ingestion of nanoplastics (0.05 μm [50 nm]), resulting in enhanced toxicity of BDE-47 and TCS on the rotifer *B. koreanus*. This study provides a better understanding of the synergic effects of microplastics and POPs.

MATERIALS AND METHODS

Rotifer Culture. The monogonont rotifer *B. koreanus* was collected at Uljin, South Korea (36°58′43.01″N, 129°24′28.40″E) and maintained in the laboratory since 2011. A single rotifer was isolated, reared, and maintained in filtered artificial seawater (ASW; TetraMarine Salt Pro, Tetra, Cincinnati, OH, U.S.A.). The seawater temperature was maintained at 25 °C, and the rotifers were maintained under a light:dark 12:12 h photoperiod with 15 practical salinity units (psu). The green alga *Tetraselmis suecica* was used as a live diet (approximately 6×10^4 cells/mL). Species identification of the rotifer was confirmed via morphological analysis and sequencing of the mitochondrial DNA gene *CO1*.[18,19]

Microbeads and Chemicals. Three different sizes of nonfunctionalized polystyrene microbeads (0.05, 0.5, and 6 μm) were purchased from Polysciences (Warrington, PA, U.S.A.). Polystyrene was chosen since it is one of the most abundant polymers in marine plastic debris.[20] For ingestion experiments, fluorescently labeled polystyrene microbeads (excitation of 441 nm/emission of 486 nm) of the same diameters were used.

BDE-47 and TCS were dissolved in 99.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, U.S.A.) to prepare the stock solution. To conduct experiments, stock solutions of all chemicals were redissolved in DMSO. The solvent concentration in control and treatment groups did not reach over 0.001% DMSO (v/v).

Microbeads Ingestion. To demonstrate microplastic ingestion in *B. koreanus*, approximately 200 adult rotifer individuals were exposed to 10 μg/mL of fluorescently labeled microbeads for 24 h. After exposure, the rotifers were washed with clean ASW to remove the residual microbeads, and then fixed in 4% formaldehyde. The ingested microbeads remaining in the rotifers were observed using confocal laser scanning microscopy (LSM 510 META; Zeiss, Oberkochen, Germany) with a 20× lens at excitation of 441 nm and emission of 486 nm.

Microplastic Accumulation Rate. To measure the accumulation rate of microbeads in rotifers, rotifers ($n \approx 2000$) were exposed to different sizes of fluorescently labeled microbeads (0.05, 0.5, and 6 μ m) for 24 h and transferred into clean seawater for 24 h to allow egestion. For the control groups, rotifers were exposed to different sizes of fluorescently labeled microbeads for 24 h and used for the analysis without the egestion process. Rotifers were homogenized with a Teflon homogenizer to measure the fluorescence of accumulated microbeads and then subsequently sonicated for 1 min to dissociate any possible microbead aggregations. Homogenized samples were used for the microbeads detection using Varioskan Flash (Thermo Electron, Vantaa, Finland) at an excitation of 441 nm and emission of 486 nm. For the normalization, homogenized samples were centrifuged at 10 000*g* for 10 min, and the supernatants were used for the protein quantification by the Bradford method.[21] The accumulation rate was expressed by the relative percentage with respect to the control groups. The experiment was performed in triplicate.

Reactive Oxygen Species (ROS) and Malondialdehyde (MDA) Assays. Approximately 2000 adult rotifer individuals were exposed to different concentrations of 0.05 μm microbeads (0, 0.1, 1, 10, and 20 μ g/mL) for 24 h, washed, and then homogenized in a buffer containing 0.32 M sucrose, 20 mM HEPES, 1 mM $MgCl_2$, and 0.5 mM PMSF (pH 7.4) with a Teflon homogenizer. Homogenates were centrifuged at 10 000*g* for 10 min, and the supernatants were used for the analysis.

Intracellular reactive oxygen species (ROS) levels were measured using 2′7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probes, Eugene, OR, U.S.A.), which was oxidized to fluorescent dichlorofluorescein (DCF) by intracellular ROS. A 96-well black plate was filled with 170 μL PBS buffer, 10 μL probe (H_2DCFDA at a final concentration of 40 μM), and 20 μL supernatant fraction to a final volume (200 μL). Measurements were obtained with an excitation wavelength at 485 nm and emission wavelength at 520 nm using Varioskan Flash (Thermo Electron).

The lipid peroxidation level was measured using a commercially available kit (Abcam, Cambridge, U.K.). Briefly, lipid peroxidation was detected by measuring the level of malondialdehyde (MDA), a final product of lipid peroxidation. To detect MDA, thiobarbituric acid (TBA) was used to generate MDA-TBA adducts, which were quantified with an excitation wavelength at 532 nm and emission wavelength at 553 nm using Varioskan Flash (Thermo Electron). All experiments were performed in triplicate. Measured ROS and MDA levels were normalized by total protein and represented as a percentage relative to the control group. Total protein was determined by the Bradford method.[21]

MXR Activity Measurements. The activity of P-gp and MRP was measured according to Rhee et al. (2012) with a minor modification.[22] To examine whether microbead ingestion influences MXR activity in *B. koreanus*, rotifers ($n \approx 200$) were exposed to 10 μg/mL of different sizes of microbeads (0.05, 0.5, and 6 μm) for 24 h, and then subsequently transferred into clean ASW containing 0.5 μM rhodamine B (Sigma-Aldrich) or 1 μM calcein AM (Sigma-Aldrich), which are fluorescent substrates for P-gp and MRP, respectively. After exposure for 2 h in the dark, the rotifers

DOI: 10.1021/acs.est.8b03211
Environ. Sci. Technol. 2018, 52, 11411−11418

were washed with clean ASW and fixed by 4% formalin. The fluorescence of substrate dyes were observed using confocal laser scanning microscopy (LSM 510 META; Zeiss) with a 20× lens at excitation of 535 nm and emission of 590 nm for rhodamine B, and excitation of 485 nm and emission of 535 nm for calcein AM.

To further investigate the effects of nanoplastic pre-exposure on the MXR activity in response to BDE-47 and TCS, rotifers ($n \approx 2000$) were exposed to 25 μg/L BDE-47 or 50 μg/L TCS for 24 h with or without pre-exposure to nanoplastic (10 μg/mL) for 24 h, and incubated for 2 h in clean ASW containing 0.5 μM rhodamine B (Sigma-Aldrich) or 1 μM calcein AM (Sigma-Aldrich), which are fluorescent substrates for P-gp and MRP, respectively.[22] After incubation, the rotifers were washed and homogenized in phosphate-buffered saline (PBS), and then centrifuged at 10 000g for 10 min. Approximately 200 μL of supernatant was used for the measurement of accumulated fluorescence in the rotifers using Varioskan Flash (Thermo Electron) over a calibration curve of rhodamine B (excitation 535 nm; emission 590 nm) and calcein AM (excitation 485 nm; emission 535 nm). The fluorescent intensities were normalized by the protein quantity of each sample determined by the Bradford method.[21]

In Vivo Experiments. To compare the acute toxicity of BDE-47 and TCS in rotifers pre-exposed to nanoplastic versus healthy rotifers, 10 healthy individuals as well as the rotifers pre-exposed to 10 μg/mL of 0.05 μm microbeads for 24 h were transferred into a 12 well culture plate (SPL, Seoul, South Korea) with 4 mL of ASW containing 0, 100, 250, 500, 750, and 1000 μg/L BDE-47 or 0, 100, 200, 300, and 400 μg/L TCS. After 24 h exposure, the numbers of dead and live rotifers were counted under a stereomicroscope (SZX-ILLK200, Olympus Corporation, Tokyo, Japan).

To measure population growth and reproduction rates as a chronic exposure test, an individual of rotifer pre-exposed to nanoplastic (10 μg/mL) and healthy rotifer was transferred into 2 mL ASW containing 25 μg/L BDE-47 or 50 μg/L TCS. The numbers of rotifers were counted daily under a stereomicroscope (Olympus Corporation) for population growth rate measurement, and the numbers of newborn rotifers were counted every 12 h until the matured rotifer died as a readout of fecundity, after which the newborn rotifers were removed. During the chronic experiments, half of the media was renewed daily with the supply of the green alga *T. suecica* as a diet (approximately 6×10^4 cells/mL). Three biological replicates were performed for all experiments.

Statistics. SPSS ver. 17.0 (SPSS Inc., Chicago, IL, U.S.A.) was used for all statistical analyses. Data are expressed as the mean ± SD. The significance of the differences between the control and experimental groups was analyzed using Student's paired *t*-test and one-way and/or multiple-comparison analysis of variance (ANOVA) followed by Tukey's test. Any difference with a P value <0.05 was considered significant.

RESULTS

Microplastic Accumulation. Fluorescence of all microbead sizes was observable in the rotifers exposed to different sizes of microbeads for 24 h (Figure 1). Particularly, the fluorescence of 0.5 and 6 μm microbeads were observed in the digestive tracts, whereas 0.05 μm microbeads were dispersed in the surrounding organs, indicating that 0.05 μm microbeads are possibly membrane permeable, leading to bioaccumulation in rotifers. This hypothesis was further

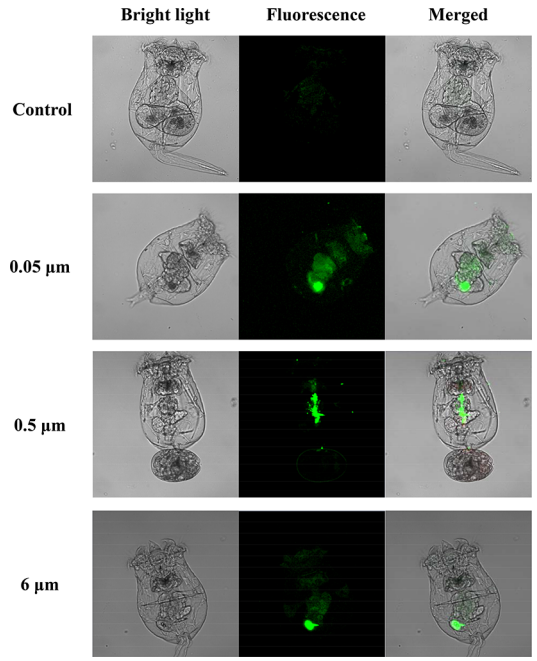


Figure 1. Images of ingested fluorescently labeled polystyrene microbeads (0.05, 0.5, and 6 μm) according to rotifer.

supported by the size-dependent egestion rates of the microbeads. Compared to 0.05 μm microbeads, 6 and 0.5 μm microbeads were more easily egested, as the accumulated amount of microbeads in the rotifers increased in a size-dependent manner with respect to the microbeads (Figure 2).

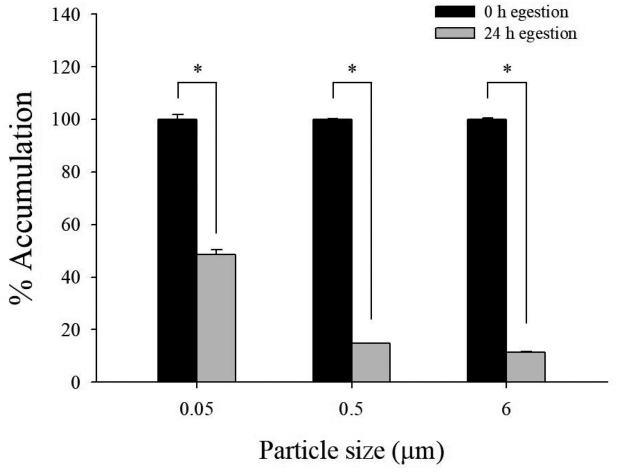


Figure 2. Quantification of accumulated polystyrene microbeads (0.05, 0.5, and 6 μm) in the rotifers after egestion. The 0 h egestion groups were considered as the control group. Asterisk above columns indicates significant differences ($P < 0.05$).

Oxidative Stress and Lipid Peroxidation Measurements. The ROS and MDA levels increased with a concentration-dependent tendency up to 10 μg/mL of nanoplastic, and then decreased at the highest concentration (20 μg/mL) (Figure 3). The strongest oxidative stress and lipid peroxidation were induced at 10 μg/mL, indicating that nanoplastic generates oxidative stress and leads to the induction of oxidative damages on lipid components.

DOI: 10.1021/acs.est.8b03211
Environ. Sci. Technol. 2018, 52, 11411−11418

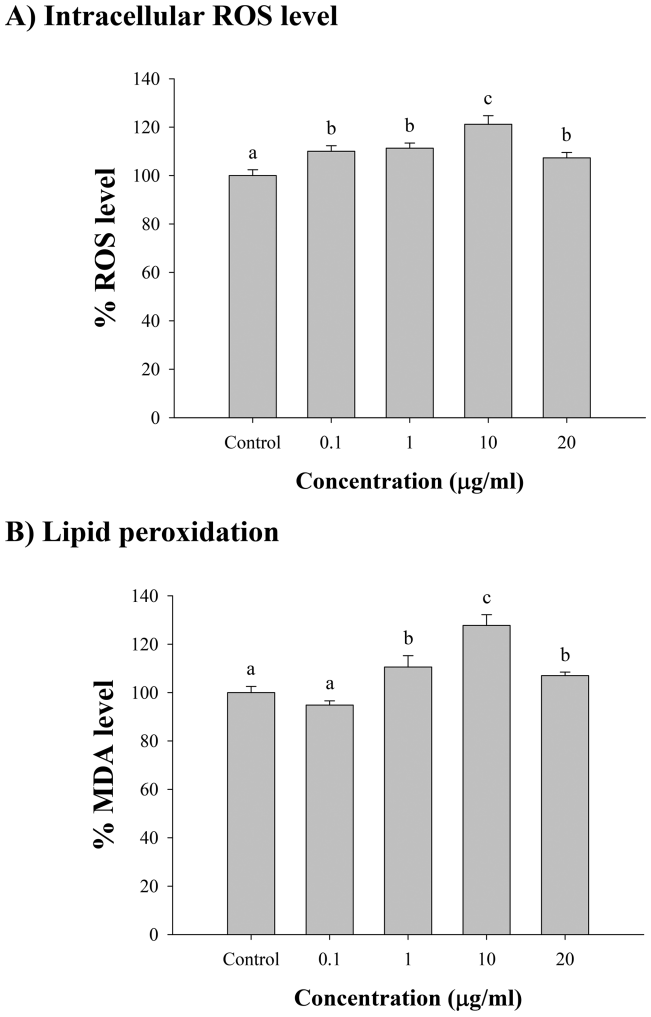


Figure 3. Level of ROS (A) and MDA (B) after exposure to different sizes of polystyrene microbeads (0.05, 0.5, and 6 μ m). Different letters above columns indicate significant differences ($P < 0.05$).

MXR Activities. The activities of P-gp and MRP were both decreased in rotifers exposed to 0.05 μm microbeads (Figure 4), while the fluorescent intensity of accumulated substrates (rhodamine B for P-gp; calcein AM for MRP) was the most increased by 0.05 μm microbeads compared to the control. The red rhodamine B signal was observed in the entire body, while the green calcein AM signal was mostly observed in the digestive organs.

To verify whether MXR is capable of effluxing BDE-47 and TCS out of rotifers, the activities of P-gp and MRP were measured as described in Rhee et al. (2012) with a minor modification. The activities of P-gps and MRPs were significantly increased ($P < 0.05$) in response to BDE-47 and TCS exposures compared to the control (Figure 5A), suggesting that BDE-47 and TCS are likely a substrate for efflux activity of P-gps and MRPs. In contrast, their activities were significantly decreased ($P < 0.05$) when rotifers were pre-exposed to the nanoplastics (Figure 5B). In addition, these transporters had different substrate specificities for BDE-47 and TCS, as the detected intensity differed depending on the transporter and chemical tested.

In Vivo Effects. Since the nanoplastics showed the most destructive effects on MXR functions, the acute and chronic toxicity of BDE-47 and TCS was measured with rotifers pre-exposed to nanoplastics and compared with healthy rotifers. The GC/MS analysis detected the actual concentration of BDE-47 and TCS in the exposure media as 22.8 and 23.0 μg/L, respectively, which is lower than nominal values owing their high lipophilicity.[23] Rotifers in each group exhibited different survival rates, showing higher tolerance to each pollutant in the nonpre-exposed rotifers (i.e., healthy rotifers) (Figure 6A). The estimated median lethal concentration (LC50) values for BDE-47 and TCS were 205.03 and 307.42 μg/L, respectively, with lower values in rotifers pre-exposed to nanoplastics (146.01 μg/L for BDE-47 and 179.19 μg/L for TCS) (Table 1). The acute toxicity values for the healthy rotifers were lower than those previously reported,[24,25] probably due to the food restrictions of healthy rotifers during the 24 h exposure time in the pre-exposure group. In addition to acute toxicity, the population growth and reproduction rates were measured as a chronic toxicity test. Rotifers exposed to BDE-47 and TCS alone did not show significant differences, compared to the control, whereas rotifers exposed to BDE-47 and TCS, treated with nanoplastic pre-exposure, have shown the decreased tendency (Figure 6B). These results indicate that the pre-exposure of nanoplastics negatively affected the tolerance against BDE-47 and TCS in the rotifers.

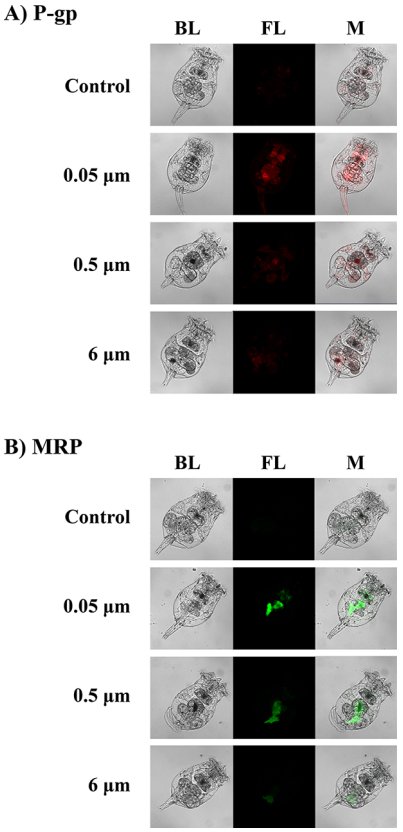


Figure 4. Fluorescence images of accumulated fluorescent substrates for P-gp (A) and MRP (B) in response to different sizes of polystyrene microbeads (0.05, 0.5, and 6 μ m).

DISCUSSION

Ingestion of microplastics in marine zooplanktons has been demonstrated in many previous studies and is considered as a primary exposure pathway of microplastics to zooplanktons, leading to adverse effects on their life parameters such as growth and reproduction.[4,6,7,10] Consistent with previous

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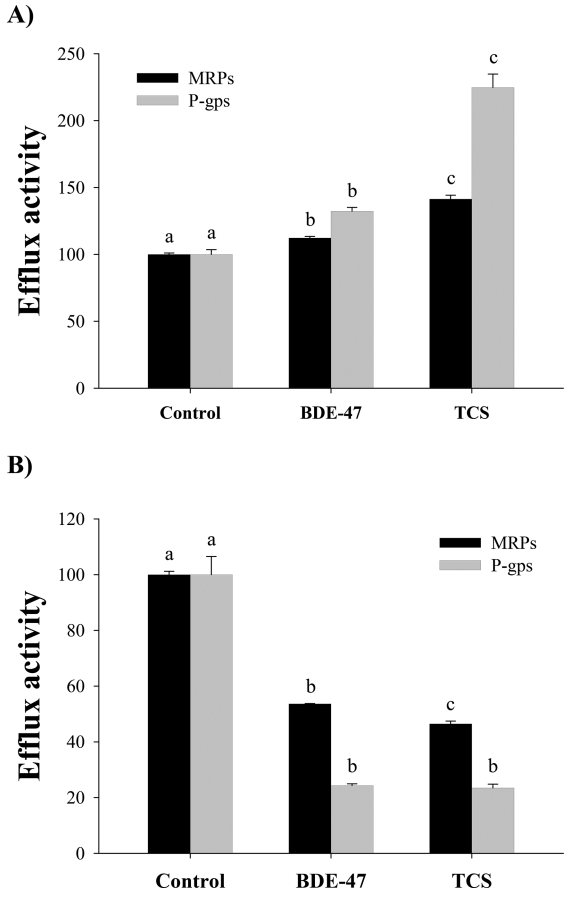

Figure 5. Relative P-gp and MRP activities in response to 25 μg/L BDE-47 and 50 μg/L TCS in the absence (A) or presence (B) of nanoplastic pre-exposure. Different letters above columns indicate significant differences ($P < 0.05$).

studies, the fluorescence of microbeads was strongly detected in the digestive tracts of rotifers, indicating that all different sizes of microbeads were ingested. Interestingly, the smallest microbeads (0.05 μm) were dispersed in the ambient organs of digestive tracts, possibly leading to longer retention time of microbeads in the rotifers. Similar result was also shown by Snell and Hicks, as the nanosized polystyrene microbeads (0.37 μm) were distributed in whole body with their ability to penetrate the gut wall, while larger microbeads were remained in the digestive tracts.[9] According to previous studies, a stronger tendency for nanosized particles to penetrate into cells has been shown as particle size decreases compared to microsized particles.[26] For example, in mouse red blood cells, nanosized (0.078 μm) polystyrene microbeads accumulated much more via membrane penetration compared to microsized (1 and 2 μm) polystyrene microbeads.[27] Therefore, the dispersion of nanoplastics in the rotifers observed in the present study is possibly associated with the membrane permeability of nanoplastics. Furthermore, the nanoplastics seemed to have longer retention time in the rotifers compared to 0.5 and 6 μm microbeads, possibly due to the excretion of nanoplastics out of digestive tracts, which act as a pathway for egestion. To verify cellular accumulation of nanoplastic via membrane penetration, we applied transmission electron microscopy (TEM) image analysis, but it was not possible to distinguish polystyrene microbeads from the embedding resins because of their similar physicochemical properties. Nanoparticles are known to become more "bioreactive" as their size decreases, as the increased total surface area facilitates chemical reactivity with higher surface area to volume ratios.[28] In this regard, it has been reported that the degree of adverse outcomes (e.g., retardation in growth rate and reproduction) of polystyrene microbead exposure is closely related to particle size,[4,9,10] implying the potential nanotoxicity of retained nanoplastics in the rotifers in the present study. On the basis of these results, we focused on the nanotoxicity of nanoplastics with subsequent experiments, as they cause more serious influences at the cellular level than microsized microbeads, leading to more potential harmful impacts at the individual level.

One of the principal mechanisms behind the toxicity of microplastics is related to the generation of oxidative stress, which negatively influences biological homeostasis in an organism.[29,30] In the present study, nanoplastic exposure to rotifers significantly increased ($P < 0.05$) the level of intracellular ROS as a possible consequence of the strong accumulation of nanoplastics observed in the present study. Along with ROS induction, the level of MDA for lipid peroxidation also increased with the level of ROS. MDA is a final product of lipid peroxidation, which is related to oxidative damage on the lipid membranes. Therefore, our results suggest that oxidative stress was induced by nanoplastics, which is associated with dysfunction of lipid membranes. In consistent, multiple evidence have been reported for oxidative damages on membranes of human cell lines from silica nanoparticles exposure.[31] These results suggest that nanosized particles are capable of inflicting direct oxidative damages on lipid membranes, causing deleterious effects on biological processes including defense impairments. In addition to lipid peroxidation as a main pathway for nanoplastic-induced damage on the membranes, the direct interaction between the membranes and particles may be another important route leading to membrane damage. Using a model membrane system, Rossi et al. (2014) stimulated the effects of nanosized polystyrene particles on lipid membranes and alteration of membrane structures were found due to particle accumulation and penetration along the membrane, resulting in the membrane dysfunction.[32] Therefore, the dispersed fluorescence and stronger accumulation of nanoplastic shown in the present study imply that nanoplastics cause membrane dysfunction in chemical and/or physical ways.

MXR is a phenomenon of xenobiotic efflux out of cells conferred by a set of ABC transporters, including P-gps and MRPs. Since these transporters are located in the cellular membrane with important roles as efflux transporters, we further examined the activity of MXR-mediated ABC transporters, namely P-gps and MRPs. The conserved function of P-gps and MRPs conferring MXR in aquatic invertebrates, including the rotifer *B. koreanus*, have been proposed as a first line of defense against environmental toxicants;[33,34] this suggests that nanoplastic-induced membrane disruption would cause inhibition of MXR activities, leading to decreases in tolerance against environmental toxicants. In particular, lipid peroxidation increased membrane permeability and fluidity, leading to disorders in MXR functions.[35,36] To verify this hypothesis, we measured the activities of P-gps and MRPs with or without different sizes of microbead pre-exposure to determine whether the efflux capacity of P-gps and MRPs is affected by microbead ingestion. As a result, the fluorescence of P-gp and MRP substrates was most strongly detected in

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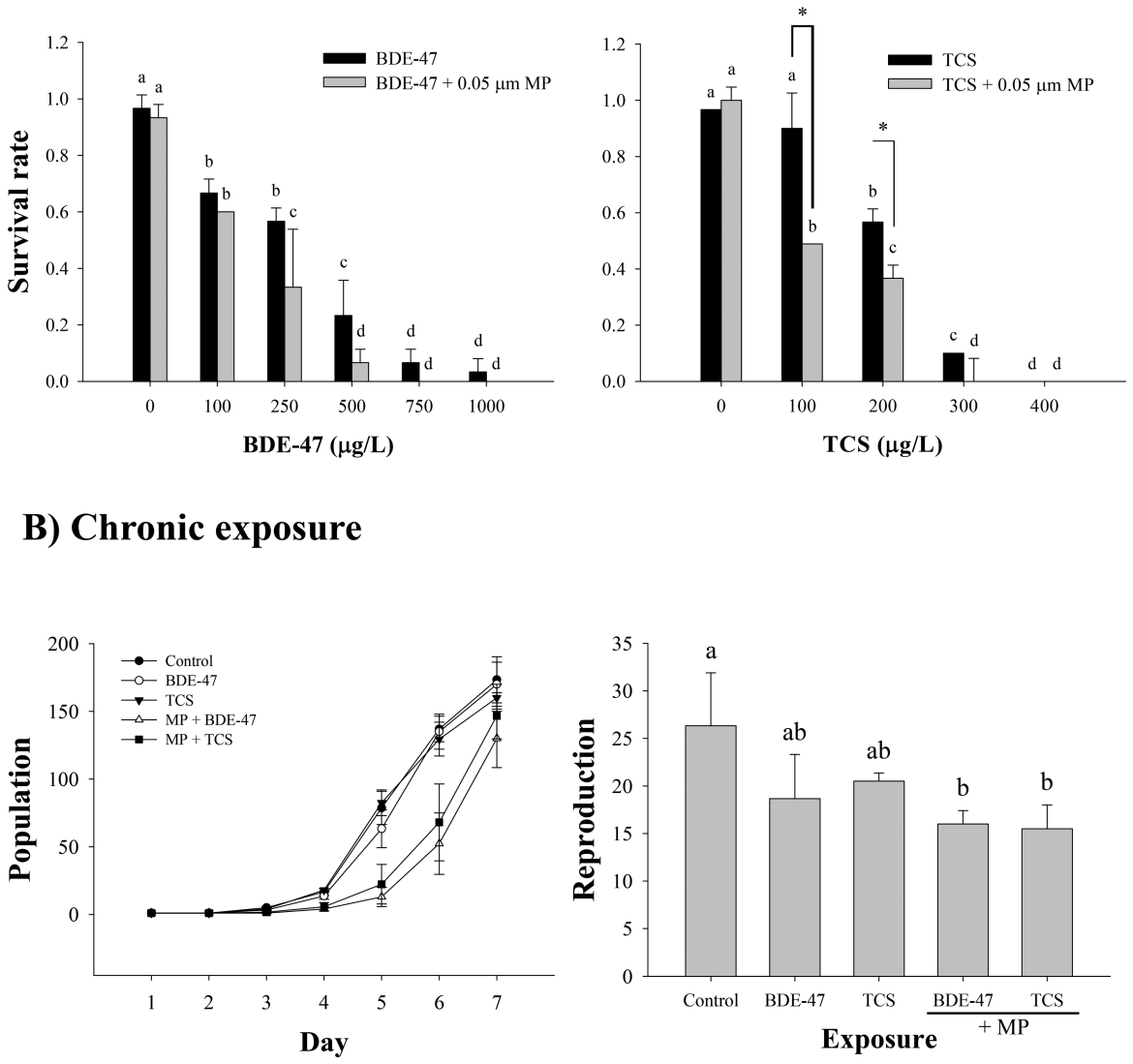

Figure 6. Acute (A) and chronic (B) toxicity of BDE-47 and TCS in the rotifers with or without nanoplastic pre-exposure. Asterisk and different letters above columns indicate significant differences ($P < 0.05$).

Table 1. LC10, LC50, and 95% Confidence Intervals (CI) for BDE-47 and TCS in *B. koreanus* in the Presence or Absence of Pre-Exposure to 0.05 μm Microbeads

chemical	pre-exposure	LC10 (μg/L)	LC50 (μg/L)
BDE-47	none	53. 31 (28.77−98.78)	205.03 (153.63−273.62)
	0.05 μm microbeads	48.97 (25.90−92.56)	146.01 (105.10−202.85)
TCS	none	248.53 (212.25−284.82)	307.42 (267.88−355.90)
	0.05 μm microbeads	93.83 (11.74−147.34)	179.19 (82.83−257.21)

rotifers exposed to nanoplastics, indicating that the activity of P-gps and MRPs was inhibited in a size-dependent manner with respect to the microbeads, in parallel to the stronger accumulation of nanoplastics in the rotifers compared to microsized microbeads, as shown in our study.

The inhibitions of P-gps and/or MRPs have previously enhanced the toxicity of environmental toxicants to organisms. For example, in the mosquito *Aedes aegypti*, co-treatments of P-gp inhibitor and RNA interference of P-gp encoding gene have increased the toxicity of temephos by 24% and 57%, respectively.[37] In addition, the survival rate of the rotifer *B. koreanus* was significantly decreased by inhibition of P-gp and MRPs in response to four different biocides exposure, implying the critical role of P-gps and MRPs in defense systems.[34] In the present study, the protective role of P-gps and MRPs in response to BDE-47 and TCS was confirmed as their efflux activity was induced by BDE-47 and TCS. The decreased activities observed with pre-exposure to nanoplastic suggest

DOI: 10.1021/acs.est.8b03211
Environ. Sci. Technol. 2018, 52, 11411−11418

that nanoplastic is able to disrupt MXR functions in response to BDE-47 and TCS. Furthermore, the survival rate and lethal concentration values (LC10 and LC50) with further population growth and reproduction rates of rotifers were decreased in the nanoplastic pre-exposure groups compared to the chemical only exposure groups. As the increased sensitivity of rotifers to BDE-47 and TCS was further demonstrated using inhibitors specific to P-gp and MRP as the positive controls (Figure S1 of the Supporting Information, SI), our results clearly imply the enhanced toxicity of BDE-47 and TCS to the rotifers via MXR disruption. In nature, complex mixtures of toxicants are present and can be transferred by absorption on microplastics, causing synergic interactions between them to enhance toxicity in the organism. In fact, there are several reports that microplastics can be a vector for environmental toxicants and provide synergic effects. For example, in the amphipod *Allorchestes compressa*, polybrominated diphenyl ethers (PBDEs) derived from microplastic assimilated into amphipod tissues via microplastic ingestion.[38] The combined exposure of microplastics and phamarceuticals, including procainamide and doxycycline, reduced the growth rate and chlorophyll *a* concentrations in the algae *Tetraselmis chuii*, even though microplastics alone did not exhibit any toxicity.[39] Similarly, in the common goby *Pomatoschistus microps*, the activity of isocitrate dehydrogenase was decreased by combined exposure to pyrene and polyethylene microbeads, whereas no significant changes were observed when the two stressors were separately exposed.[40] These results suggest that toxicological interactions between microplastics and other environmental toxicants lead to enhanced toxicity of environmental toxicants or at least disruption of biological processes. In this regard, to our knowledge, we first provide a mechanistic view that provides a better understanding of the synergetic toxicity of microplastics and POPs. Taken together, nanoplastics had longer retention time, compared to microplastics, in the rotifer *B. koreanus* and induced membrane dysfunction via oxidative damages and penetration, leading to disruption of MXR functions (Figure 7). As the increased mortalities in response to BDE-47 and TCS have shown a possible consequence of those subsequent molecular events, we have shown how nanosized microplastics affect aquatic invertebrates at the molecular level with a mechanistic insight into a joint toxicity of microplastics and environmental toxicants.

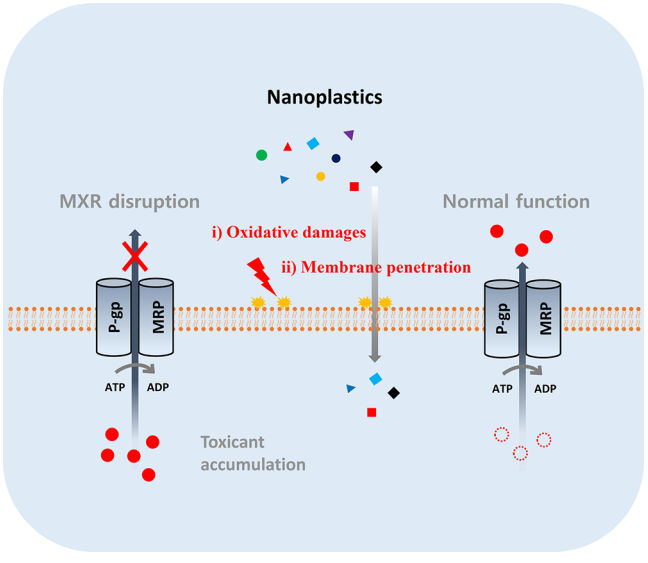

Figure 7. Schematic representation of the proposed mechanism for MXR disruption via nanoplastic ingestion in the rotifer *B. koreanus*. Modified from Lee et al. (2018).[33]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03211.

Figure S1. Survival rate of rotifers exposed to BDE-47 and TCS in the absence or presence of inhibitors that are specific to P-gp and MRP (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Postdoctoral Research Program of Sungkyunkwan University (2017).

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