



Leaching of endocrine disrupting chemicals from marine microplastics and mesoplastics under common life stress conditions

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ABSTRACT

Microplastics (MPs) and mesoplastics are able to sorb harmful substances and often contain additives, e.g., endocrine disrupting chemicals (EDCs), that can cause adverse effects to organisms. The present study aims to determine EDC concentrations and their endocrine activities in leachates of field-collected marine MPs and mesoplastics under stress conditions that are known to occur during the plastic life cycle. Estrogens were the dominant EDCs on plastic particles and were either concentrated from the surrounding water or originated from plastic manufacturing. Bisphenol A had the highest detection frequency (75%) with an average concentration of $475 \pm 882 \mu\text{g}/\text{kg}$, followed by bisphenol S, octylphenol and nonylphenol. Moreover, smaller marine MPs leached greater quantities of EDCs because the sorption from surrounding seawater is more efficient for smaller particles. It was found that normal life stresses such as microwaving (MW) and autoclaving (AC) can decrease EDC concentrations, but solar irradiation (solar) can increase EDC concentrations in leachates. Even though organisms with higher metabolic ability exhibited greater estrogenic effects, the comprehensive toxicity of plastic leachates after common life treatments was still limited (below the EC_{10} value) if 0.1% is taken as the EDC uptake from plastic. In future studies, the accurate contribution of plastic bound EDCs needs to be further explored, and the monitoring of MPs and mesoplastics in the human diet remains important because the concentrations of these plastics may change in the future.

1. Introduction

The issue of marine plastic debris has received growing global attention (Guzzetti et al., 2018). This has raised concerns about their potential effects to marine ecosystems (Alimba and Faggio, 2019; Savoca et al., 2019). Plastic debris can be classified as nanoplastics ($< 0.001 \text{ mm}$), microplastics (MPs, $\geq 0.001 \text{ mm}$ and $< 5 \text{ mm}$), mesoplastics ($\geq 5 \text{ mm}$ and $< 25 \text{ mm}$), and macroplastics ($\geq 25 \text{ mm}$) (Shim et al., 2018). Both polar and nonpolar compounds can sorb onto plastics depending on solution chemistry alterations (Seidensticker et al., 2018). Highly hydrophobic organic contaminants (HOCs) have been detected on commonly found marine plastics (Chen et al., 2018; Chen et al., 2019; Endo et al., 2005; Hirai et al., 2011). However, research on the presence and effects of plastic-associated moderately HOCs is relatively limited. Among the moderately HOCs, endocrine disrupting chemicals (EDCs) such as bisphenol A (BPA), nonylphenol, and octylphenol are widely incorporated into plastics and primary MP pellets

and abrasion beads produced directly from industry as plastic anti-oxidants and plastic packaging stabilizers or their reaction reagents (Chen et al., 2017; Hansen et al., 2013; Nizzetto et al., 2016). Endocrine disrupting chemicals are a group of endocrine disruptors, which are exogenous substances or mixtures that alter function(s) of the endocrine system and cause adverse health effects in organisms (Damstra et al., 2002). Little research has been done so far to explore the occurrence and concentrations of different kinds of EDCs on marine plastics.

It is noteworthy that EDCs are more easily released from plastic debris than highly hydrophobic chemicals, because the K_p values (an organic pollutant partitioning coefficient between plastic and water) for EDCs are much lower than highly HOCs (Liu et al., 2019; Lohmann, 2012). Bisphenol A, an important plasticizer (Oehlmann et al., 2008; Sajiki and Yonekubo, 2003), can leach into the aqueous phase from plastic bottles and the average migration rates of BPA can reach $1.84\text{--}4.83 \text{ ng}/\text{cm}^2/\text{h}$ at $70 \text{ }^\circ\text{C}$ (Cao and Corriveau, 2008). Other plastic additives such as nonylphenol and octylphenol can also quickly migrate

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from plastic to water or milk (Bittner et al., 2014a; Loyo-Rosales et al., 2004). Excess EDCs can disrupt normal functioning of endocrine systems in human beings as well as in wildlife, affecting reproductive systems and metabolism and causing obesity (Bergman et al., 2012; Kabir et al., 2015). Therefore, the leaching of associated EDCs from plastics may pose a potential ecological risk.

Microplastics and mesoplastics are dominant plastic pieces in the marine environment and could reenter our daily life (Lebreton et al., 2018; Ter Halle et al., 2016). In the Great Plastic Garbage Patch (GPGP), MPs account for 94% of the plastic pieces floating in the area. The MP and mesoplastic concentrations in GPGP have reached 678,000 and 22,000 pieces/km², respectively (Lebreton et al., 2018). It is comparable or higher than the reported data in the North Atlantic Garbage Patch (358,000–537,000 pieces/km² for MP and 99,000–150,000 pieces/km² for mesoplastic) and that in the Mediterranean Sea (0–4480 pieces/km² for MP) (Ter Halle et al., 2016; Zeri et al., 2018). Plastic litter is frequently mistaken for food by marine life (Efferth and Paul, 2017). Examples of marine areas with extensive plastic pollution include The North Pacific Subtropical Gyre, which is a fishing place (Polovina and Woodworth-Jefcoats, 2013), and the well-known GPGP (Moore, 2003). Plenty of studies have reported the occurrence of plastic in zooplankton (Desforges et al., 2015), barnacles (Goldstein and Goodwin, 2013) and various species of fishes (Davison and Asch, 2011; Jantz et al., 2013) within the GPGP. The vast majority of plastic fragments found in barnacles in the GPGP are within the range of 0.5–15 mm (Goldstein and Goodwin, 2013). These aquatic organisms could be prepared into feed or food stuffs and subsequently consumed by humans. In that case, the pollutants on plastics would enter into human daily life system. While salinity and dissolved organic matter have minor effects on the release of EDCs, high temperature has an enhanced effect on some EDCs (such as BPA) leaching process (Le et al., 2008; Liu et al., 2019). In the course of everyday cooking at home, common cooking stresses such as microwaving (dry heat) and autoclaving (moist heat) may change the potential risks of leachable EDCs.

We hypothesize that EDCs associated with MPs and mesoplastics can leach out under normal life stresses, and toxic consequences and risks may occur if the leached EDCs are exposed to biota. Hence, to test these hypotheses, we examined the concentrations of EDCs on marine MPs and mesoplastics under different normal life stresses such as microwaving, autoclaving and solar irradiation. We also tested the potential toxicity of contaminants leached out of the plastics by *in vitro* bioassays and analyzed the potential risk to human health.

2. Materials and methods

2.1. Sampling and identification of marine microplastics and mesoplastics

Marine MP and mesoplastic samples were collected from the North Pacific Subtropical Gyre while aboard the RV Ocean Starr in August 2015 by a manta trawl with a mouth opening of 15 cm × 90 cm and a mesh size of 500 μm. Sample collection was conducted by The Ocean Cleanup Foundation and was reported previously by Chen et al. (2018). In the present study, three size classes of small (≥0.5 mm and < 1.5 mm), medium (≥1.5 mm and < 5 mm), and large (≥5 mm and < 15 mm) plastics were separated. All of the plastics selected for study were hard plastics (fragments and objects made of plastic with thick walls of ~1–3 mm) consisting of polyethylene, which are the dominant type of MPs and mesoplastics (Chen et al., 2018), as verified by micro-Fourier Transform Infrared Spectroscopy (Bruker, LUMOS FTIR, UK).

2.2. Extraction of EDCs

In the present study, we did not use dichloromethane (DCM) to achieve a complete extraction, but EDCs on plastic particles were extracted with 1 mL of 100% ethanol or a saline-based solution (saline:

DMEM/F-12 medium without phenol red, Gibco, Cat. 21041025) in order to simulate common life extraction scenarios. We chose saline as a cell culture medium to simulate the cooking of soup. The saline medium contains several amino acids, vitamins, salts and sugar (Table S1, Supporting Information (SI) pages S8–S9), whose components are similar to those in seafood soup. A pilot experiment was conducted for the comparison of EDC extraction efficiency between saline and ethanol. There were no significant differences in the concentrations of most EDCs extracted by ethanol or saline (Fig. S1, SI page S18). Since the chemical and bioassay analyses took several months, we used ethanol for extractions in the formal experiments to decrease the risk of bacterial contamination. Detailed explanation can be found in the SI (pages S3–S4).

In each extraction treatment, 100 mg of plastic was soaked in ethanol or saline and gently shaken at 300 rpm (equals to 0.3 g) on a well-plate shaker at 30 ± 1 °C for 24 h. The extraction time was based on a pilot experiment, which suggested that equilibria of leached EDCs can be reached after 24 h (Fig. S2, SI page S19). The glass tubes containing the plastic particles were rinsed twice with 100 μL of methanol before use. After shaking, the liquid phase was transferred to clean glass vials with glass pipettes. For each ethanol extract (the liquid phase), one half was preserved for further chemical analysis, and the other half was dried under a stream of nitrogen and redissolved in dimethyl sulfoxide (DMSO) for further bioassay analysis. Each saline extract (the liquid phase) was filtrated through a 0.2 μm filter (Whatman, PTFE) for chemical analysis and comparison.

2.3. Quantification of estrogens, androgens, progestogens, and glucocorticoids

Endocrine disrupting chemicals were quantified using a Xevo TQ triple-quadruple Mass Spectrometer (Waters, USA) fitted with an electrospray ionization (ESI) source. Chromatographic separation was conducted using an Acquity UPLC system fitted with a BEH C18 column (1.7 μm, 2.1 × 100 mm) maintained at 30 °C according to (Zhang et al., 2011) with slight modification. Internal standards were spiked into procedural blanks and all samples to automatically compensate for the loss of compounds during sample preparation. Recoveries and detection limits of the analytes are shown in Table S2 (SI page S10). Detailed information can be found in the SI pages S4–S5.

The 29 measured EDCs include the estrogens estrone (E1), 17β-estradiol (E2), estriol (E3), diethylstilbestrol (DES), dienoestrol (Dieno), hexestrol (Hex), estradiol benzoate (E2-ben), bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), 4-n-nonylphenol (NP), and 4-tert-octylphenol (OP), the androgens testosterone (TES), methyl testosterone (Me-TES), 19-nortestosterone (Nortes), trenbolone (Tren), nandrolone phenylpropionate (Nan-phen), testosterone propionate (TES-pro), and boldenone (Bold), the progestogens progesterone (Proges), norgestrel (Norges), medroxyprogesterone (Me-pro), megestrol acetate (Me-ace), and hydroxyprogesterone (Hydrop), and the glucocorticoids prednisone (Predn), cortisone (Corti), dexamethasone (Dexa), prednisolone (Prednl), and methyl prednisolone (Me-prednl).

2.4. Cytotoxicity analysis by MTT bioassay

The cell viability bioassays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were performed to estimate the cytotoxicity of plastic extracts under normal life stresses prior to conducting the Estrogen Receptor mediated Chemical Activated Luciferase gene expression (ER-CALUX) and Lyticase Yeast Estrogen Screen (LYES) bioassays according to (Brinkmann et al., 2014).

2.5. *In vitro* analysis of estrogenic effects

The ER-CALUX assays were performed according to (Legler et al., 1999) with previously reported modifications (Kuckelkorn et al., 2018;

Maletz et al., 2013; Valitalo et al., 2016). T47D human breast adenocarcinoma cells (T47Dluc) expressing the endogenous estrogen receptors alpha that have been stably transfected with an estrogen-responsive luciferase reporter gene (pERE_{tata}-Luc) were used. The estrogenic effects were quantified by measuring the luciferase activity with a luminescence counter (Berthold, Germany). Detailed description can be found in the SI pages S6–S7.

The LYES assays were conducted as described by (Routledge and Sumpter, 1996) with modifications according to (Kunz et al., 2017). Genetically modified yeast cells were used that contain the DNA sequence of hER α and expression plasmids carrying the reporter gene *lacZ* encoding the enzyme β -galactosidase (β -gal) under the control of an estrogen-responsive element (ERE), thus allowing quantification of receptor activation. Yeast cell density was measured at 595 nm and the amount of converted chlorophenol red- β -D-galactopyranoside (CPRG) was measured at 540 nm. Detailed description can be found in the SI pages S6–S7.

2.6. Estrogenic effect equivalence calculations

Data analysis of both ER-CALUX and LYES bioassays were conducted following the recommendations of (Villeneuve et al., 2000). Average luminescence and absorbance values of plastic leachate samples and 17 β -estradiol (E2) standards were corrected for the response of the solvent controls. As there were various kinds of EDCs on marine plastics, we adopted a mass balance approach (Wolz et al., 2008) based on the concentration addition mechanism (Kortenkamp, 2007) to evaluate the toxicity of mixtures of EDCs leached from plastic particles. Estrogenic effects determined in the bioassays were expressed as biological toxicity equivalency concentrations of estradiol (Bio-EEQ) (Wolz et al., 2008).

$$\text{Bio-EEQ} = EC_{20}(E2)/EC_{20}(\text{plastic extract}) \quad (1)$$

where EC_{20} refers to the concentration of the extracted mixture which induces a response that is 20% of that of E2 standard.

The chemical equivalency concentrations (Chem-EEQ) were calculated according to Eq. 2,

$$\text{Chem-EEQ} = \sum_{i=1}^n \text{REP}_i \cdot C_i \quad (2)$$

$$\text{REP}_i = EC_{50}(E2)/EC_{50}(i) \quad (3)$$

where REP represents the relative estrogenic potential, i represents a chemical, EC_{50} refers to the concentration of the extracted mixture which induces a response that is 50% of that of E2 standard. REP_i was calculated using Eq. (3) with the EC_{50} value of the reference compound estradiol and the matching EC_{50} value of the detected estrogen. The EC_{50} values were obtained from the literature to calculate REP (Bovee et al., 2004; Molina-Molina et al., 2013; Pillon et al., 2005).

2.7. EDC concentrations alteration under normal life stresses

The stress application methods were adopted from (Bittner et al., 2014b) with slight modifications. First, for simulating the real-life stress of exposure to dry heat, microwave radiation (MW treatment) was applied. Glass tubes with plastic particles were incubated in a microwave at 1200 W for 2 min followed by a 30 min pause. This microwave-pause cycle was repeated five times. Second, for simulating the real-life stress of moist heat, an autoclaving process (AC treatment) was applied by heating plastic samples in a pressure cooker (Perfect Plus, WMF, Germany) to 120 °C (resulting in a pressure of 1.8 atm) and maintained for 20 min. Third, for simulating natural solar irradiation, an irradiation process (solar treatment) was applied by irradiating under artificial sunlight for 1 month (Atlas SUNTEST XXL + ST, USA). Meanwhile, a no treatment (NT) group was also included by putting plastics in methanol-rinsed glass tubes without any further stresses. Procedural

blanks were using clean glass tubes without plastic particles throughout the experiment.

2.8. Statistics

Data in the present study was processed using SPSS (version 22.0). Endocrine disrupting chemical concentrations among different plastic treatments were first tested for normality and homogeneity of variance. Subsequently, one-way ANOVA tests or t -tests were conducted when parametric assumptions were met, otherwise nonparametric multivariate rank tests were conducted. The ANOVA tests were applied to compare the concentrations of estrogens, androgens, progesterones, and glucocorticoids, to test the MP and mesoplastic size effects on EDC concentrations, to compare the EEQ values among different samples, and to compare the effects of different treatments (NT, MW, AC, and solar) on the release of EDCs. The t -tests were applied to compare the differences in extracted EDC concentrations between ethanol and saline. Figures containing bars or symbols with an asterisk or lowercase letters above them represent significant differences ($p < 0.05$).

3. Results and discussion

3.1. Estrogens are dominant EDCs on plastics

The results suggested that estrogens had the highest concentrations on MPs and mesoplastics among all categories of the measured EDCs. The concentration of estrogens ($525.6 \pm 912.2 \mu\text{g}/\text{kg}$) was even significantly higher than the sum of androgens ($16.2 \pm 38.8 \mu\text{g}/\text{kg}$), progesterones ($138.1 \pm 269.3 \mu\text{g}/\text{kg}$) and glucocorticoids ($31.8 \pm 91.4 \mu\text{g}/\text{kg}$) combined ($p < 0.05$) (data in the Fig. 1). Among all the estrogens, the highest detection frequency was found for BPA (75%), followed by bisphenol S (BPS, 68%), octylphenol (63%) and nonylphenol (49%); the average concentrations of these chemicals were $475.1 \pm 881.5 \mu\text{g}/\text{kg}$, $7.3 \pm 25.9 \mu\text{g}/\text{kg}$, $2.5 \pm 8.7 \mu\text{g}/\text{kg}$, and $3.7 \pm 7.7 \mu\text{g}/\text{kg}$, respectively. Bisphenol A and nonylphenol are important stabilizers or antioxidants for plastics (Hermabessiere et al., 2017; Loyo-Rosales et al., 2004), and therefore they are added during plastic manufacturing. Consistent with earlier findings, BPA and nonylphenol are frequently detected EDCs on PE or polypropylene (PP) MPs collected from the Pacific Ocean (Hirai et al., 2011). Bisphenol S is often used to replace BPA in 'BPA-free' plastic products, whose usage is increasing rapidly, and octylphenol is used as an antioxidant in plastic stabilizers (Hansen et al., 2013). Thus, the high detection frequencies of these two kinds of estrogens on marine MPs and mesoplastics are reasonable due to the additive usage during manufacturing. Similarly, high BPA, octylphenol, and nonylphenol concentrations in MPs ($34.4 \pm 123.1 \mu\text{g}/\text{kg}$, $9.9 \pm 25.2 \mu\text{g}/\text{kg}$, and $238.1 \pm 642.0 \mu\text{g}/\text{kg}$, respectively) and in seawater ($38.9 \pm 7.6 \text{ ng}/\text{L}$, $24.6 \pm 29.5 \text{ ng}/\text{L}$, and $59.2 \pm 48.7 \text{ ng}/\text{L}$, respectively) have been reported previously (Hirai et al., 2011; Staniszewska et al., 2014). The high concentrations of estrogens on marine plastic particles can either be derived from the surrounding water or be leftovers from plastic manufacturing (Hermabessiere et al., 2017).

3.2. Effects of size on EDC concentrations

It is noteworthy that the average detection frequencies of EDCs for the small-sized MPs (59%) were much higher than those for the medium- and large-sized plastics (34% and 50%, respectively). Correspondingly, the estrogen concentration in the small-sized group was $1093.0 \pm 1607.5 \mu\text{g}/\text{kg}$, which was significantly higher than that in the medium-sized ($250.0 \pm 445.3 \mu\text{g}/\text{kg}$, $p = 0.001$) and the large-sized ($270.6 \pm 557.6 \mu\text{g}/\text{kg}$, $p = 0.002$) groups (data in the Fig. 1). Similarly, total EDC concentrations on marine plastics were also reported to range from sub $\mu\text{g}/\text{kg}$ to mg/kg (Hirai et al., 2011; Teuten et al., 2009). With further analysis, we found that for most of the EDCs

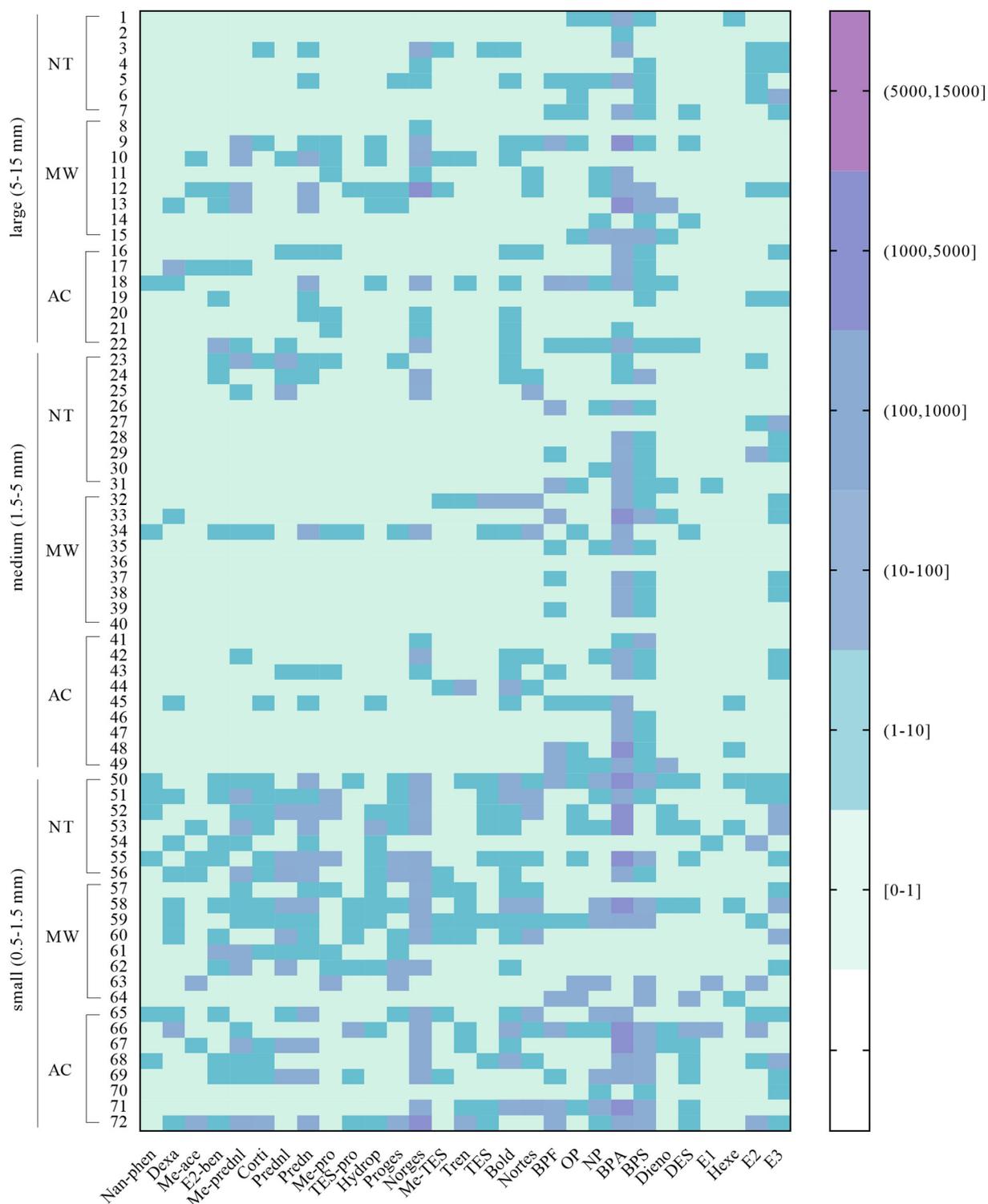


Fig. 1. Heatmap of leached EDCs from marine MPs and mesoplastics under common life stresses. Plastic particles were all composed of PE and classified as small (0.5–1.5 mm), medium (1.5–5 mm), or large (5–15 mm). Treatments included no treatment (NT), microwaving treatment (MW), and autoclaving treatment (AC).

analyzed, concentrations on plastic particles increased with decreasing particle size.

In general, small sized particles yielded higher EDC concentrations than medium and large sized particles (Fig. 2). Five estrogens, one androgen, four progesterones, and two glucocorticoids had significantly higher concentrations in small MPs (0.5–1.5 mm) than that in large mesoplastics (5–15 mm) ($p < 0.05$) (Fig. 2). The size of the plastic pellet or fragment strongly affects the rate at which sorbed HOCs are

taken up (Teuten et al., 2009). The small plastics had higher EDC concentrations, which is probably because they can adsorb EDCs from seawater faster than the larger plastic particles. Even though strong weathering on the small MPs may lead to lower K_p values for EDCs, faster sorption still outcompetes a lower K_p at a sufficiently long enough scale, and the residence times of these marine MPs and mesoplastics in the ocean are very long (Koelmans et al., 2016).

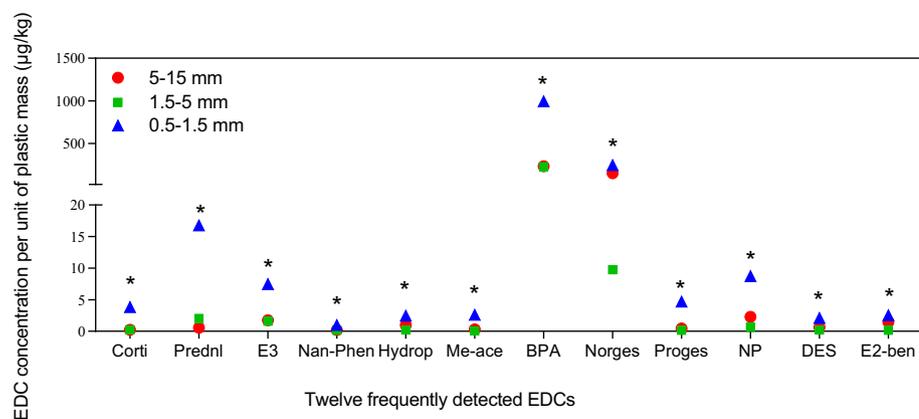


Fig. 2. The mean values of twelve EDCs on different sizes of plastic particles. The smallest particles (0.5–1.5 mm, $N = 23$) showed significantly higher concentrations than those on larger particles (1.5–5 mm, $N = 27$) and (5–15 mm, $N = 22$). These EDCs had relatively higher measured EDC concentrations. Asterisks represent that the small plastic group had significant differences from the medium (1.5–5 mm) and large (5–15 mm) plastic groups ($p < 0.05$).

3.3. Different estrogenic effects in ER-CALUX and LYES bioassays

Previous studies have reported that nonylphenol- or phthalate-containing leachates from plastic products can lead to immobility of *Daphnia magna* and 11–60% lethality of coral reef fish *Pseudochromis fridmani* (Hamlin et al., 2015; Lithner et al., 2009). However, in the present study, we did not find strong toxic effects using mammalian and yeast cells. The LYES bioassay showed comparatively weaker estrogenic effects to those in ER-CALUX bioassay (Fig. 3A). This trend is because the metabolic abilities for EDCs of yeast cells in the LYES bioassay were much lower than those of mammalian cells (T47Dluc) in the ER-CALUX bioassay (Brinkmann et al., 2014). The elevated estrogenic activity in the ER-CALUX bioassay may be attributed to increased formation of phenolic moieties (which is a good predictor of chemical estrogenic activity) during the exposure (Yang et al., 2011). Benzene moieties are often converted to phenolic moieties when they are exposed to mammalian cell lines. This conversion occurs because the oxidation reaction is one of the most important phase I biotransformations, which can introduce –OH and –COOH functional groups into the xenobiotics (Yu et al., 2016), and therefore more phenolic hydroxyl groups could be formed in the ER-CALUX bioassay.

3.4. Causal links between chemical and biological signals

We compared the bioassay results with the identified causative chemical compounds by calculating the Chem-EEQ values (Fig. 3B). The contribution percentages (Chem-EEQ/Bio-EEQ) of estrogenic chemicals for biological effects were plotted (Fig. S3, SI page S20). The plot suggested that Chem-EEQ values were generally lower than Bio-EEQ values, as most contribution percentages were lower than 100%. This

result may be due to metabolism effects during exposure especially for the ER-CALUX bioassay, and the limited number of quantified estrogenic chemicals in the present study.

However, the Chem-EEQ values for a small fraction of samples (large-NT, large-MW, large-AC, and medium-NT) were higher than their Bio-EEQ values (contribution% > 100%) (Fig. S3, SI page S20). It was thought that the estrogenic effects can be affected by other EDCs. One possibility is that some steroids (e.g., estrone and estradiol) are aryl hydrogen (Ah) receptor ligands (Umbuzeiro et al., 2011), which can enhance catabolism of cellular estrogens via induction of cytochrome P450 1A1; therefore, the presence of these EDCs could decrease the net estrogenic effects (Kortenkamp, 2007). Moreover, these steroids are also related to the transcription of estrogen response genes, and thus the ligand-Ah receptor complex can down regulate estrogen receptor expression after their binding (Chen et al., 2001). Another explanation is that negative modulation can be observed at relatively low concentrations by Ah receptor agonists. For example, PCB-126 and benzo[a]pyrene showed negative modulation starting at 10 nM and 1 µM, respectively, even though an actual estrogen was present at higher concentrations (Evans et al., 2012).

3.5. Normal life treatment effects on the release of EDCs

Endocrine disrupting chemical concentrations appeared to differ for the different treatments applied, namely, no treatment (NT), microwaving (MW), autoclaving (AC), and solar irradiation (solar) (Fig. 4). Four chemicals from each group (estrogens, androgens, and progestogens) were plotted to compare the treatment effects. In general, the solar treatment increased, whereas MW or AC treatments decreased the release of EDCs compared with the NT treatment.

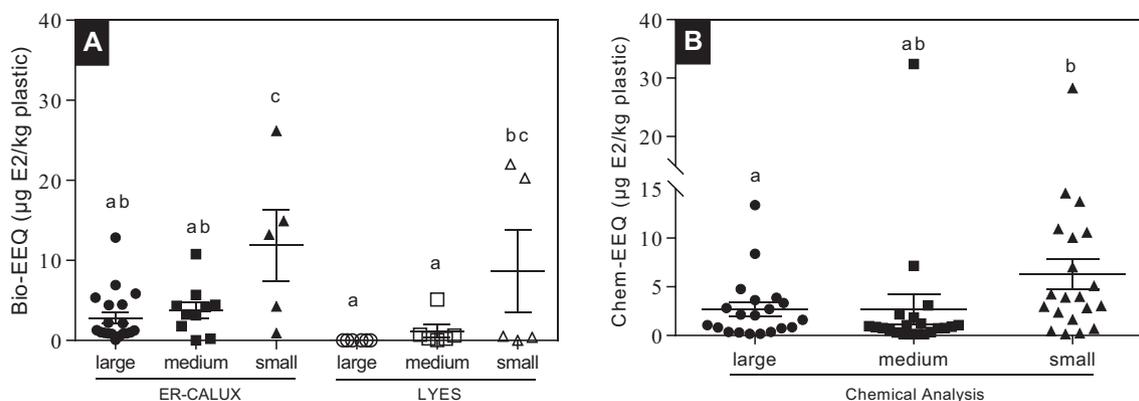


Fig. 3. (A) Bio-EEQ values and (B) Chem-EEQ values for EDC extracts of plastic particles. Small: 0.5–1.5 mm plastic particles; medium: 1.5–5 mm plastic particles; large: 5–15 mm plastic particles. E2: 17β-estradiol, as a reference compound for estrogenic effect. Different lowercase letters represent significant differences between treatment groups ($p < 0.05$).

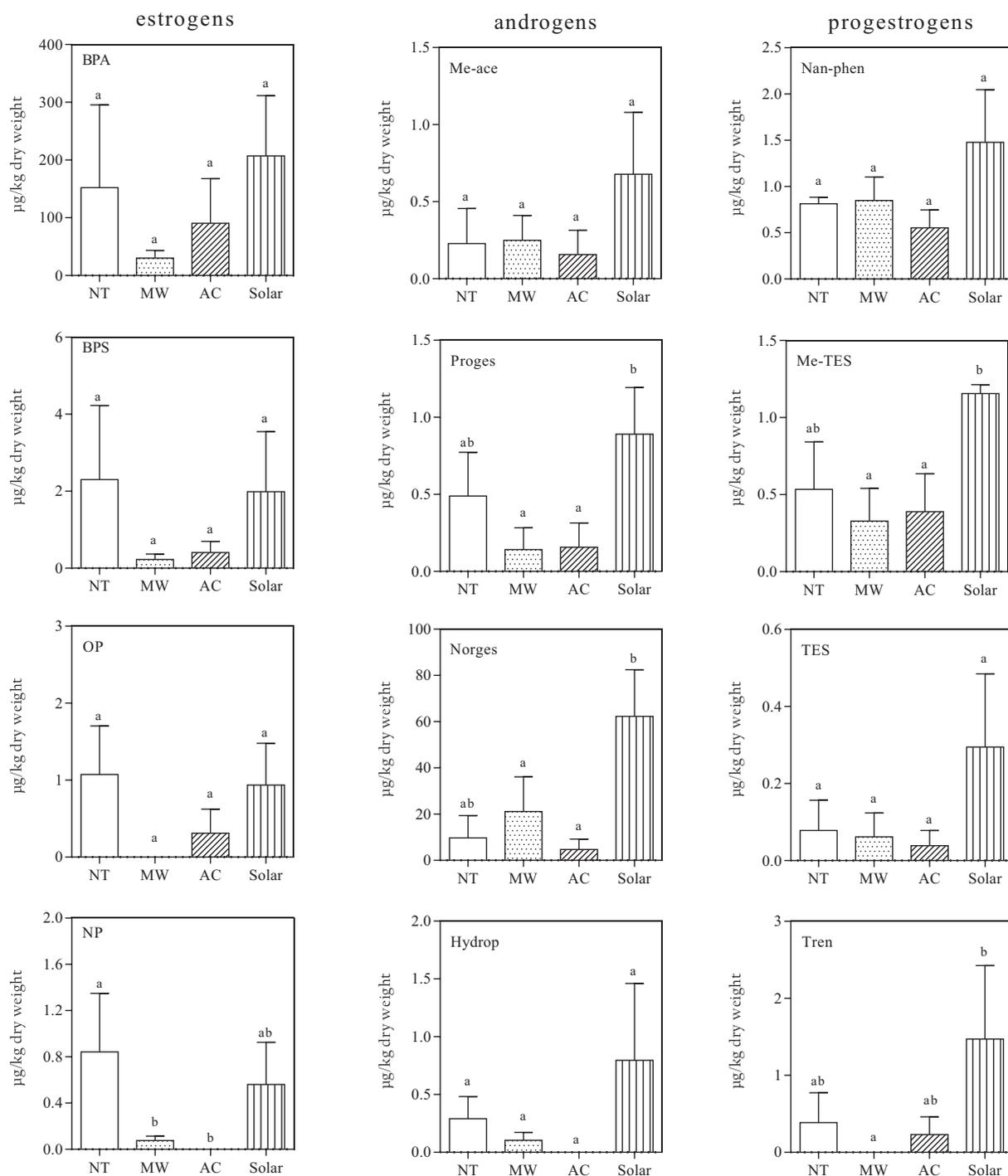


Fig. 4. Comparisons of twelve EDCs released into ethanol (normalized to µg EDC/kg plastic unit) from marine MPs and mesoplastics after 4 h of extraction. No treatment (NT) (N = 6), microwaving (MW) (N = 16), autoclaving (AC) (N = 14), and solar irradiation (Solar) (N = 6). Different lowercase letters represent significant differences ($p < 0.05$). BPA: bisphenol A, BPS: bisphenol S, OP: 4-tert-octylphenol, NP: 4-n-nonylphenol, Me-ace: megestrol acetate, Proges: progesterone, Norges: norgestrel, Hydrop: hydroxyprogesterone, Nan-phen: nandrolone phenylpropionate, Me-TES: methyl testosterone, TES: testosterone, Tren: trenbolone.

There are several possible reasons for the relatively higher leaching of EDCs upon solar irradiation. Photodegradation of the polymer surface can accelerate the increase in plastic surface area (Holmes et al., 2012). An increase in the size of pores on plastic may increase effective diffusivities of the EDCs (Mato et al., 2001). Moreover, solar weathering also introduces oxygen-containing groups and consequently may have increased the polarity of the polymer surfaces (Mato et al., 2001). This change may decrease the sorption capacity of plastic for hydrophobic pollutants. In this case, the chemical diffusion gradient is from plastic to

the ethanol/saline phase, and therefore the dissolved concentrations of EDCs increased after solar irradiation. It has also been reported that UV photolysis can degrade EDCs including BPA, ethinyl estradiol, and estradiol; however, this requires strong UV irradiation, such as pressure UV lamps at 254 nm (Rosenfeldt and Linden, 2004). In the present study, we used natural solar irradiation, which contained low intensity UV light, therefore leading to negligible degradation of EDCs.

In contrast, MW and AC treatments decreased the release of EDCs relative to the control, even though they have been considered as

attractive alternative methods for the extraction of hormones (Hibberd et al., 2009; Labadie and Budzinski, 2005). It was shown that most EDC concentrations after MW and AC treatments were lower than those for the NT control and were significantly lower than those in solar treatment leachates ($p < 0.05$) (Fig. 4). There are two possible influences of MW and AC treatments. On the one hand, both MW and AC can increase the sample extraction temperature, which could have affected EDC leaching rates and increased EDC vaporization. However, previous studies showed that different treatment temperatures showed no positive linear effects for most EDCs (Hamid and Eskicioglu, 2013). Furthermore, the AC method also had negligible effects on the vaporization of EDCs because the temperature was controlled at 120 °C, whereas the melting points of most EDCs are much higher than 120 °C (Table S3 SI pages S9–S15) even under the autoclaving pressure, except for nonylphenol and octylphenol (41–42.5 and 84.5 °C, respectively). The boiling points of nonylphenol and octylphenol are 293–297 and 206.3 °C (at 1 atm), respectively. Thus, some of these two chemicals were possible to evaporate out of glass tubes at high temperatures and under high pressure. On the other hand, MW and AC have been used as a way of EDC removal in engineering and sewage disposal processes (Carballa et al., 2006; Hamid and Eskicioglu, 2013). In general, the MW treatment decreased hormone concentrations (estrone, testosterone and androstenedione) in the soluble phase (Hamid and Eskicioglu, 2013). Similarly, the AC process was also reported to efficiently remove estrone and 17 β -estradiol (Carballa et al., 2006). The decrease in EDC concentrations by either MW or AC could have been caused by the abiotic transformation of hormones. This transformation is attributed partly to autoxidation. As shown in Table S3 (SI, pages S11–S17), many EDCs have ring structures similar to those of sterols, which have been well documented in the literature as being susceptible to autoxidation and denaturation by heating (Hamid and Eskicioglu, 2013).

Hence, solar irradiation can increase the leaching of EDCs, but other common normal life stresses (MW and AC) led to lower EDC leaching compared to no treatment (NT). For some compounds (e.g., nonylphenol), a substantial decrease was observed (Fig. 4), indicating that normal life stresses (frequently used in cooking) have the ability to remove plastic-associated EDCs to some extent.

3.6. Potential estrogenic effects of plastic-containing seafood

Among the measured EDCs, only BPA has a suggested tolerable daily intake (TDI) value (Bang et al., 2012). Thus, we first compared uptake with the TDI value for BPA, which is 50 $\mu\text{g}/\text{kg}$ body weight/day (Bang et al., 2012). The plastic extracts were aimed to simulate plastic-containing seafood (such as mussels and barnacles) soup. According to the literature, in the sampling area, barnacles and mussels contain 1–30 and 0.2–0.36 MPs and mesoplastics per organism (only “particle” data), respectively (Goldstein and Goodwin, 2013; Van Cauwenberghe et al., 2015; Van Cauwenberghe and Janssen, 2014). If a person (62 kg) (Walpole et al., 2012) takes in BPA through 1 kg seafood with a cooking density of approximately 50 barnacles in 0.5 L soup after boiling according to a common recipe (CookingLisbon, 2019), and we use the average BPA concentrations on plastics, calculations show that a person would need to consume thousands to millions of barnacles per day (corresponding to 1,111,485, 313,647, and 76,494 particles for 0.5–1.5 mm, 1.5–5 mm, and 5–15 mm marine plastics, respectively), to reach the TDI threshold. These calculated quantities far outnumber the estimated consumption of MPs of 11,000 pieces per person per year by eating seafood (Van Cauwenberghe and Janssen, 2014). Thus, it can be seen that plastic-containing seafood cooking will lead to a negligible intake of BPA with respect to the TDI threshold. However, these calculations may underestimate BPA uptake because EDCs can also be accumulated along the food chain, as barnacles and mussels prey upon aquatic plankton (Wieters et al., 2008). In addition, we did not account for the temperature, pH, BPA diffusion efficiency in biota, organism conditions in this calculation, which can also change the BPA uptake

amounts in human beings and other organisms (Hu et al., 2006; Kang et al., 2007).

Additionally, we utilized the same recipe (50 barnacles in 0.5 L soup) to predict comprehensive biological effects based on the ER-CALUX bioassay results for the plastic extract mixtures. Here, we assume that 0.1% of the EDCs in the plastic extracts could be accumulated by biota, because the uptake of additives via plastic is thought to be $< 0.1\%$ of the total dietary exposure (Lusher et al., 2017). The EC_{10} value can replace the No Observed Effect Concentration (NOEC) (Hung et al., 2006), which is a commonly used parameter in microbial tests. Following this criterion, none of the NT, MW, or AC samples have potential estrogenic effects (all below the EC_{10} value).

What needs illustration is that the bioavailability of EDCs on MPs and mesoplastics ($< 0.1\%$) calculated above depends on temperature, pH and the concentration gradients between plastics and the surrounding water or organism (Lusher et al., 2017). However, in our present study, there is a difference with respect to exposure of EDCs. We used plastic extracts (the liquid phase) under normal life stress conditions for exposure. Therefore, the EDCs were present in the liquid phase and that may change the bioavailability to some extent. For example, the use of extracts negates the time necessary for EDCs to diffuse from polymers to the liquid phase and is likely increases the EDC bioavailability to $> 0.1\%$. Therefore, in future studies, the actual contribution of plastic-bound EDCs through seafood cooking needs to be further explored. Then, a more realistic estimate of comprehensive estrogenic effects through normal life stresses can be approached.

In conclusion, with evidence from the present study, we found a size effect: the smaller the particle size is, the greater the resulting EDC concentrations are. Marine MPs had higher concentrations of estrogens than mesoplastics, but estrogenic effects mediated by both MPs and mesoplastics may be negligible. Uptake of EDCs through MP- and mesoplastic-bearing seafood cooking may not raise a safety concern. However, if plastic particles become even smaller, estrogenic effects could probably no longer be ignored, and EDCs on nanoplastics need to be explored in future studies. Moreover, even though no obvious risks are indicated at present, monitoring of seafood that contains MPs and mesoplastics remains important because the concentrations of these particles may change in the future.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.104938>.

References

Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: current trends in

- environmental pollution and mechanisms of toxicological profile. *Environ. Toxicol. Pharmacol.* 68, 61–74.
- Bang, D.Y., Kyung, M., Kim, M.J., Jung, B.Y., Cho, M.C., Choi, S.M., Kim, Y.W., Lim, S.K., Lim, D.S., Won, A.J., Kwack, S.J., Lee, Y., Kim, H.S., Lee, B.M., 2012. Human risk assessment of endocrine-disrupting chemicals derived from plastic food containers. *Compr. Rev. Food Sci. Food Saf.* 11, 453–470.
- Bergman, A., Heindel, J., Jobling, S., Kidd, K., Zoeller, R.T., 2012. State-of-the-science of endocrine disrupting chemicals, 2012. *Toxicol. Lett.* 211, S3.
- Bittner, G.D., Denison, M.S., Yang, C.Z., Stoner, M.A., He, G.C., 2014a. Chemicals having estrogenic activity can be released from some bisphenol A-free, hard and clear, thermoplastic resins. *Environ. Health-Glob.* 13, 103.
- Bittner, G.D., Yang, C.Z., Stoner, M.A., 2014b. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. *Environ. Health-Glob.* 13, 41.
- Bovee, T.F.H., Helsdingen, R.J.R., Rietjens, I.M.C.M., Keijer, J., Hoogenboom, R.L.A.P., 2004. Rapid yeast estrogen bioassays stably expressing human estrogen receptors alpha and beta, and green fluorescent protein: a comparison of different compounds with both receptor types. *J. Steroid Biochem.* 91, 99–109.
- Brinkmann, M., Maletz, S., Krauss, M., Bluhm, K., Schiwy, S., Kuckelkorn, J., Tiehman, A., Brack, W., Hollert, H., 2014. Heterocyclic aromatic hydrocarbons show estrogenic activity upon metabolism in a recombinant transactivation assay. *Environ. Sci. Technol.* 48, 5892–5901.
- Cao, X.L., Corriveau, J., 2008. Migration of bisphenol A from polycarbonate baby and water bottles into water under severe conditions. *J. Agric. Food Chem.* 56, 6378–6381.
- Carballa, M., Omil, F., Alder, A.C., Lema, J.M., 2006. Comparison between the conventional anaerobic digestion of sewage sludge and its combination with a chemical or thermal pre-treatment concerning the removal of pharmaceuticals and personal care products. *Water Sci. Technol.* 53, 109–117.
- Chen, I., Hsieh, T., Thomas, T., Safe, S., 2001. Identification of estrogen-induced genes downregulated by AhR agonists in MCF-7 breast cancer cells using suppression subtractive hybridization. *Gene* 262, 207–214.
- Chen, Q.Q., Yin, D.Q., Jia, Y.L., Schiwy, S., Legradi, J., Yang, S.Y., Hollert, H., 2017. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. *Sci. Total Environ.* 609, 1312–1321.
- Chen, Q.Q., Reisser, J., Cunsolo, S., Kwadijk, C., Kotterman, M., Proietti, M., Slat, B., Ferrari, F.F., Schwarz, A., Levivier, A., Yin, D.Q., Hollert, H., Koelmans, A.A., 2018. Pollutants in plastics within the North Pacific Subtropical Gyre. *Environ. Sci. Technol.* 52, 446–456.
- Chen, Q.Q., Zhang, H.B., Allgeier, A., Zhou, Q., Ouellet, J.D., Crawford, S.E., Luo, Y.M., Yang, Y., Shi, H.H., Hollert, H., 2019. Marine microplastics bound dioxin-like chemicals: model explanation and risk assessment. *J. Hazard. Mater.* 364, 82–90.
- Cooking** Lisbon, 2019. Recipe of goose barnacles, Portugal, website: <https://cookinglisbon.com/recipes/fish/goose-barnacles/>.
- Damstra, T., Barlow, S., Bergman, A., Kavlock, R., Van Der Kraak, G., 2002. Global Assessment of the State-of-the-Science of Endocrine Disruptors. World Health Organization, Geneva, pp. 11–32.
- Davison, P., Asch, R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific subtropical gyre. *Mar. Ecol. Prog. Ser.* 432, 173–180.
- Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch. Environ. Contam. Toxicol.* 69, 320–330.
- Effrth, T., Paul, N.W., 2017. Threats to human health by great ocean garbage patches. *The Lancet Planetary Health* 1, e301–e303.
- Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R., Date, T., 2005. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. *Mar. Pollut. Bull.* 50, 1103–1114.
- Evans, R.M., Scholze, M., Kortenkamp, A., 2012. Additive mixture effects of estrogenic chemicals in human cell-based assays can be influenced by inclusion of chemicals with differing effect profiles. *PLoS One* 7, e43606.
- Goldstein, M.C., Goodwin, D.S., 2013. Gooseneck barnacles (*Lepas* spp.) ingest microplastic debris in the North Pacific subtropical gyre. *PeerJ* 1, e184.
- Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: environmental and toxicological effects. *Environ. Toxicol. Pharmacol.* 64, 164–171.
- Hamid, H., Eskicioglu, C., 2013. Effect of microwave hydrolysis on transformation of steroidal hormones during anaerobic digestion of municipal sludge cake. *Water Res.* 47, 4966–4977.
- Hamlin, H.J., Marciano, K., Downs, C.A., 2015. Migration of nonylphenol from food-grade plastic is toxic to the coral reef fish species *Pseudochromis fridmani*. *Chemosphere* 139, 223–228.
- Hansen, E., Nilsson, N., Lithner, D., Lassen, C., 2013. Hazardous Substances in Plastic Materials, 1st ed.
- Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: a review. *Chemosphere* 182, 781–793.
- Hibberd, A., Maskaoui, K., Zhang, Z., Zhou, J.L., 2009. An improved method for the simultaneous analysis of phenolic and steroidal estrogens in water and sediment. *Talanta* 77, 1315–1321.
- Hirai, H., Takada, H., Ogata, Y., Yamashita, R., Mizukawa, K., Saha, M., Kwan, C., Moore, C., Gray, H., Laursen, D., Zettler, E.R., Farrington, J.W., Reddy, C.M., Peacock, E.E., Ward, M.W., 2011. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. *Mar. Pollut. Bull.* 62, 1683–1692.
- Holmes, L.A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.* 160, 42–48.
- Hu, X.-l., Peng, J.-f., Liu, J.-f., Jiang, G.-b., Jönsson, J.Å., 2006. Evaluating the impacts of some environmentally relevant factors on the availability of bisphenol A with negligible-depletion SPME. *Chemosphere* 65, 1935–1941.
- Hung, Y.-T., Wang, L.K., Lo, H.H., Yapjijkas, C., 2006. Hazardous Industrial Waste Treatment, 1 ed. CRC Press.
- Jantz, L.A., Morishige, C.L., Bruland, G.L., Lepczyk, C.A., 2013. Ingestion of plastic marine debris by longnose lancetfish (*Alepisaurus ferox*) in the North Pacific Ocean. *Mar. Pollut. Bull.* 69, 97–104.
- Kabir, E.R., Rahman, M.S., Rahman, I., 2015. A review on endocrine disruptors and their possible impacts on human health. *Environ. Toxicol. Pharmacol.* 40, 241–258.
- Kang, J.-H., Aasi, D., Katayama, Y., 2007. Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. *Crit. Rev. Toxicol.* 37, 607–625.
- Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported re-interpretation of empirical studies. *Environ. Sci. Technol.* 50, 3315–3326.
- Kortenkamp, A., 2007. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. *Environ. Health Perspect.* 115, 98–105.
- Kuckelkorn, J., Redelstein, R., Heide, T., Kunze, J., Maletz, S., Waldmann, P., Grummt, T., Seiler, T.B., Hollert, H., 2018. A hierarchical testing strategy for micropollutants in drinking water regarding their potential endocrine-disrupting effects-towards health-related indicator values. *Environ. Sci. Pollut. Res.* 25, 4051–4065.
- Kunz, P.Y., Simon, E., Creusot, N., Jayasinghe, B.S., Kienle, C., Maletz, S., Schifferli, A., Schonlau, C., Ait-Aissa, S., Denslow, N.D., Hollert, H., Werner, I., Vermeirssen, E.L.M., 2017. Effect-based tools for monitoring estrogenic mixtures: evaluation of five *in vitro* bioassays. *Water Res.* 110, 378–388.
- Labadie, P., Budzinski, H., 2005. Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). *Environ. Sci. Technol.* 39, 5113–5120.
- Le, H.H., Carlson, E.M., Chua, J.P., Belcher, S.M., 2008. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicol. Lett.* 176, 149–156.
- Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., 2018. Evidence that the great Pacific garbage patch is rapidly accumulating plastic. *Sci Rep-UK* 8, 4666.
- Legler, J., van den Brink, C.E., Brouwer, A., Murk, A.J., van der Saag, P.T., Vethaak, A.D., van der Burg, P., 1999. Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line. *Toxicol. Sci.* 48, 55–66.
- Lithner, D., Damberg, J., Dave, G., Larsson, Å., 2009. Leachates from plastic consumer products—screening for toxicity with *Daphnia magna*. *Chemosphere* 74, 1195–1200.
- Liu, X., Xu, J., Zhao, Y., Shi, H., Huang, C.-H., 2019. Hydrophobic sorption behaviors of 17β-estradiol on environmental microplastics. *Chemosphere* 226, 726–735.
- Lohmann, R., 2012. Critical review of low-density polyethylene's partitioning and diffusion coefficients for trace organic contaminants and implications for its use as a passive sampler. *Environ. Sci. Technol.* 46, 606–618.
- Loyo-Rosales, J.E., Rosales-Rivera, G.C., Lynch, A.M., Rice, C.P., Torrents, A., 2004. Migration of nonylphenol from plastic containers to water and a milk surrogate. *J. Agric. Food Chem.* 52, 2016–2020.
- Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety. In: *FAO Fisheries and Aquaculture Technical Paper*.
- Maletz, S., Floehr, T., Beier, S., Klumper, C., Brouwer, C., Behnisch, P., Hügley, E., Giesy, J.P., Hecker, M., Gebhardt, W., Linnemann, V., Pinnekamp, J., Hollert, H., 2013. *In vitro* characterization of the effectiveness of enhanced sewage treatment processes to eliminate endocrine activity of hospital effluents. *Water Res.* 47, 1545–1557.
- Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ. Sci. Technol.* 35, 318–324.
- Molina-Molina, J.M., Amaya, E., Grimaldi, M., Saenz, J.M., Real, M., Fernandez, M.F., Balaguer, P., Olea, N., 2013. *In vitro* study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. *Toxicol. Appl. Pharmacol.* 272, 127–136.
- Moore, C., 2003. Trashed - across the pacific ocean, plastics, plastics, everywhere. *Nat. Hist.* 112, 46–51.
- Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environ. Sci.: Processes Impacts* 18, 1050–1059.
- Oehlmann, J., Oetken, M., Schulte-Oehlmann, U., 2008. A critical evaluation of the environmental risk assessment for plasticizers in the freshwater environment in Europe, with special emphasis on bisphenol A and endocrine disruption. *Environ. Res.* 108, 140–149.
- Pillon, A., Boussioux, A.M., Escande, A., Ait-Aissa, S., Gomez, E., Fenet, H., Ruff, M., Moras, D., Vignon, F., Duchesne, M.J., Casellas, C., Nicolas, J.C., Balaguer, P., 2005. Binding of estrogenic compounds to recombinant estrogen receptor-alpha: application to environmental analysis. *Environ. Health Perspect.* 113, 278–284.
- Polovina, J.J., Woodworth-Jefcoats, P.A., 2013. Fishery-induced changes in the subtropical Pacific pelagic ecosystem size structure: observations and theory. *PLoS One* 8, e62341.
- Rosenfeldt, E.J., Linden, K.G., 2004. Degradation of endocrine disrupting chemicals bisphenol A, ethinyl estradiol, and estradiol during UV photolysis and advanced oxidation processes. *Environ. Sci. Technol.* 38, 5476–5483.
- Routledge, E.J., Sumpter, J.P., 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* 15, 241–248.
- Sajiki, J., Yonekubo, J., 2003. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. *Chemosphere* 51, 55–62.
- Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio,

- C., D'Angelo, G., Spanò, N., 2019. Microplastics occurrence in the Tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. *Environ. Toxicol. Pharmacol.* 67, 35–41.
- Seidensticker, S., Grathwohl, P., Lamprecht, J., Zarfl, C., 2018. A combined experimental and modeling study to evaluate pH-dependent sorption of polar and non-polar compounds to polyethylene and polystyrene microplastics. *Environ. Sci. Eur.* 30, 30.
- Shim, W.J., Hong, S.H., Eo, S., 2018. Marine microplastics: Abundance, distribution, and composition. In: *Microplastic Contamination in Aquatic Environments*. Elsevier, pp. 1–26.
- Staniszewska, M., Falkowska, L., Grabowski, P., Kwaśniak, J., Mudrak-Cegiołka, S., Reindl, A.R., Sokołowski, A., Szumiło, E., Zgrundo, A., 2014. Bisphenol A, 4-tert-octylphenol, and 4-nonylphenol in the Gulf of Gdańsk (Southern Baltic). *Arch. Environ. Con. Toxicol.* 67, 335–347.
- Ter Halle, A., Ladirat, L., Gendre, X., Goudouneche, D., Pusineri, C., Routaboul, C., Tenailleau, C., Duployer, B., Perez, E., 2016. Understanding the fragmentation pattern of marine plastic debris. *Environ. Sci. Technol.* 50, 5668–5675.
- Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Pham, H.V., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhang, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. T. R. Soc. B* 364, 2027–2045.
- Umbuzeiro, G., Machala, M., Weiss, J., 2011. *Diagnostic Tools for Effect-Directed Analysis of Mutagens, AhR Agonists, and Endocrine Disruptors, Effect-Directed Analysis of Complex Environmental Contamination*. Springer, pp. 69–82.
- Valitalo, P., Perkola, N., Seiler, T.B., Sillanpää, M., Kuckelkorn, J., Mikola, A., Hollert, H., Schultz, E., 2016. Estrogenic activity in Finnish municipal wastewater effluents. *Water Res.* 88, 740–749.
- Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* 193, 65–70.
- Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* 199, 10–17.
- Villeneuve, D.L., Blankenship, A.L., Giesy, J.P., 2000. Derivation and application of relative potency estimates based on *in vitro* bioassay results. *Environ. Toxicol. Chem.* 19, 2835–2843.
- Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., Roberts, I., 2012. The weight of nations: an estimation of adult human biomass. *BMC Public Health* 12, 439.
- Wieters, E.A., Gaines, S.D., Navarrete, S.A., Blanchette, C.A., Menge, B.A., 2008. Scales of dispersal and the biogeography of marine predator-prey interactions. *Am. Nat.* 171, 405–417.
- Wolz, J., Engwall, M., Maletz, S., Takner, H.O., van Bavel, B., Kammann, U., Klempt, M., Weber, R., Braunbeck, T., Hollert, H., 2008. Changes in toxicity and ah receptor agonist activity of suspended particulate matter during flood events at the rivers Neckar and Rhine - a mass balance approach using *in vitro* methods and chemical analysis. *Environ. Sci. Pollut. Res.* 15, 536–553.
- Yang, C.Z., Yaniger, S.I., Jordan, V.C., Klein, D.J., Bittner, G.D., 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ. Health Perspect.* 119, 989–996.
- Yu, M.H., Yu, M.H., Tsunoda, H., Tsunoda, M., Tsunoda, Humio, Masashi, 2016. *Environmental Toxicology: Biological and Health Effects of Pollutants*, 3rd ed. CRC Press/Taylor & Francis.
- Zeri, C., Adamopoulou, A., Varezić, D.B., Fortibuoni, T., Viršek, M.K., Kržan, A., Mandić, M., Mazziotti, C., Palatinus, A., Peterlin, M., 2018. Floating plastics in Adriatic waters (Mediterranean Sea): from the macro-to the micro-scale. *Mar. Pollut. Bull.* 136, 341–350.
- Zhang, H.C., Yu, X.J., Yang, W.C., Peng, J.F., Xu, T., Yin, D.Q., Hu, X.L., 2011. MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai. *J. Chromatogr. B* 879, 2998–3004.