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Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea

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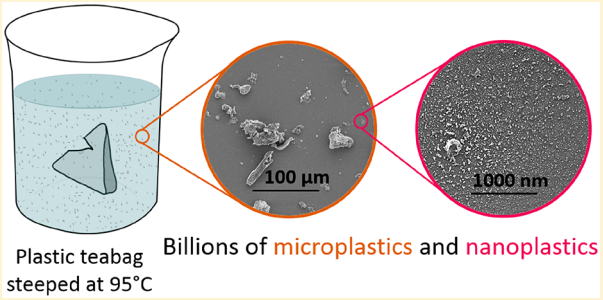
Supporting Information

ABSTRACT: The increasing presence of micro- and nano-sized plastics in the environment and food chain is of growing concern. Although mindful consumers are promoting the reduction of single-use plastics, some manufacturers are creating new plastic packaging to replace traditional paper uses, such as plastic teabags. The objective of this study was to determine whether plastic teabags could release microplastics and/or nanoplastics during a typical steeping process. We show that steeping a single plastic teabag at brewing temperature (95 °C) releases approximately 11.6 billion microplastics and 3.1 billion nanoplastics into a single cup of the beverage. The composition of the released particles is matched to the original teabags (nylon and polyethylene terephthalate) using Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The levels of nylon and polyethylene terephthalate particles released from the teabag packaging are several orders of magnitude higher than plastic loads previously reported in other foods. An initial acute invertebrate toxicity assessment shows that exposure to only the particles released from the teabags caused dose-dependent behavioral and developmental effects.



INTRODUCTION

The widespread use and mismanagement of plastics has led to a significant environmental burden of growing concern.[1] Plastic from consumer goods can break down into microplastics and nanoplastics complicating their detection and quantification.[2−4] The nano-sized fraction of plastic is particularly difficult to identify in complex organic matrices such as soils and foods. Previous studies have used different definitions for the size range of microplastics and nanoplastics,[5−9] but for the purpose of this work, we define microplastics as particles ranging from 100 nm to 5 mm in size and nanoplastics as particles ≤100 nm in size. This definition of nanoplastics is in agreement with the definition of nanomaterials by the environmental nanoscience research community.[10−12]

Plastic is commonly used in food packaging and increasingly detected in our food supply.[13] Microplastics identified as poly(ethylene) and poly(ethylene terephthalate) were detected in table salt,[14] at levels up to 681 particles/kg.[15] Several studies report the presence of microplastics in fish (pelagic and demersal),[16−18] with up to a third of the sampled fish containing ingested microplastics at detection levels between 0.2 to 1.9 particles/fish.[17−19] Others have shown that mussels can contain between 0.3 and 0.5 microplastics/g (wet weight) at the time of consumption.[83] Recent studies reported finding plastic microparticles and fibers in tap waters[20] and in 240 water bottles sold around the world.[21,22] A recent study estimates that the annual consumption of microplastics ranges between 39000 and 52000 particles depending on sex and age.[23]

Attempts are being made to curb the proliferation of plastic pollution by phasing out its use in consumer goods[24,25] such as drinking straws,[26] facial scrubs, and toothpaste; yet, new applications of plastic are being introduced in the food industry. For instance, some teas are sold in plastic teabags instead of the traditional paper teabags. This raises concern as water is frequently at or above 95 °C when brewing tea, and even "food grade" plastics may degrade or leach toxic substances when heated above 40 °C.[27]

The primary objective of this study was to evaluate whether steeping of plastic teabags under conditions that mimic those used when brewing a cup of tea causes release of plastic micro- and nanoparticles into the beverage. Empty plastic teabags were steeped in reverse osmosis (RO) water for 5 min at 95 °C and the resulting teabag leachate was analyzed for the presence of particles by scanning electron microscopy (SEM). The composition of the particles was confirmed by X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR).

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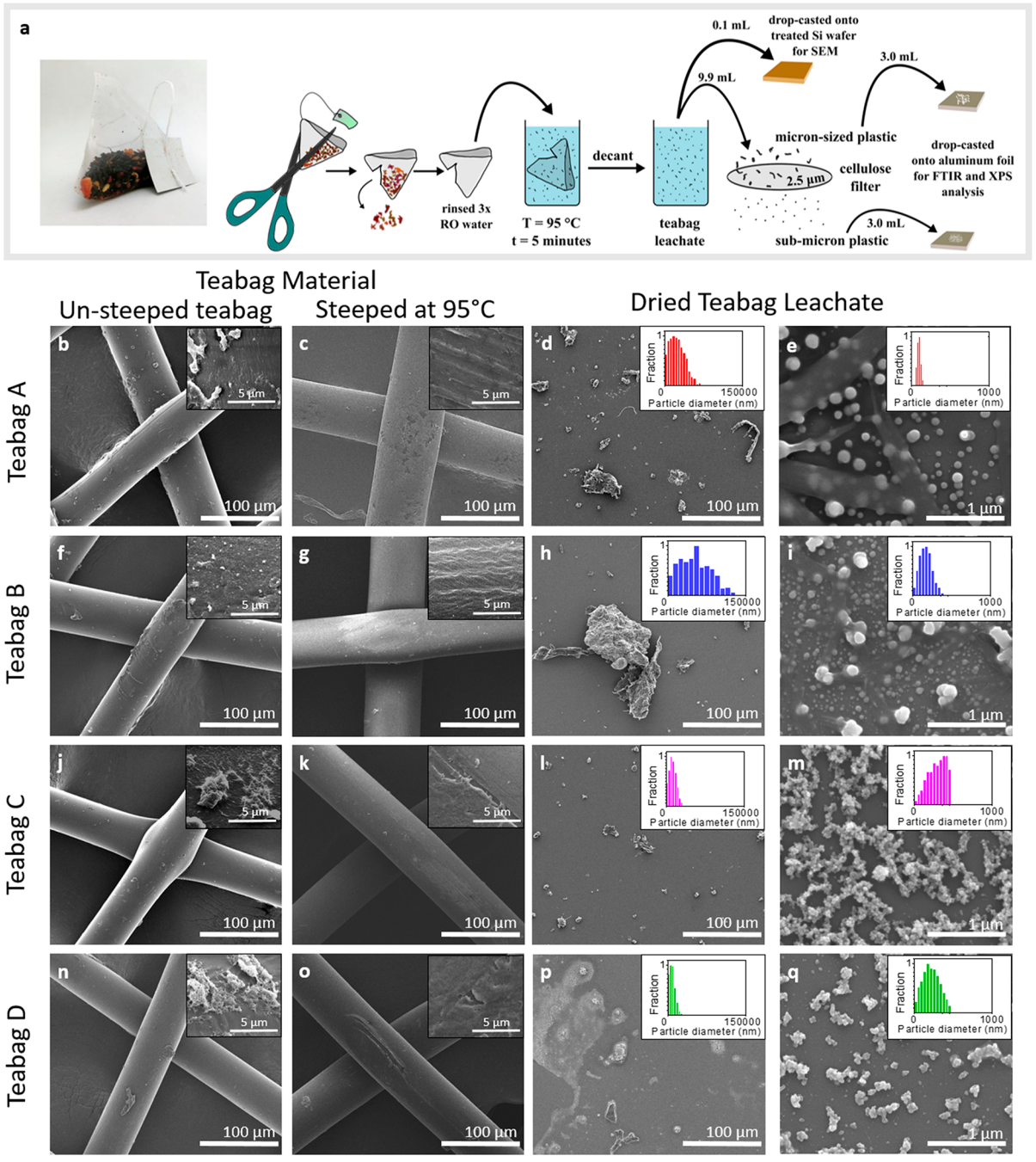

Figure 1. Teabag and leachate preparation for analysis with SEM, FTIR, and XPS. (a) Schematic showing the preparation of teabag samples. (b–q) SEM images of original teabags and their leachates after steeping. Empty plastic teabags were fixed onto carbon tape and the leachates (100 μL) were drop cast onto silicon wafers. (b, f, j, n) Imaging at 1000× of the original teabags before steeping shows a net-like structure (~30–70 μm) that appears to have a smooth surface, whereas at higher magnification (30000× insets) surface roughness and small particles (~200–1000 nm) are observed. (c, g, k, o) Imaging at 1000× of the teabags after steeping reveals a rougher surface, at higher magnification (30000× insets) dents and fractures are observed. (d, h, l, p) Teabag leachates: irregularly shaped microparticles (~1–200 μm) are observed at 1000×. Micron-sized particle size distribution is shown in the insets. (e, i, m, q) Increasing the magnification to 100000× confirms the presence of submicron and nano-sized particles in the teabag leachates. Submicron particle size distribution is shown in the insets.

MATERIALS AND METHODS

Release of Particles from Teabags. Four different commercial loose-leaf teas packaged in individual plastic teabags were purchased from grocery stores and coffee shops in Montreal, Canada (January 2016). The plastic teabags were cut with steel scissors and the tea leaves were removed. The teabags were emptied to enable determination of the number and composition of the particles released from the teabag material itself and not from the tea. The empty teabags (referred to as teabags A–D) were thoroughly washed three times using room temperature RO water to remove any tea or plastic debris and subsequently dried under a stream of nitrogen. Glass vials containing 10 mL of RO water were heated to 95 °C using a DigiPREP block digestion system. For each of the teabags A–D, three empty teabags were inserted into a single heated vial and left to steep for 5 min. After

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steeping, the water was decanted into an empty clean vial. This decanted water was referred to as the leachate from teabags A–D (Figure 1a). Triplicate samples were prepared for all experiments.

Characterization of Debris Leached from Teabags. Electron microscopy was the method chosen for observation of the leachates, as it provides the possibility of observing nanoparticles which are too small for observation with conventional imaging methods. Samples of the empty teabags (A–D) and tea leachates (A–D) were imaged using a FEI Inspect F50 SEM, as this equipment can observe particles down to 3 nm at 1 kV in SE mode. The plastic empty teabags were imaged before and after steeping by fixing them onto carbon tape. The teabag leachates (100 μL) were carefully drop cast and dried onto silicon wafers for imaging. Silicon wafers were first washed with ethanol to render the surface hydrophilic, and only 10 μL of leachate was drop-casted at a time to avoid the so-called coffee ring effect during drying of the leachate (Figure S1). The carbon tape (with the attached teabag sample) and the silicon wafers (coated with tea leachate) were coated with a 2 nm layer of platinum (Leica Microsystems EM ACE600 Sputter Coater) for SEM imaging. Triplicates were analyzed for each teabag and leachate. SEM images of the dried leachates were used to estimate the numbers and sizes of particles released into the leachates. ImageJ analysis software was used to estimate the particle sizes in the leachates of teabags A–D. Because of the polydispersity of the leachates, two average sizes were determined, the first at 1000× magnification (micro-sized particles) and the second at 100000× magnification (submicron particles). This yields a bimodal size distribution for each leachate. The mean particle diameter in each dried leachate was determined by averaging measured particle sizes from 30 images taken randomly at each magnification for each of three replicates (total of 90 images at each magnification per leachate type).

Nanoparticle Tracking Analysis (NTA) can be used to count the number of submicron particles. NTA (LM14 instrument with 532 nm green laser, NanoSight Ltd.) was used to confirm the SEM count of submicron particles in leachates diluted at a 1:15 ratio with RO water to fall within the detection range of the instrument. Results for over 200 tracked submicron particles per sample were analyzed to determine the leachate concentration (repeated in triplicate for each teabag type).

The chemical composition of all teabags and leachates was determined by FTIR and XPS. The leachates were first separated into two fractions: micron-sized and submicron particles. For this, leachates were filtered through a grade 5 Whatman filter (cellulose filter with 2.5 μm pore size) (Figure 1a). A 3 mL portion of the filtrate containing the submicron fraction was dried over aluminum foil in a desiccator yielding a thin powder film. The fraction of particles retained on the Whatman filter was resuspended in 10 mL of RO water. A 3 mL portion of this micron-sized leachate fraction was also dried over aluminum foil. The dried films of leachate and the original teabags were characterized using a Spectrum TWO FTIR instrument with a single-bounce diamond (PerkinElmer) in attenuated total reflection (ATR) mode and a Kα X-ray photoelectron spectrometer (Thermo Scientific, using a monochromatic Al Kα X-ray source and a flood gun in a 10^{-8} mbar vacuum). Additionally, commercial nylon-6,6 and PET (McMaster Carr) were analyzed using FTIR and XPS and used as references to confirm the composition of the teabags and their leachates.

Control Experiments with Teabags. In this study, all experiments were conducted with cut, emptied, and washed teabags to ensure that the enumerated particles originated from the steeped teabag itself and to avoid interference from tea organics in the SEM, FTIR, and XPS analyses. A control experiment with uncut teabags was conducted to confirm that cutting of the teabag did not cause leaching of particles (i.e., to confirm that particles were released even when the plastic teabag was uncut). These leachates contain tea and therefore require two additional steps before analysis, as the organics in tea interfere with SEM, FTIR, and XPS characterization. A 0.45 μm EMD Millipore Millex poly(ether sulfone) sterile syringe filter was used to remove small tea leaves and twigs, and a 50 mL Amicon stirred cell (UFSC05001 model) with a 30 kDa filter was used to remove dissolved organics (Figure S2a). These processed leachates were then characterized by SEM, FTIR, and XPS (Figures S2–S4).

An experiment was conducted to investigate the effect of heat on the release of particles from the plastic teabags. Briefly, teabags from brands B and D were opened, emptied, rinsed, and dried with N_2 as previously described. Subsequently, three empty teabags were inserted into a single glass vial containing 10 mL of RO water at 22 °C. After 5 min, the water was decanted into an empty clean vial. This decanted water is referred to as the "unheated leachate" from teabags B and D. The "unheated leachate" was observed by SEM in the same manner as the heated leachate (Figure S2d,e). Moreover, 3 mL of "unheated leachate" were dried over aluminum foil but no film was formed due to the lack of particulate matter in the leachate; therefore, characterization by XPS and FTIR was not possible.

A negative control was performed by processing RO water through the process described in Figure 1a. Briefly, 10 mL of RO water in a vial were heated to 95 °C for 5 min and transferred to another vial (decantation step). Subsequently, 100 μL of the negative control were carefully drop-casted onto a silicon wafer for SEM imaging (Figure S 2f). To verify that the size separation did not introduce any artifacts to the FTIR and XPS analyses, the remaining leachate of the negative control was filtered through a grade 5 Whatman filter. A 3 mL portion of the micro-sized fraction (retentate resuspended in 10 mL of RO water) and a 3 mL portion of the submicron fraction (filtrate) were dried separately over aluminum foil. The chemical analysis on the negative control using FTIR and XPS yielded only background noise due to the absence of particles, indicating that the sample processing in Figure 1a did not introduce particles or artifacts into the teabag leachates.

An additional negative control was carried out by repeating the process in Figure 1a using a metallic steeper (IKEA, Canada) with loose tea leaves (from the same supplier as teabag B) to confirm that plastic particles were not leaching from the tea leaves themselves. The resulting tea was processed to remove twigs and dissolved organics using a 0.45 μm syringe filter and a 50 mL Amicon stirred cell with a 30 kDa filter (Figure S2a). The processed leachate was characterized by SEM (Figure S2g). This control confirmed that the tea leaves did not release any particles sized between 0.45 μm and ~2 nm.

Lastly, a control to verify that the system in Figure S2a was not discharging plastic was performed by processing RO water through the Amicon stirred cell and observing the dried retentate for the presence of particles using SEM (Figure S2h). The leachates from all control experiments conducted with RO

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water were not characterized using XPS or FTIR as it was not possible to drop-cast a film of these leachates due to the absence of particulate matter. This control confirms that the apparatus in Figure S2a did not introduce particles into the leachates.

Control experiments with plastic teabags that have never been in contact with tea were not possible, as empty plastic teabags are not commercially available.

Concentrated Leachate Preparation for Preliminary Toxicity Assays. Teabags B and D were used for toxicity assays as representative nylon and PET teabags, respectively. The number of teabags needed to prepare the concentrated leachates was calculated taking into account the density of each plastic, with the objective of maintaining an equivalent total mass of plastic. Concentrated leachates for toxicity experiments were prepared by using 3.55 teabags/mL of RO water and 2.95 teabags/mL of RO water for teabags B and D, respectively (these concentrations are referred to as "100% leachates"). All teabags were opened with stainless steel scissors, all tea leaves were carefully removed, and the empty teabags were washed three times with RO water and dried with ultrapure N_2. Ten clean teabags at a time were steeped in glass vials containing RO water at 95 °C for 5 min and subsequently removed from the vials. Sequentially, another 10 teabags were steeped in the same water at 95 °C for 5 min. The process was repeated until the final target number of teabags per mL was achieved. Concentrated leachates mimic the amount of plastic ingested when drinking 1 cup of tea every other day for 1 year. The 100% leachate was sterilized by exposing the suspension to UV light (wavelength 254 nm, 4.6 mW cm^{-2}) for 20 min.

Selected toxicity tests with *D. magna* were conducted with dialyzed leachate to isolate the effect of the micro- and nanoplastics. To remove any dissolved metal(loid)s, each concentrated (100%) leachate was dialyzed for 7 days using 3.5 kDa Spectra-Por regenerated cellulose dialysis tubing. RO water for the dialysis process was exchanged every 2 h for the first 12 h and every 8 h for the remaining 7 days. This is referred to as dialyzed 100% leachate.

Characterization of Leachates and Teabags Using ICP-MS. Inductively coupled plasma mass spectrometry (ICP-MS NexION 300, PerkinElmer) was used to quantify trace levels of Al, As, Cr, and Pb in digested samples of 100% leachates (before and after dialysis), empty plastic teabags (prewashed with RO water), and full teabags. Three negative control samples and three positive control samples (spiked with a known concentration of arsenic) were digested using the same method to account for possible contamination. Finally, because arsenic is a volatile compound and the mixture of organics and acids can cause the release of vapor, the third triplicate of all the samples was spiked with a known concentration of arsenic. Details of sample preparation, digestion, and data analysis are provided in the Supporting Information.

Toxicity Assays Using *D. magna*. *Daphnia magna* used for this study was provided by Environment and Climate Change Canada (Montreal) and maintained under controlled conditions in the laboratory for more than 1 year in moderately hard reconstituted water (MHRW), at room temperature, and a 16 h light and 8 h dark cycle. They were fed daily with cultured green algae (*Chlamydomonas reinhardtii*) grown in an algae growth incubator (INFORS HT - Multitron Pro). The acute testing procedure was conducted in accordance with the OECD guideline *D. magna* Acute Immobilization Test.[28] Briefly, the 100% leachates B and D as representative of nylon and PET teabag leachates were converted to MHRW by adding calcium chloride (Fisher Scientific, Certified ACS grade), magnesium sulfate (Fisher Scientific, Certified grade), sodium bicarbonate (Sigma-Aldrich, ACS reagent, ≥ 99.7%), and potassium chloride (Fisher Scientific, BP/EP/FCC/JP/USP Purity grade) and then diluted to 50%, 5%, and 0.5% with MHRW. Neonates younger than 24 h were exposed for up to 48 h to 50%, 5%, and 0.5% nondialyzed or dialyzed leachate as well as MHRW as a control. Each 50 mL glass beaker contained 10 mL of test solution and five animals. The experiment was done in triplicates for each leachate concentration and the control. All test beakers were placed in a random order at light/dark conditions of 16 h/8 h. The temperature (21−22 °C), pH (7−8), and dissolved oxygen (9.9−10.6) of the beakers were recorded, which were in accordance with the OECD guideline. The neonates were not fed during the 48 h exposure, and the immobile neonates were counted and removed at 24 and 48 h. The swimming behavior of *D. magna* was measured according to the method described by Bownik et al.[29] with some modifications. Experimental details of the assay, analysis of the swimming behavior, and data processing are provided in the Supporting Information. After swimming assessment, a subsample of *D. magna* was randomly selected for X-ray computed tomography scan (CT scan) and optical microscopy. Experimental details of CT scan and optical microscopy are provided in the Supporting Information.

RESULTS

Plastic Teabags and Their Leachates Contain Micro- and Nanoparticles. The original teabags (Figure 1b,f,j,n) show changes after steeping at 95 °C (Figure 1c,g,k,o). At 30000× (insets), small particles can be observed on the teabag before steeping (Figure 1b,f,j,n) whereas after steeping (Figure 1c,g,k,o), these particles disappear, and dents and fractures appear.

SEM was used to determine the shape and size of particles in the dried leachates (Figure 1d−q). Figure 1e,i shows similar morphologies of large (∼100 nm) and small (∼20 nm) spherical nanoparticles in the leachates of teabags A and B. In contrast, irregularly shaped, larger agglomerates (∼100−1000 nm) are observed in the leachates of teabags C and D (Figure 1m,q). Some smaller (∼20−30 nm) individual particles are also identified in proximity to the agglomerates. At 1000× , the particle sizes measured range from 520 nm to 270 μm, whereas at 100000× , particles show characteristic lengths between ∼17 nm and 1260 nm (large aggregates). The overlap in these ranges suggests that the determined bimodal distribution is representative of the overall leachate sample. The analysis of more than 2000 particles (and aggregates) in images taken at 1000× and 100000× yielded mean particle diameters of 24.3 ± 14.4 μm and 102 ± 22 nm, 52.3 ± 29.3 μm and 171 ± 78 nm, 12.6 ± 6.7 μm and 357 ± 156 nm, and 8.6 ± 5.2 μm and 229 ± 116 nm for leachates A, B, C, and D, respectively. The particle size distributions in Figure 1 clearly show a micro-sized particle population (imaged at 1000×; particle sizes mostly between ∼1−150 μm) and a submicron particle population (imaged at 100000×; particle sizes mostly between 1 and 1000 nm).

To estimate the number of particles in each population (imaged at 1000× and 100000×), the average number of particles in 90 images was determined for each dried leachate

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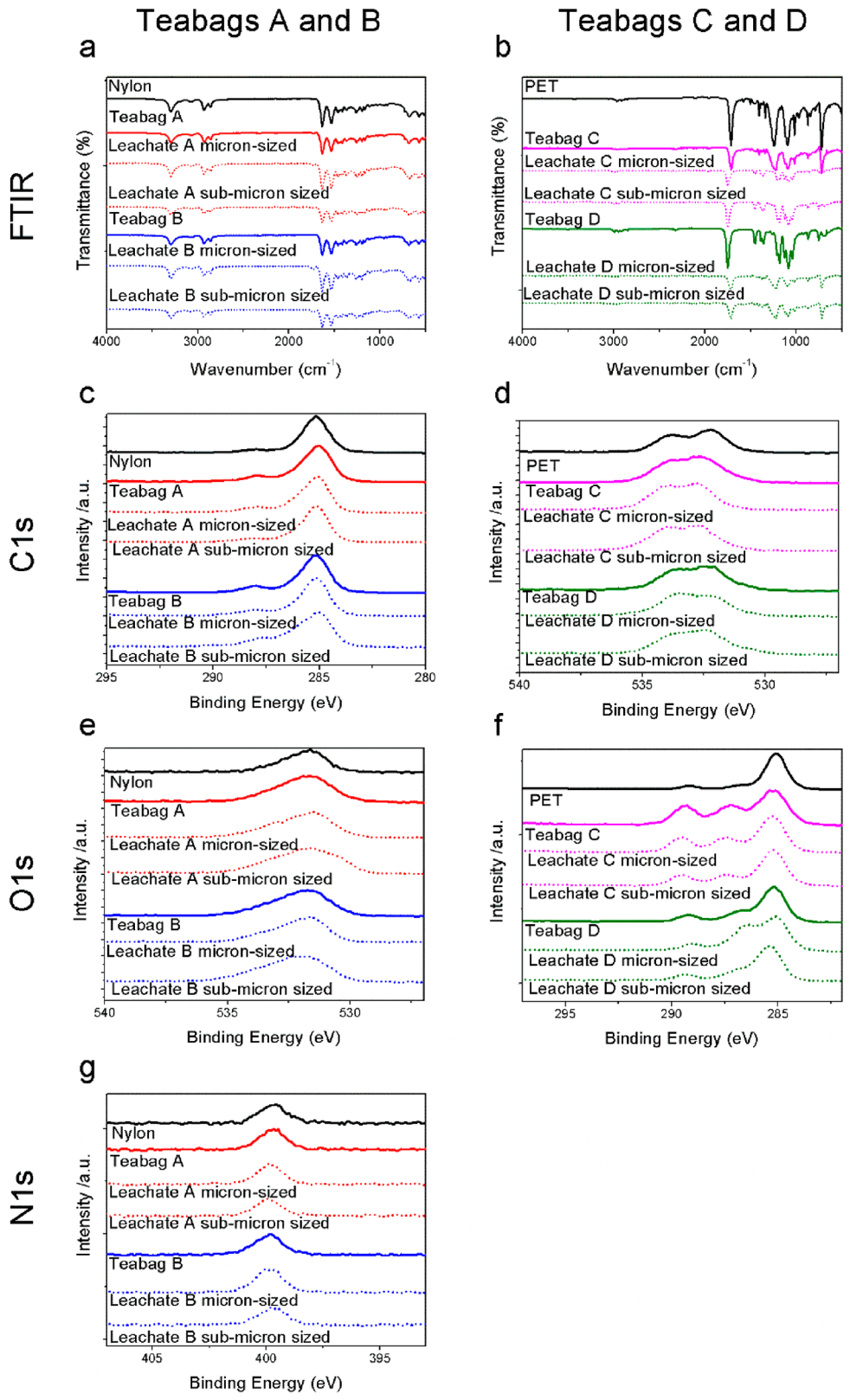

Figure 2. FTIR spectra and XPS scans for nylon-6,6, PET, the original teabags and their corresponding leachates. (a) The FTIR peak at 3289 cm^{-1} corresponds to the stretching vibration frequency of N−H groups in nylon-6,6 and the peaks detectable at 2932 and 2860 cm^{-1} can be associated with those of the ethylene sequence in nylon-6,6 (CH_2 asymmetric stretching). Teabags A and B and their leachates also present characteristic peaks of nylon-6,6 in the fingerprint region of their FTIR spectra (2000 to 500 cm^{-1}): 1634 cm^{-1} (amide I band, having a main contribution of the C=O stretching), 1535 cm^{-1} (amide II band, bending vibration frequency of N−H), 1371 cm^{-1} (amide III band, CH_2 wagging), and 681 cm^{-1} (bending vibration frequency of N−H). (b) Peaks can be observed at 1748 cm^{-1} (acid ester C = O group), 1375 and 1347 cm^{-1} (CH_2 wagging of glycol, sample D specifically), 1226 and 1089 cm^{-1} (broad bands, asymmetric C−C−O and O−C−C stretching, respectively), 1025 cm^{-1} (in-plane vibration of benzene), and 730 cm^{-1} (C−H wagging vibrations from the aromatic structure, out of plane of benzene group). (c, e, g) C 1s, O 1s,

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Figure 2. continued

and N 1s XPS spectra of teabags A and B and their corresponding leachates. The peaks observed confirm that the composition of these teabags is nylon. (d, f) C 1s and O 1s XPS spectra of teabags C and D and their corresponding leachates. The observed peaks confirm that the composition of these teabags is PET.

sample (note: each sample consisted of 100 μL of dried leachate). The number of particles per unit area was then calculated using the number of particles per image and the size of the area scanned at each magnification. The surface coverage of the dried leachate at 1000× yielded an average count of 1200 micro-sized particles/mm^2, whereas the surface coverage at 100000× resulted in an average count of 7 million submicron particles/mm^2. The overall particle count in 100 μL of leachate was estimated from the total area of the dried droplet (note: no coffee-ring effect was observed in any of the dried leachates indicating a homogeneous distribution of the particles on the imaged surface). These particle counts were used to determine the particle load in the 10 mL of leachate that had been prepared with three teabags. Finally, the result was divided by three to estimate the number of particles released from a single teabag. Hence, we estimate that when a single cup of tea prepared using one plastic teabag is consumed, a person could ingest approximately 2.3 million micro-sized (>1 μm) and 14.7 billion submicron particles (<1 μm in size). On the basis of the particle size distributions for the submicron population shown in Figure 1e,i,m,q, we can further determine that, on average, ∼21% of this population consists of nano-sized particles (<100 nm in size). Thus, we estimate that teabags A−D release an average of 3.1 billion nanoparticles per steeped teabag. NTA analysis of over 600 tracked submicron particles per leachate sample shows that teabags A−D release 8.9, 7.8, 19.1, and 21.4 billion particles, respectively. The average particle count by NTA (14.3 billion submicron particles) is very close to the average determined from SEM image analysis (14.7 billion submicron particles), thereby validating the quantitative imaging approach used.

Particles Originate from Plastic Teabags. To verify the composition of the micro-sized and submicron particles, filtration was used to separate the micro-sized fraction from the submicron fraction in the leachates (Figure 1a). Regardless of the type of teabag tested, the FTIR spectra of the micro-sized and the submicron fractions in the leachates are nearly identical to that of the corresponding original teabags, with detection of the same characteristic peaks from 500 to 4000 cm^{-1} (Figure 2a,b). The FTIR spectra of teabags A and B and their respective leachates are similar to that of a poly(hexamethylene adipamide) (such as nylon-6,6).[30] The FTIR spectra of samples C and D (teabags and their leachates) present the characteristic vibrations of poly(ethylene terephthalate) (PET).[31] Thus, the FTIR results indicate that teabags A and B are composed of nylon-6,6 and teabags C and D are made of PET. To verify the results obtained, commercial PET and nylon-6,6 samples were analyzed, and the resulting spectra were in agreement with the characterization of the teabags and their leachates, as seen in Figure 2a,b.

To further confirm the composition of the teabags and their leachates, XPS was used to characterize the elemental composition and electronic configuration of the elements. Figure 2c,e,g shows C 1s, O 1s, and N 1s XPS spectra of teabags A and B and their corresponding leachates. C 1s spectra of samples A and B exhibit two peaks (Figure 2c). A major peak is observed at 285−286 eV which corresponds to three carbon-containing groups of nylon-6,6: C−C, C−N, and C−O/C−OH. A minor peak ranging from 287 to 288 eV corresponds to the fourth carbon-containing group, namely CONH, characteristic of nylon-6,6.[32] A monomodal peak, reaching a maximum at ∼532 eV, is observed for each O 1s spectrum of the teabags and leachates A and B (Figure 2e). CONH/COOH oxygen-containing groups are likely major contributors to this peak. A slight tailing is observed to the left of the peak which is related to C−O/C−OH groups. Lastly, the N 1s spectral region was also explored and a peak at 399−400 eV is detected (Figure 2g), revealing the presence of nitrogen-containing groups (N−H). These assignments were corroborated by the XPS analysis of a nylon-6,6 sample, providing very similar spectra. Thus, XPS analysis confirms the presence of nylon-6,6 in teabags A and B and their leachates.[32]

XPS characterization of teabags C and D and their leachates (Figure 2d,f) points to the presence of PET. The C 1s spectral region (Figure 2d) is split into three main peaks that are clearly identified on the spectrum: ∼284 eV (intense peak) attributed to the C−(CH) bond, ∼287 eV (smaller peak at the foot of the intense peak) corresponding to the C−O bond, and ∼290 eV attributed to carbon ester groups. Two main components are noted in the O 1s spectra of teabags C and D (Figure 2f). A bimodal distribution with peaks at 534 eV (C−O−C groups) and 532 eV (COO groups) is observed, confirming that the two types of oxygen of the ester functional group are present in teabags and leachates C and D.[33] A commercial PET sample was also characterized by XPS and the resulting spectra matched those of the teabags and their leachates. Thus, the XPS data support the FTIR results for all four types of teabags, confirming that the material of the micro-sized and submicron particles in the leachate match the parent plastic teabag. Considering the density of PET and nylon, the average size of the particles observed, and the estimated particle count per cup of tea, it was estimated that when drinking a single cup of tea prepared with one plastic teabag, a person might ingest 13−16 μg of plastic micro- and nanoparticles.

SEM images of the leachates from uncut teabags B and D (Figure S2b,c) show that a significant number of particles are released even when teabags are uncut (Figure 1d,h,l,p). FTIR and XPS analyses of the tea leachates from the uncut teabags correspond to those of the original emptied teabags B and D (Figures S3a and S4). Thus, plastic micro- and nanoparticles leached out of the teabags even when they were uncut and contained tea, confirming that the plastic particles were not simply formed as a result of cutting the teabag. Since these leachates from uncut teabags required additional processing to remove the tea, it was not deemed appropriate to quantify the number of particles released into these leachates.

SEM imaging of the leachates for the unheated teabags (Figure S2d,e) shows drastically fewer particles than the teabags steeped at 95 °C (Figure 1i,q). Analysis of these SEM images shows that the leachates from unheated teabags contain an average of 300 times less particles than leachates prepared at 95 °C. This suggests that the material of the teabag may be fragile such that high temperature can enhance the release of particles from the teabag.

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An additional control experiment was performed by processing only RO water through the procedure described in Figure 1a. SEM imaging of the dried sample (Figure S2f) showed that neither the RO water nor the vials were a source of micro- or nanoparticles. An additional experiment was performed using a metallic steeper containing loose-leaf tea to verify whether the detected plastic particles were leaching from the tea. A representative SEM image of the leachate from the tea-filled metallic steeper showed no particles (Figure S2g). A final control experiment conducted by processing RO water through the system in Figure S2a showed that no particles were released from the filters or stirred cell (Figure S2h).

Adverse Biological Effects of Dialyzed Leachates of the Plastic Teabags. In an effort to isolate the biological impact of the micro- and nanoplastic particles, the concentrated leachates of teabags B and D (as representative nylon and PET teabags, respectively), were dialyzed for 7 days to remove any dissolved species released from the tea or plastic (e.g., metal(loids)). The biological effects of the released micro- and nanoplastics in the aquatic model species, *D. magna*, were assessed using a series of dilutions of the dialyzed leachate (50%, 5%, and 0.5%). No immobility was observed with teabag B or D leachates that had been dialyzed or controls. Nonetheless, a number of micro-sized foreign particles were observed inside the bodies of 5% and 50% leachate-exposed *D. magna* but not in controls, and the shape and size of the particles were similar to those observed in the raw leachates (Figure 3a). Some of the *D. magna* also exhibited anatomical abnormalities that were observed on the third day of development (Figure 3b). The most notable malformation was the failure of the carapace to develop properly into the lateral shields that were present in the controls. For example, in the dialyzed 50% teabag B and D leachates, the carapace shields were fused dorsally and inflated with fluid, producing a large ballooned sack that was suspended over the rest of the animal (Figure 3b).

Sublethal behavioral effects of dialyzed teabag leachate were observed in *D. magna*. Representative images in Figure 3c show a dose-dependent increase of track density for *D. magna* exposed to teabag D leachate. Significantly longer swimming distances were observed at 5% and 50% concentrations of the dialyzed leachates (Figure 3d,e). In addition, the track density was significantly increased in both 5% and 50% teabag B and D leachates (Figure S5c,d).

Identification of Metal(loid)s in the Teabag Leachate. It is well-known that tea can contain several metal(loid)s such as arsenic (As),[34] aluminum (Al),[35] lead (Pb),[35,36] and chromium (Cr).[35] Also, micro- and nanoplastics can sorb a wide variety of contaminants, including heavy metals such as Co, Cr, Cu, Ni, Pb, and Zn.[37,38] ICP-MS was used to quantify the levels of selected metal(loid)s (Al, As, Cr, and Pb) in the concentrated (100%) leachates before and after dialysis (Figure S6). The nondialyzed concentrated leachates generally have higher levels of metal(loid)s than the corresponding dialyzed leachates, with the exception of Cr in teabag D (Figure S6). The residual amount of metal(loids) in the dialyzed leachate may be due to sorption to the plastic particles. Finally, the total amount of metal(loid)s in full teabags (containing tea leaves) is compared to empty teabags (Figure S7). The results suggest that most or all of these metal(loid)s originated from the tea leaves and not the plastic teabags, with the exception of Cr in teabag D.

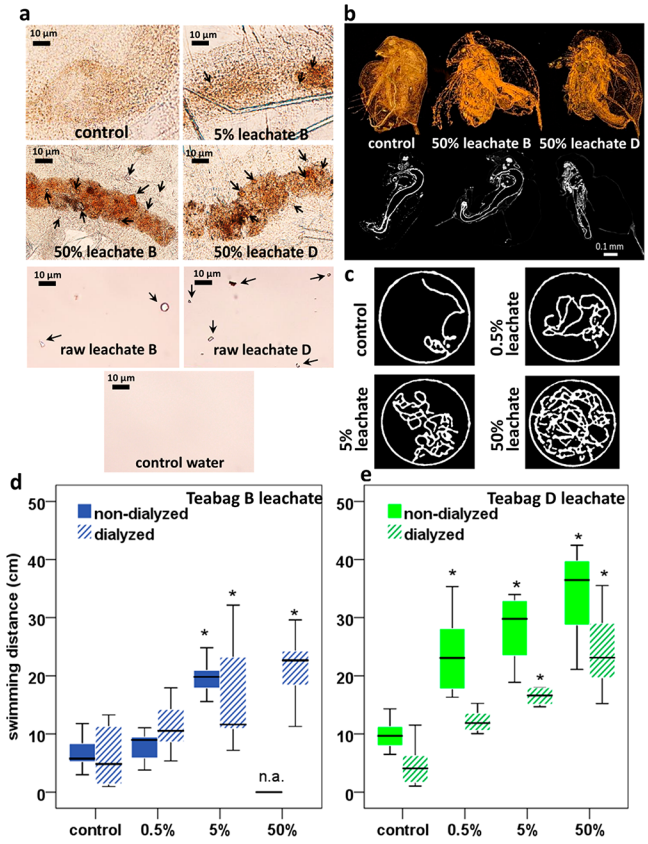

Figure 3. Teabag leachates affect the morphology and swimming behavior of *Daphnia magna*. (a) Microparticles identified in *D. magna* exposed to dialyzed teabag B and D leachates as well as in raw leachates are marked with arrows. Optical images of *D. magna* were taken using an Olympus DP80 microscope digital camera at 60× magnification focusing on the intestine. (b) CT images of *D. magna* exposed to dialyzed teabag B and D leachates. Top images show the 3-D morphology and bottom images show 2-D internal sections. (c) Representative swimming tracks of *D. magna* exposed to dialyzed teabag D leachate. (d, e) The swimming distance of *D. magna* after exposure to teabag B and teabag D leachates. Asterisk indicates that the measurement is statistically significantly different (p-value <0.05, one-way analysis of variance (ANOVA) followed by the posthoc Tukey's multiple comparison test) from the control; $n = 9$ for dialyzed teabag B, dialyzed leachate D, and nondialyzed leachate B, and $n = 6$–9 for nondialyzed teabag D leachate.

Biological Effects of Nondialyzed Leachates. In an effort to begin to evaluate the contribution of the dissolved metal(loids) to the toxicity of teabag leachate, additional experiments were conducted using dilutions of nondialyzed leachates (50%, 5%, and 0.5%) of teabags B and D. In contrast to the observations with the dialyzed leachates, during the first 24 h of *D. magna* exposure, the 50% nondialyzed teabag B leachate caused significantly higher immobility than the control (Figure S8a). After 48 h, higher immobility was observed in both nondialyzed teabag B and D leachates with statistical significance at 50% teabag B leachate and 5% teabag D leachate (Figure S8a,b). The nondialyzed leachate had a more significant impact on the morphology of *D. magna* than the dialyzed leachate, with the tissues and organs in individuals exposed to 50% leachate being poorly defined, especially in the head and intestine (Figure S9). A similar "ballooned" carapace developed, but in leachate concentrations as low as 5%. As noted with the dialyzed leachate, exposure of *D. magna* to the

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nondialyzed leachate led to the uptake of particles (Figure S10) and affected swimming behavior (Figures 3d,e and S5), but the body size was not affected (Figure S8c,d).

DISCUSSION

The mechanism by which nylon and PET degrade to form nanoparticles is yet to be studied. The polymer science literature suggests that these polymers degrade at temperatures higher than 95 °C at which the polymer undergoes disturbances in the molecular structure.[39] Others have studied the thermal degradation of these polymers under environmental conditions.[40−42] However, none of these studies have considered the possibility of polymers breaking down into nanoparticles. Some studies have shown that polystyrene breaks down to nanoparticles, but no mechanism was suggested.[43]

Polymer hydrolysis might be a mechanism by which the degradation is occurring. Hydrolytic degradation is the scission of chemical functional groups by reaction with water.[44] Chain scission is a reduction in the molecular weight of the macromolecules of a polymer, causing the polymer to become more fragile.[45] Nylon is susceptible to hydrolysis; therefore contact with water at high temperature will produce degradation and fractures.[46] PET is more resistant to hydrolysis; however, studies have shown that in the absence of oxygen and at high temperatures, hydrolytic aging may occur.[47]

Interestingly, the micro and nanoparticles released from teabags A and B have similar shape and size distributions. The microparticles are large (∼50−100 μm) irregular pieces, whereas the sub-micron particles are small (∼10−400 nm) spheres. Micro and nanoparticles released from teabags C and D are also similar. The microparticles are small (∼1−50 μm) and irregular, whereas the sub-micron particles are larger (50−600 nm) and agglomerated. Such high similarities in the shape and size distributions of particles suggest that similar materials were used in the manufacture of teabags A and B or teabags C and D. Although the two plastics used in the manufacture of the teabags we tested were considered food grade materials,[48,49] their degradation into micro- and nano-sized particles presents an unknown risk. Several studies detected the presence of microplastics in the food chain at relatively low concentrations.[17,18,50] However, in this study, we report that the level of plastic potentially ingested when drinking tea packaged in plastic teabags is several orders of magnitude higher than levels previously reported in foods. The plastic load per cup of tea prepared with one plastic teabag is estimated at 16 μg, which is in contrast to the highest level reported in table salt (0.005 μg/g of salt).[14,15] Interestingly, far fewer particles are released when the teabag is steeped at room temperature, showing the impact of packaging utilization conditions on exposure risks. The World Health Organization launched a health review in March 2018 into the potential risks of plastic in drinking water after a study reported that bottled water contained microplastics on the order of only a few tens to a few hundred particles per liter.[21] In contrast, we report here that 2.3 million micron-sized particles (∼1−150 μm) and 14.7 billion submicron plastic particles (<1 μm), which were estimated as 16 μg, can be released into 1 cup of tea. The annual load of plastic particles can be initially predicted as $L \times N \times P \times 365$, where L is plastic load per cup of tea (16 μg), N is the number of cups of tea/per day that is suggested to be safe in healthy adults (2−5,[51]), and P is the population of the tea drinkers in America (159 million[52]). On the basis of this rough estimation, 1.9 to 4.6 tons of micron and submicron plastic particles would be generated annually during tea steeping process if only plastic teabags were used. The released plastic particles may not only be ingested/excreted by humans[53] but may also enter waterways through domestic drainage systems and sewage treatment plants, contributing to microplastic pollution in the environment. The discarded single-use plastic teabags themselves further contribute to plastic waste.

D. magna is a common model species in both environmental and pharmaceutical toxicology with the advantage that it has high sensitivity to a wide range of toxic chemicals, and its transparency facilitates the imaging of uptaken particles. *D. magna* also contains similar toxin targets (e.g., eyes and heart) or molecular pathways as humans.[54] Moreover, the ease of culture and handling, short life cycle, and low cost of maintenance make *D. magna* a simple, fast, and suitable model for this study.[54−59] We observed no immobility in *D. magna* that were exposed to the plastic particles (i.e., dialyzed leachate) released from the teabags. In contrast, we noted significant acute toxicity of the nondialyzed teabag leachate to *D. magna*, likely due to the presence of metal(loid)s (Al, As, Cr, and Pb). However, in both exposure scenarios, *D. magna* swimming behavior was significantly affected in a similar dose-dependent manner (Figure 3d,e), suggesting that the altered behavior can be attributed to the micro- and nanoplastics. The increased swimming distance and track density can lead to an increase in energy expenditure and predation risk which can negatively impact the *D. magna* population.[60−62] Similar results were also noted in previous studies in *D. magna* and fish, showing disrupted locomotor activity postexposure to micro- and nanoparticles.[60,63,64] Although the answers to how the micro- and nanoplastics disrupt the swimming behaviors of *D. magna* remain elusive, some potential mechanisms may be implicated. We hypothesize that the deformed carapace in individuals with 50% dialyzed and 5% and greater nondialyzed leachates likely altered normal swimming behavior. This malformation might also lead to reproductive failure as eggs and early hatchlings are normally housed in a brooding pouch between the carapace and body. In addition, increases in locomotor activity may correspond to *D. magna* attempts to clean the particles off their appendages (see example of particles on appendages in Figure S10).

Although the *Daphnia* assay cannot be directly related to human ingestion, it has been used as a first screening method for assessing the toxic potency of a wide range of chemicals to humans (e.g., pharmaceuticals, heavy metals, pesticides).[65,66] A high correlation between the acute toxicity of chemicals to *Daphnia* and the corresponding toxicity values for mice and humans has been confirmed.[67,68] Furthermore, it has been shown that the predictive screening potential of aquatic invertebrate tests for acute oral toxicity in humans is better than that of the rat LD50 (median lethal dose) test for some chemicals.[69] Guilhermino et al. also concluded that the *Daphnia* test is more sensitive as an indicator of toxicity to rats.[70] Moreover, the oral reference dose (RfD), a preferred approach by USEPA for characterizing the noncancer health risks,[71] is significantly correlated with toxicity values for *Daphnia*, suggesting that acute toxicity assays with *Daphnia* can give important and relevant information concerning possible human oral chronic intoxication.[68]

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To date, the health effects of consuming micro- and nanoplastics to humans are still unknown, while the sublethal effects observed in the present study and in other animals (e.g., algae, zooplankton, fish, mice)[64,72−75] give an early warning of both environmental risk and possible human health risk. One of the main potential human exposure pathways of micro- and nanoplastics is likely via ingestion, and particle uptake may occur in the digestive tract.[1] Once inside the digestive tract, cellular uptake and subcellular translocation or localization of the ingested particles may occur. Translocation of various types of microparticles (particle size 0.03 to 100 μm) across the mammalian gut has been demonstrated in multiple studies involving rodents, rabbits, and dogs.[76] Potential biological responses include genotoxicity, apoptosis, and necrosis, which could lead to tissue damage, fibrosis and carcinogenesis.[1] The only in vitro human evidence showed generation of reactive oxygen species in cerebral and epithelial human cell lines after exposure to micro- and nanoplastic particles.[77]

The scarce body of data on nanoplastics, on both human exposure and potential toxicity, cannot predict the health risk of consuming nanoplastics. However, experience from nanotoxicological studies on engineered nanoparticles might be extrapolated to advance our current understanding on the uptake kinetics, potential toxicity, and mechanisms of nanoplastic particles.[13] Among different engineered nanoparticles, TiO_2 is one of the most widely studied. On the basis of the available toxicity data from oral exposures,[78−80] nano-TiO_2 seems to have low toxicity following oral exposure. For example, mice exposed to nano-TiO_2 (25 and 80 nm) at a very high dose (5 g/kg) for 2 weeks showed particle translocation from guts to spleen, lungs, kidneys, and injured liver.[81] Lower doses (1−2 mg/kg in vivo and <36 μg/mL in vitro) of nano-TiO_2 showed neither cellular toxicity nor oxidative stress in rats, even though they penetrated intestinal cells.[82] In a comparison of the doses used in these studies with the plastic particles we found here (16 μg/cup of tea), the ingested micro- and nanoplastics were not likely to present acute toxicity risks for human health. However, more subtle or chronic effects are not impossible after long-term exposure. Overall, the knowledge on adverse effects of plastic particles on human health is still lacking and there is an urgent need to investigate potential toxic mechanisms in higher vertebrates and humans, which is of vital importance when assessing the human health risk of micro- and nanoplastics.[1,77]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02540.

Information on control experiments; details for *Daphnia magna* swimming assay, immobility and body size, X-ray CT scanning, and optical imaging; details on ICP-MS measurements of aluminum, arsenic, chromium and lead in leachates and teabags (PDF)

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Notes

The authors declare no competing financial interest.

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"For instance, some tea manufacturers have shifted to using plastic teabags instead of the traditional paper teabags" was removed from the introduction section in the version published on September 25, 2019. The revised version was published on October 22, 2019.

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