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Article

Assessing the Release of Microplastics and Nanoplastics from Plastic Containers and Reusable Food Pouches: Implications for Human Health

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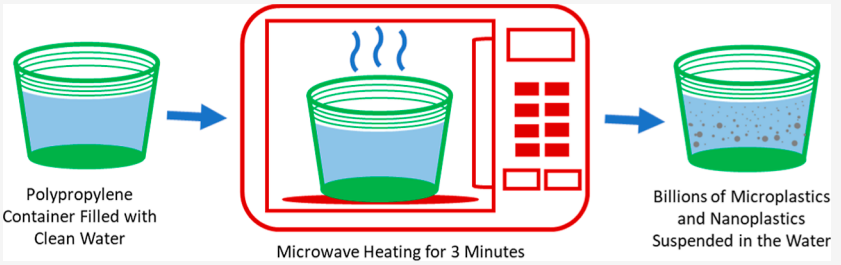

ABSTRACT: This study investigated the release of microplastics and nanoplastics from plastic containers and reusable food pouches under different usage scenarios, using DI water and 3% acetic acid as food simulants for aqueous foods and acidic foods. The results indicated that microwave heating caused the highest release of microplastics and nanoplastics into food compared to other usage scenarios, such as refrigeration or room-temperature storage. It was found that some containers could release as many as 4.22 million microplastic and 2.11 billion nanoplastic particles from only one square centimeter of plastic area within 3 min of microwave heating. Refrigeration and room-temperature storage for over six months can also release millions to billions of microplastics and nanoplastics. Additionally, the polyethylene-based food pouch released more particles than polypropylene-based plastic containers. Exposure modeling results suggested that the highest estimated daily intake was 20.3 ng/kg·day for infants drinking microwaved water and 22.1 ng/kg·day for toddlers consuming microwaved dairy products from polypropylene containers. Furthermore, an in vitro study conducted to assess the cell viability showed that the extracted microplastics and nanoplastics released from the plastic container can cause the death of 76.70 and 77.18% of human embryonic kidney cells (HEK293T) at 1000 μg/mL concentration after exposure of 48 and 72 h, respectively.

KEYWORDS: *plastic food containers, reusable food pouches, microplastics, nanoplastics, in vitro study, cell viability, HEK293T*

INTRODUCTION

The extensive use of plastic-based products in food preparation, storage, and handling raises the risk of directly releasing microplastics and nanoplastics into food, which are plastic particles with diameters on the scales of several micrometers and nanometers.[1,2] The presence of microplastics has been detected in table salt, bottled water, tap water, fish, and mussels.[3−7] Additionally, a study found that plastic teabags released billions of micro- and nano-plastics during the steeping process at 95 °C.[8] This has raised concerns about human exposure to micro- and nano-plastics via food. It has been estimated that using take-out food 4−7 times per week can result in a person ingesting 12−203 pieces of micro-plastics.[9] Furthermore, it has been reported that an individual consuming an American diet can ingest between 39,000 and 52,000 pieces of microplastics from food and beverages each year.[10]

Infants and toddlers are more susceptible to potential health impacts of micro- and nanoplastics than adults,[11] making their exposure to these particles and associated health risks a significant concern. A recent study focused on the release of microplastics from polypropylene-based baby feeding bottles during formula preparation.[12] The findings suggest that by the age of one year, babies can ingest anywhere from 14,600 to 4,550,000 microplastic particles from polypropylene feeding bottles, not including silicone-rubber teats. Additionally, another study revealed that silicone-rubber baby teats could lead to the ingestion of more than 0.66 million microplastics by

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a one-year-old baby.[13] Despite the prevalence of other plastics-based baby products, such as plastic containers and food pouches, there is currently no research available on whether they can also serve as sources of micro- and nanoplastic exposure to infants and toddlers.

Although microplastics have been found in various parts of the human body, including the human placenta and meconium,[14,15] their effects on human health are not yet fully understood. In animal studies, exposure to microplastics has been linked to gut microbiota dysbiosis and lipid metabolism disorder,[12] as well as brain damage and blood disorder in fish.[12] There is also evidence to suggest that microplastics can cause cytotoxicity in various human cell lines, such as gastrointestinal, lung, immune, nerve, and kidney cells.[16−21] A meta-analysis of existing studies found that the Caco-2 cells, i.e., human adenocarcinoma cells, are the most susceptible to microplastics' cytotoxicity.[22] However, the majority of cytotoxicity data available in the literature comes from studies using engineered microplastics, and it remains unclear whether similar effects would be observed from exposure to micro- and nanoplastics directly released from plastic food containers and food pouches. Factors such as particle morphology,[23] size,[17,24] and concentration[17] and the exposure time[22,24,25] can all influence the degree of cytotoxicity observed.

The objective of this study was to assess the potential risks associated with the use of plastic baby food containers and reusable food pouches by investigating the release of micro- and nanoplastics, estimating their potential exposure for infants and toddlers, and evaluating their cytotoxicity to human embryonic kidney cells. To the best of our knowledge, this is the first study of its kind to examine these various aspects of plastic-based baby food containers and food pouches. The findings of this study have important implications for understanding the potential health risks associated with the use of such products.

MATERIALS AND METHODS

Materials and Property Characterization. From a popular US chain store, two brands of baby food containers made of polypropylene and one brand of reusable food pouch without material information on the label were purchased. The selection of polypropylene containers was based on its widespread use in baby food packaging. These choices aimed to showcase diverse types of baby food packaging.

The food containers and the food pouch were analyzed for their semicrystalline structure and thermal stability by DSC using a Q200 differential scanning calorimeter (TA Instruments, New Castle, DE). Briefly, a small sample weighing between 3 and 8 mg was taken from each container or pouch, placed in a DSC aluminum pan/lid assembly, and crimped with a press. The samples were heated and cooled at a rate of 10 °C/min under a nitrogen atmosphere, resulting in calorimetric curves that indicate the heat transfer to and from the polymer sample during the thermal cycle, which was used to monitor phase transitions.

Transmission wide-angle X-ray diffraction (WAXD) of the reusable food pouch was performed at the 12-ID-B beamline at the Advanced Photon Source (Argonne National Laboratory), using incident X-rays with energy 13.30 keV and a Pilatus 300k 2D detector mounted 0.4 m from the sample. WAXD patterns of the two plastic containers were acquired in reflection geometry with a Bruker-AXS D8 Discover equipped with a Cu Kα lab source (λ = 1.5406 Å) and a Vantec 500 area detector. In all cases, the acquired 2D patterns were radially averaged to produce 1D intensity (I) vs scattering vector (q) plots.

Release Experiments. To simulate different types of food, two different food simulants were used in the release experiments: nanopure deionized water (DI water; 18.2 MΩ cm, Barnstead Nano-pure Systems, Thermo Scientific Inc., Waltham, MA) and 3% ACS grade acetic acid (Ibis Scientific, Las Vegas, NV), as recommended by the US FDA[26] guidelines to represent aqueous and acidic food types, respectively. As controls, both DI water and acetic acid were stored in a glass beaker and analyzed separately. Before the release experiments, the baby food containers (designated as container 1 and container 2) and the baby food pouch were thoroughly cleaned using a 2% Hellmanex solution, rinsed three times with DI water, and air-dried. Container 1 and container 2 were then filled with either DI water or 3% acetic acid up to the capacity, while the pouch was filled to three-quarters of its capacity to prevent any leakage from the sealing.

The release experiments were conducted in accordance with the US FDA[26] guidelines for accelerated testing to simulate various consumer usage conditions. To replicate the release after an extended period of refrigerated storage (i.e., 6−12 months), containers and the pouch filled with DI water or 3% acetic acid were kept at 20 °C for 10 days. For the release under room temperature of extended storage, experiments were conducted at 40 °C for 10 days. To simulate the high-temperature condition, which is storage with food at temperatures above the glass transition temperature of the plastics, experiments were conducted at 70 °C for 2 h followed by storage at 20 °C for 10 days. Microwave heating was also tested by placing containers filled with food simulants into a microwave oven at maximum power (1000 W) for 3 min. The pouch was not tested for microwave heating as it was not suitable for microwave use. The effluents were collected and analyzed at the end of the release experiments.

Effluent Characterization. The effluent samples collected were directly analyzed for the number of nanoplastics present, i.e., plastic particles with a diameter smaller than 1 μm, using a Nanoparticle Tracking Analysis (NTA), NanoSight NS300 (Malvern Panalytical Ltd, UK), which is equipped with 532 nm green laser to detect and count particles in a 10 nm to 1 μm size range. It is worth noting that a previous study found comparable result for nanoplastics when using NTA and scanning electron microscopy analysis.[8] In addition to the number of nanoplastics, NTA also provided us the size distribution of nanoplastic particles. Three effluent samples were analyzed for each release experiment.

To quantify the number of microplastics in the effluent, i.e., plastic particles with a diameter on the scale of several microns, we employed an EVOS FL Auto Imaging System (Thermo Fisher Scientific, Waltham, MA) at 40× magnification with 2.7 times zoom. A glass slide was cleaned with 10% isopropanol rinse, followed by a 10% ethanol and DI water rinse. We followed a consistent procedure to analyze each effluent sample collected during the release experiment. To begin, a 20 μL droplet of the sample was placed on a clean glass slide, and we captured 15 images of the dried area for analysis using the EVOS FL Auto Imaging System. These images were processed using ImageJ (National Institutes of Health, USA), which was set up to remove background noise from each image in a consistent way. With ImageJ, we were able to determine the area of the droplet, the number of particles within it, and the

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sizes of the particles. We conducted three replicates, resulting in a total of 45 images analyzed for each effluent sample. To ensure accuracy, we accounted for background particles in the control sample, which did not come into contact with plastic food containers or the food pouch. By subtracting the control sample's particle count from the total microplastics in the effluent, we accurately calculated the microparticle quantity in the effluent while discounting the control's minimal background particles. Air drying of microplastics could potentially promote particle aggregation, potentially causing a slight underestimation of particle count and an overestimation of particle size. However, the impact is expected to be minimal as the drying occurred on a stationary flat surface.

Raman spectroscopy (Renishaw InVia Raman spectrometer) equipped with a 514 nm laser, and an optical microscope (about 1 μm resolution) was used to further characterize the released particles in the effluent. The effluent from the release experiments was first filtered through a 25 mm diameter gold-coated track-etched membrane filter with a 0.8 μm pore size (Sigma-Aldrich Inc., MA). Raman spectra of the filtered sample were recorded from 500 to 3000 cm^{-1} spectral range at 10% laser power for particles and the background, respectively.

Exposure Assessment. Although literature showed that the consumption of microplastic particles is capable of causing some toxic effects such as gastrointestinal, liver, and reproductive toxicity,[27−34] there was no adequate toxicological information to quantify the dose−response relationship between microplastic ingestion and the risk of adverse effects in humans or estimate the reference dose for microplastic particles in foods that can be compared against for risk characterization.[27] Herein, we used the relative comparison in exposure levels as a crude indicator or sentinel of the impact on public health risks.

Exposure scenarios adopted from EPA were applied to estimate the exposure of microplastics into infant/toddler foods from plastic food packaging materials.[35] The estimated daily intake (EDI; ng/kg·day) of microplastics and nanoplastics in aqueous (i.e., water) and acidic (i.e., dairy products, fruits, and vegetables) food was calculated using the following equation

$$\mathrm{EDI} = \frac{C \times \mathrm{IR}}{\mathrm{BW}} \quad (1)$$

where C is the average concentration of microplastics and nanoplastics in foods or beverages at the time of consumption (ng/g or ng/mL); IR is the per capita ingestion rate (g/day or mL/day); and BW denotes the average body weight (kg).

Infants between 6 and 12 months and toddlers between 12 and 24 months are the subpopulation groups targeted in this analysis due to their relevance to the tested plastic products. Hence, subpopulation-specific EDIs were calculated by integrating the estimates of IR and BW for infants and toddlers. Different food types were chosen following the practices of EPA by considering the foods which the tested simulants substitute for.[1] The mean ingestion of different food types and body weight for different age groups were adopted from EPA's guidelines on the exposure assessment, summarized in Table 1.[35]

Exposure assessment of microplastics was conducted by calculating EDIs in two different scenarios, i.e., at room temperature and microwave treatment, which represent the most common usage conditions of the tested products. In this analysis, there was no attempt to incorporate probabilistic distributions to consider variations in IR or BW, hence results were interpreted based on deterministic estimations.

Table 1. Estimates of Average Ingestion of Various Food Types and Mean Body Weight for the Calculation of EDI of Microplastics among Infants and Toddlers

	age group	
	infant (6−12 months)	toddler (12−24 months)
average ingestion rate (IR)		
water (mL/day)	360	271
dairy products (g/day)	91.9	488.2
fruits (g/day)	56.4	88.1
vegetables (g/day)	0.3	2.5
average body weight (BW, kg)	9.1	11.3

In Vitro Cell Viability Study. The collected effluent samples (release experiment with container 2 when in contact with DI water under microwave heating for 3 min) were freeze-dried (0.1 mbar; −50 °C) using a lyophilizer instrument (Labconco, USA) to extract the microplastics and nanoplastics. Plastic particles collected from freeze-drying were resuspended in complete media before the in vitro treatment.

HEK293T human embryonic kidney cell line was obtained from American Type Culture Collection (ATCC) and was cultured in high-glucose DMEM media (Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, California), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were cultivated in a humidified incubator at 37 °C with 5% CO_2 and harvested with 0.05% trypsin−EDTA (ethylene diamine tetraacetic acid) before subculture.

An MTT assay was conducted to evaluate in vitro viability after exposure to released plastic particles. In brief, HEK293T cells were seeded in 96-well plates at a density of 2500 cells per well for 24 h prior to treatment. Subsequently, cells were treated with different concentrations of microplastics and nanoplastics with a series of dilutions between (1−1000 μg/mL) in full medium. Following 48 h or 72 h of treatment, MTT (20 μL, 5 mg/mL) reagent was added for an additional 2 h incubation period at 37 °C. The medium was discarded, the formed formazan salt was dissolved in 200 μL of dimethyl sulfoxide (DMSO; Thermo Scientific Inc., Waltham, MA), and absorbance was measured at 510 nm wavelength in a Spectramax i3x spectrophotometer (Molecular Devices, Sunnyvale, CA). Cell survival rates were calculated as normalized to untreated control wells. Each concentration was tested in 4 wells and data presented as mean ± SEM. The mean microplastics and nanoplastics concentration required for 50% growth inhibition (IC_{50}) was determined with an AAT Bioquest IC_{50} calculator available online.

RESULTS AND DISCUSSION

Release of Microplastics and Nanoplastics. Figures 1 and 2 illustrate that a significant amount of micro- and nanoplastics were released into both aqueous and acidic food simulants from a single square centimeter area of each food container and the food pouch under various storage and usage conditions, including refrigeration, room temperature, high temperature, and microwave heating. Across all conditions, the number of nanoplastics released was generally 3 orders of magnitude higher than that of microplastics. The quantity of

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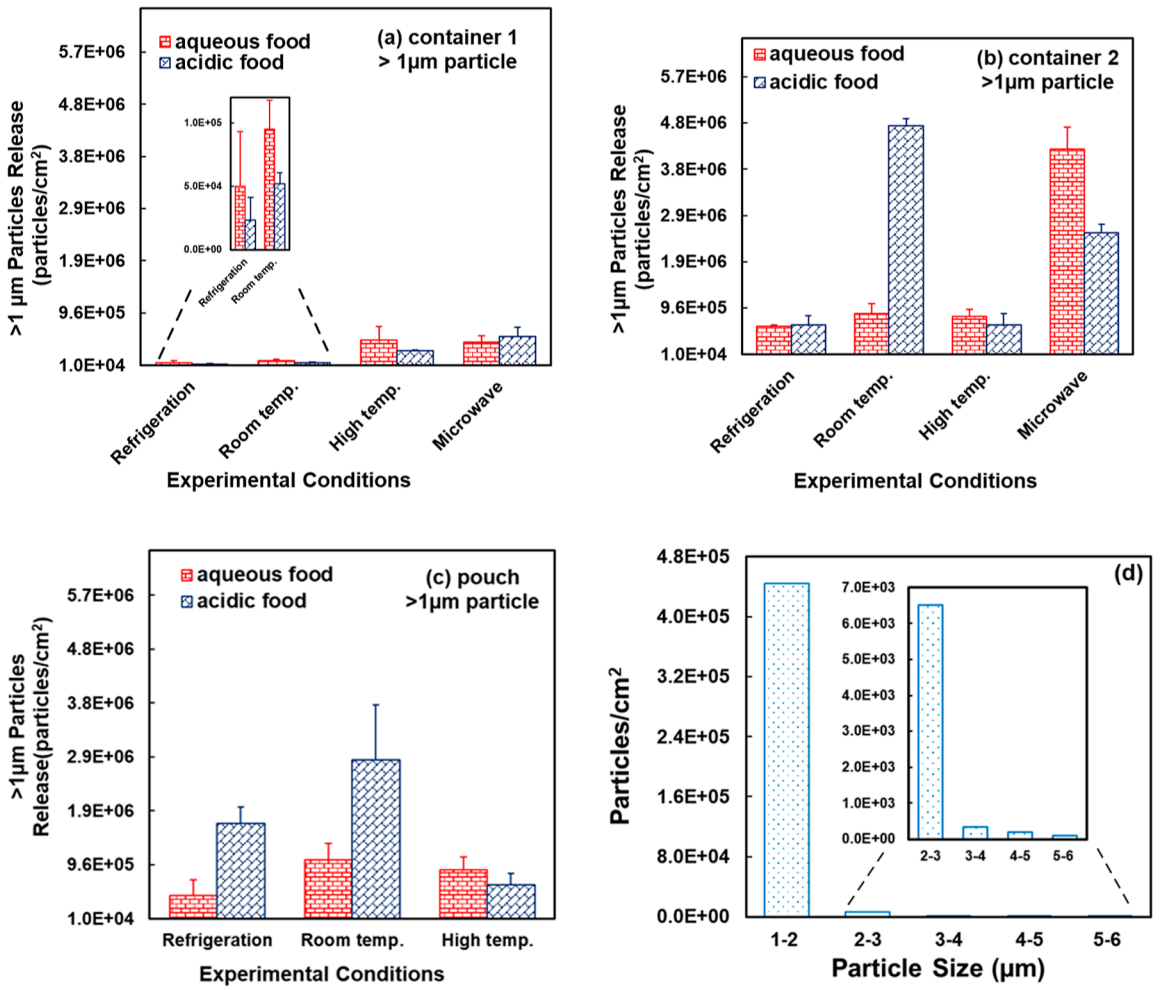

Figure 1. Microplastics released in contact with aqueous food (simulated by DI water) and acidic food (simulated by 3% acetic acid) under different usage scenarios such as refrigeration storage (replicated by experiment at 20 °C for 10 days), room-temperature storage (replicated by experiment at 40 °C for 10 days), high-temperature condition (replicated by experiment at 2 h at 70 °C followed by 20 °C for 10 days), and microwave heating for 3 min, (a) container 1; (b) container 2; and (c) reusable food pouch. (d) Representative size distribution of microplastics released from container 2 when in contact with DI water under microwave heating for 3 min.

microplastics released ranged from 23.2 thousand/cm^2 to 4.71 million/cm^2 and nanoplastics ranged from 11.5 million/cm^2 to 2.11 billion/cm^2, depending on the container and usage conditions.

Figure S1 in the Supporting Information depicts the amount of particles intercepted by a gold-coated membrane with 0.8 μm pore size, after filtering 300 mL of effluent produced from the microwave heating of container 2 for 3 min. The Raman spectroscopy analysis performed on the membrane surfaces confirmed the particles released from containers 1 and 2 as polypropylene. Raman spectral peaks ranging from 2830 to 3030 cm^{-1} are shown in Figure 3a,b, which indicate the presence of $CH/CH_2/CH_3$ groups, a representative Raman spectrum of polypropylene. Previous studies using Raman spectroscopy have shown similar results for reference polypropylene microplastics,[36] microplastic particles released from polypropylene feeding bottles,[12] a marine microplastics sample, and microplastics from tap water.[37]

Particle size distributions for micro- and nanoplastics released from all three products under all experimental scenarios are provided in the Supporting Information (Figures S2 and S3). Across all containers, microplastics generally fall within the size range of 1−14 μm. The group with the highest abundance is the microparticles between 1 and 2 μm, followed by particles between 2 and 3 μm. On the other hand, nanoparticles in the range of 10−100 nm are the most abundant group, followed by particles between 100 and 200 nm. To illustrate the size distributions of microplastics and nanoplastics released, we have included representative size distribution of particles released from microwave heating of container 2 when in contact with DI in Figures 1d and 2d.

Release of Particles into Aqueous Foods. When stored under refrigeration and in contact with aqueous food (simulated by DI water), container 1, container 2, and the pouch released 49.8 thousand, 577 thousand, and 415 thousand microplastics per centimeter square (Figure 1) and 11.5 million, 21.5 million, and 59.0 million nanoplastics/cm^2 (Figure 2), respectively. In contrast, under room-temperature storage, container 1 released 95 thousand microplastics/cm^2 and 47.9 million nanoplastics/cm^2, container 2 released 841 thousand microplastics/cm^2 and 34.9 million nanoplastics/cm^2, and the pouch released 1.05 million microplastics/cm^2

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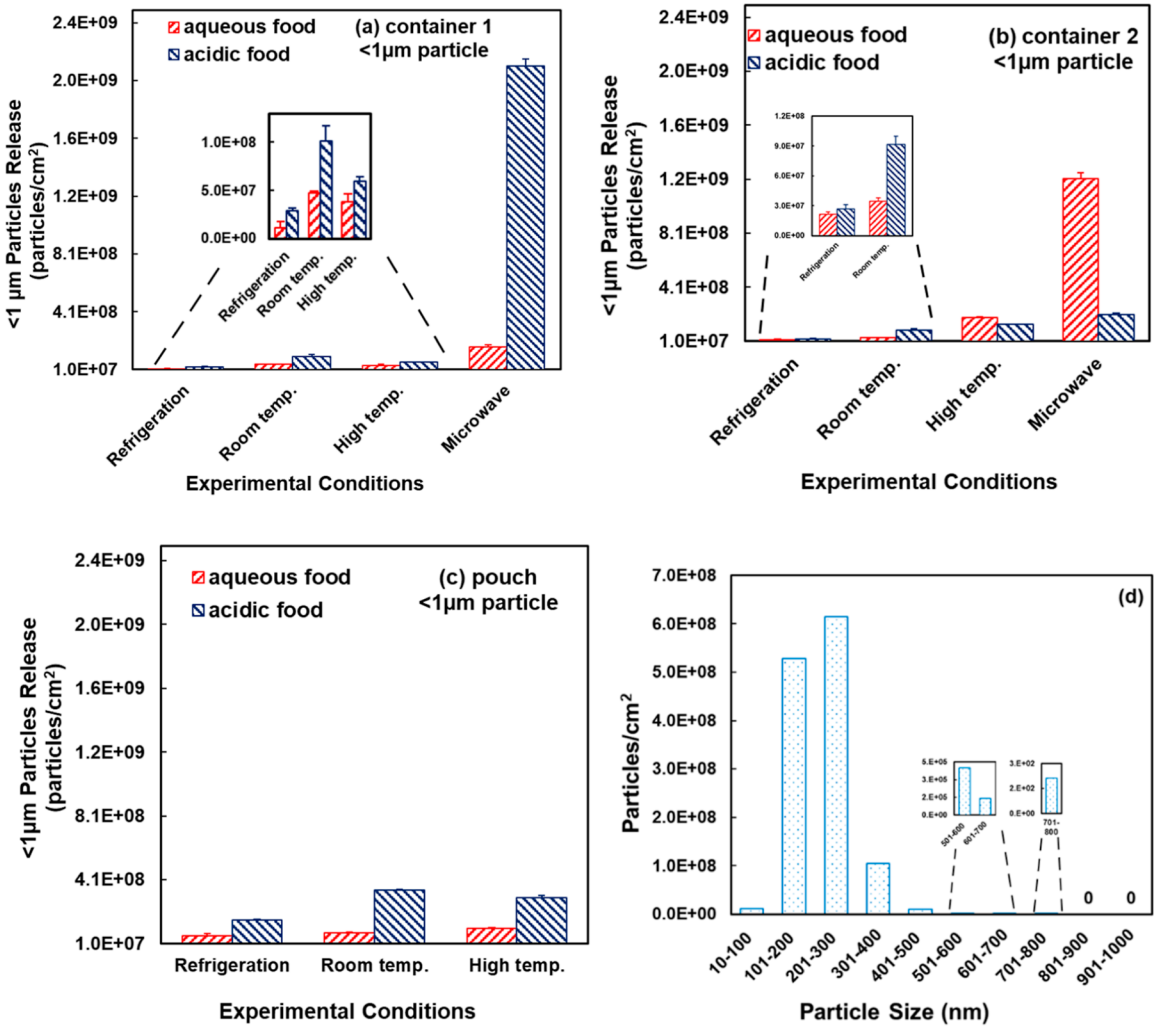

Figure 2. Nanoplastics released in contact with aqueous food (simulated by DI water) and acidic food (simulated by 3% acetic acid) under different usage scenarios such as refrigeration storage (replicated by experiment at 20 °C for 10 days), room-temperature storage (replicated by experiment at 40 °C for 10 days), high-temperature condition (replicated by experiment at 2 h at 70 °C followed by 20 °C for 10 days), and microwave heating for 3 min, (a) container 1; (b) container 2; and (c) reusable food pouch. (d) Representative size distribution of nanoplastics released from container 2 when in contact with DI water under microwave heating for 3 min.

and 78.6 million nanoplastics/cm^2 (Figures 1 and 2). The data showed that room-temperature storage caused a higher release of both microplastics and nanoplastics compared to refrigeration storage for all tested products, and high-temperature storage resulted in even more particles released. Container 1, container 2, and the pouch released 471 thousand, 783 thousand, and 873 thousand microplastics/cm^2 (Figure 1) and 38.6 million, 183 million, and 106 million nanoplastics/cm^2 (Figure 2), respectively, in the high-temperature storage condition. These findings are consistent with a previous study[12] that reported a 2 order magnitude increase in microplastics release from polypropylene infant feeding bottles into water when temperatures increased from 25 to 95 °C. Interestingly, our data showed that more particles were not necessarily released under high-temperature storage than under room-temperature conditions, indicating a nonlinear relationship between particle release and temperature.

Previous studies have linked the release of particles from plastic food containers to the degradation or breakdown of plastics.[12] This process is influenced by both the intrinsic properties of the plastics, such as material type, structure, copolymer, and size of initially released plastic particles, as well as external factors, such as pH, temperature, oxygen, and light.[38,39] Plastic breakdown generally occurs due to the formation of cracks under an applied load.[40] When in contact with water, hydrolysis—a chemical process where a water molecule is added to a substance—may cause polymer chain scission and lead to the fragmentation and release of plastic particles. An increase in temperature can accelerate hydrolysis and lead to a higher release of particles. Moreover, a higher temperature can cause plastic materials to lose strength[41] and expand unevenly, which further accelerates the breakdown process. These factors underscore the complexity of the issue and highlight the need for further research to better understand the mechanisms underlying plastic particle release.

Among all tested conditions, microwave heating—the most commonly used method for heating food in daily life—released the highest amount of plastic particles. Container 1 released 425 thousand microplastics/cm^2 and 169 million nanoplastics/cm^2, while container 2 released 4.22 million microplastics/cm^2 and 1.21 billion nanoplastics/cm^2 (Figures 1 and 2). The higher release of plastic particles during microwave heating is

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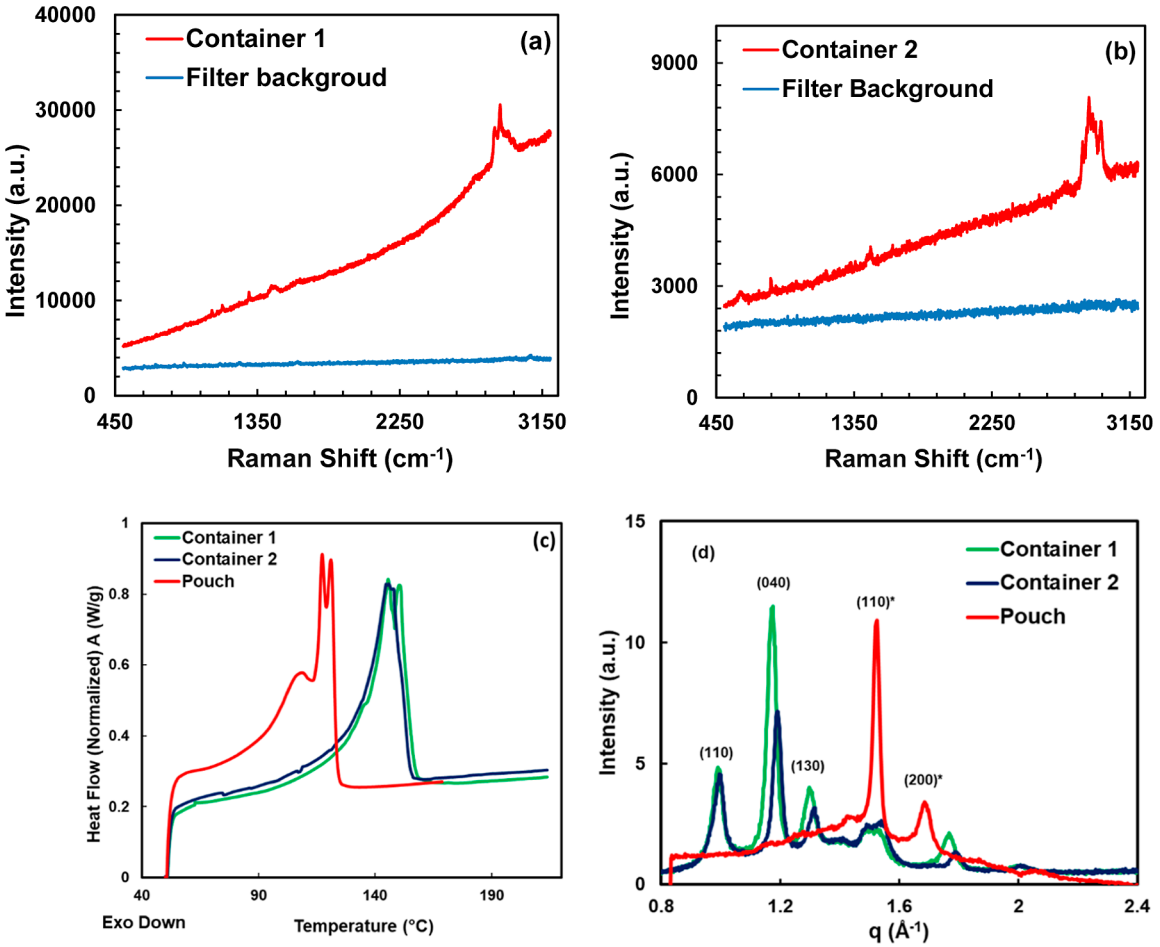

Figure 3. (a,b) Raman signal confirming the detected particles as polypropylene for (a) container 1 and (b) container 2. (c) Differential scanning calorimetry curves during first heating at 10 °C/min. The phase transition peaks are over the baseline, indicating that the samples absorbed heat during the phase transitions (i.e., during melting). Pouch exhibited lower thermal stability, and container 1 and container 2 showed similar thermal stability. (d) 1D WAXD patterns of container 1, container 2, and reusable pouch.

attributed to the simultaneous occurrence of hydrolysis, thermal degradation, and UV irradiated degradation.[42] The electromagnetic waves of the microwave can penetrate the plastic material and heat the inside of the container, while the high temperature of the food further increases the release of the micro- and nanoplastics from the plastic containers. Nevertheless, compounding the plastic materials with UV stabilizers can potentially reduce the release of plastic particles during microwave heating, according to a previous study.[43]

Impacts of Food Types on Particle Release. There were differences in the amounts of plastic particles released into aqueous foods (simulated by DI water) and acidic foods (simulated by 3% acetic acid). For container 1, more nanoplastics were released when in contact with acidic food than aqueous food in all conditions (Figure 2a). However, with the exception of microwave heating, more microplastics were released into aqueous food (Figure 1a). Container 2 released more microplastics and nanoplastics into acidic food under refrigeration storage and room-temperature conditions, whereas more particles were released in aqueous food under high-temperature conditions and during microwave heating. For the food pouch, a higher release of microplastics and nanoplastics into acidic food was observed in all three experimental conditions except for the microplastic count in acidic food, which was lower under the high-temperature condition.

A study conducted by Ariza-Tarazona et al.[40] revealed that the breakdown of high-density polyethylene (HDPE) microplastics occurred more rapidly under acidic conditions (pH 3) than neutral (pH 7) and basic conditions (pH 11). Similarly, other studies[44,45] have demonstrated that polyethylene terephthalate undergoes enhanced hydrolytic cleavage under acidic conditions compared to basic or neutral conditions. When in contact with acidic food, the release of microplastics and nanoplastics may be intensified due to acid's catalytic role in the hydrolytic breakdown of plastic. The high number of hydrogen ions in the acidic condition protonates the polymer chain, rendering it more reactive and susceptible to chain scission by hydroxide ions.

It is suspected that the breakdown of plastic materials occurs through a complex, multistep process. Primary microplastics and nanoplastics are generated when the plastic containers and the pouch initially break down. These primary particles can further break down into secondary micro- and nanoplastics. This multistep process helps to explain some unexpected findings. For instance, we observed a lower number of nanoparticles released from container 2 into acidic foods than aqueous foods when exposed to microwave heating. This can be attributed to the accelerated breakdown of primary and secondary nanoparticles under acidic conditions, which can result in particles too small to be detected.

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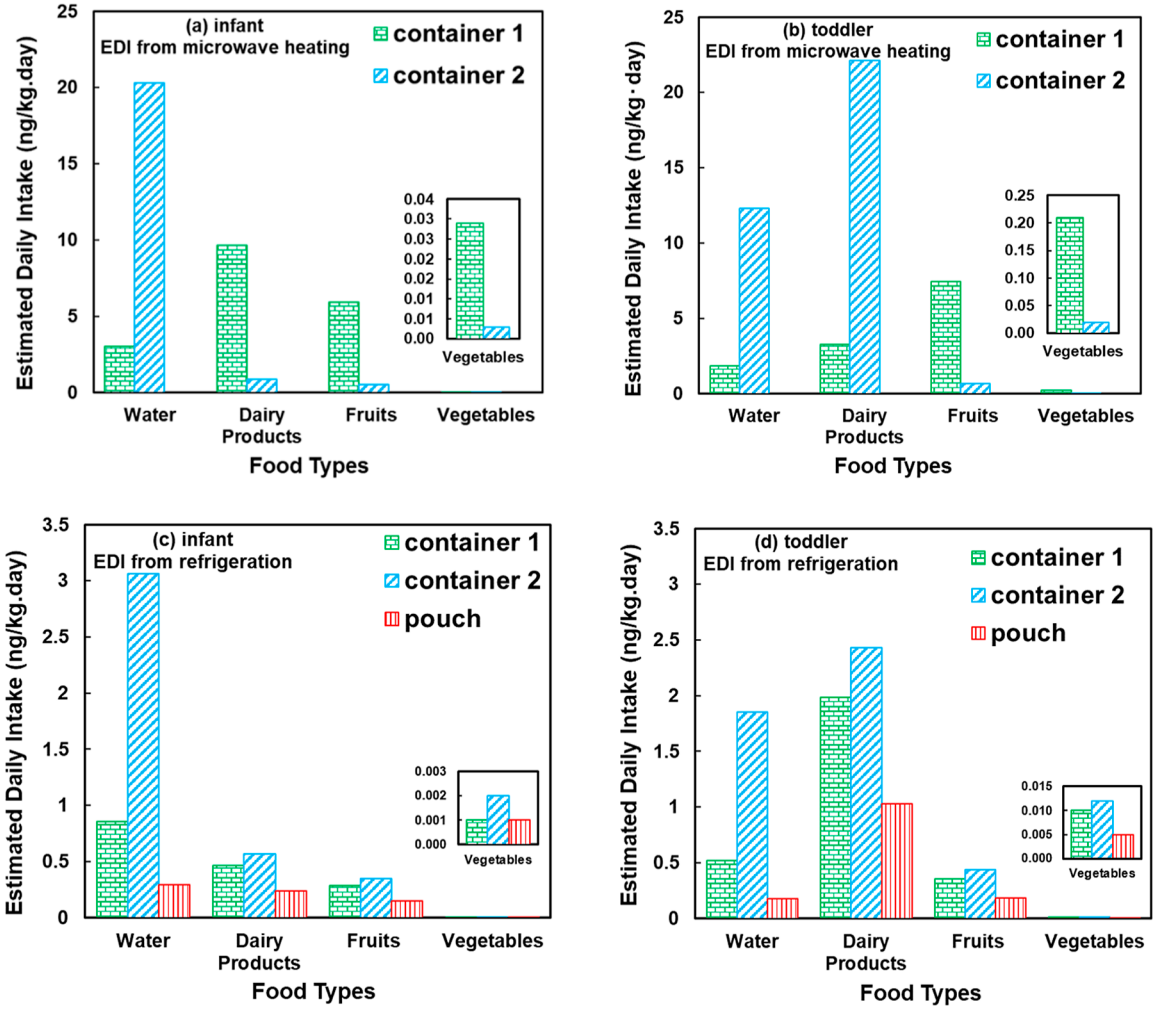

Figure 4. EDI (ng/kg·day) from the use of containers for microwave heating for (a) infant and (b) toddler. EDI (ng/kg·day) from the use of containers and the pouch under refrigeration storage for (c) infant and (d) toddler.

Impacts of Plastic Properties on Particle Release. The amount of plastic particles released from two containers and the food pouch varied. Container 1 and container 2 were made of polypropylene polymer, but calorimetric (Figure 3c) results indicated that they were not made of pure polypropylene homopolymer, which typically has a melting peak temperature of around 165 °C. Instead, they were likely made of polypropylene-based polymers with chain defects, such as copolymer units with different chemistry, introduced to lower melting temperatures and tailor processability and final properties. The release of micro- and nanoplastics from container 1 and container 2 differed under different tested conditions (Figure 1a,b and 2a,b), which may be due to the distinct chemical structures of the material used in each container. Therefore, it is critical to understand the exact characteristics of each product studied, as materials that may appear similar can have differences in chain structure that result in significantly different thermal and mechanical behavior.

The WAXD analysis confirmed that the material of the food pouch was polyethylene. Figure 3d shows the WAXD patterns obtained from the two types of plastic containers and the reusable pouch. Container 1 and container 2 exhibited strong peaks that corresponded to the α crystalline phase of isotactic polypropylene (reflections at $q \sim 0.99$, 1.17, and 1.30 Å^{-1} are indexed as (110), (040), and (130), respectively).[46] In contrast, diffraction from the pouch exhibited two main crystalline peaks at $q \sim 1.52$ and 1.68 Å^{-1}, which are consistent with the (110) and (200) reflections of the orthorhombic crystal phase of polyethylene, respectively.[47] In general, more plastic particles were released from the polyethylene-based food pouch than polypropylene-based containers. The exact reason for this difference is not certain.

The calorimetric measurements taken during the first heating ramp (Figure 3c) revealed a significant variation in thermal stability among the three plastic samples. The reusable food pouch exhibited much lower melting temperatures, with prominent melting peaks at around 107, 117, and 121 °C and significant melting occurring at even lower temperatures (between 50 and 100 °C, as indicated by the deviation of the calorimetric trace from the baseline). In contrast, container 1 and container 2 had peak melting temperatures that were approximately 30 °C higher than those of the reusable food pouch and very little melting below 80 °C (Figure 3c). The pouch, which had the lowest thermal stability, generally released more microplastics and nanoplastics compared to container 1 and container 2 in all three tested conditions.

Exposure Assessment. Figure 4 summarizes the results of EDIs for different package materials and food types calculated based on the measured concentration of micro- and nano-

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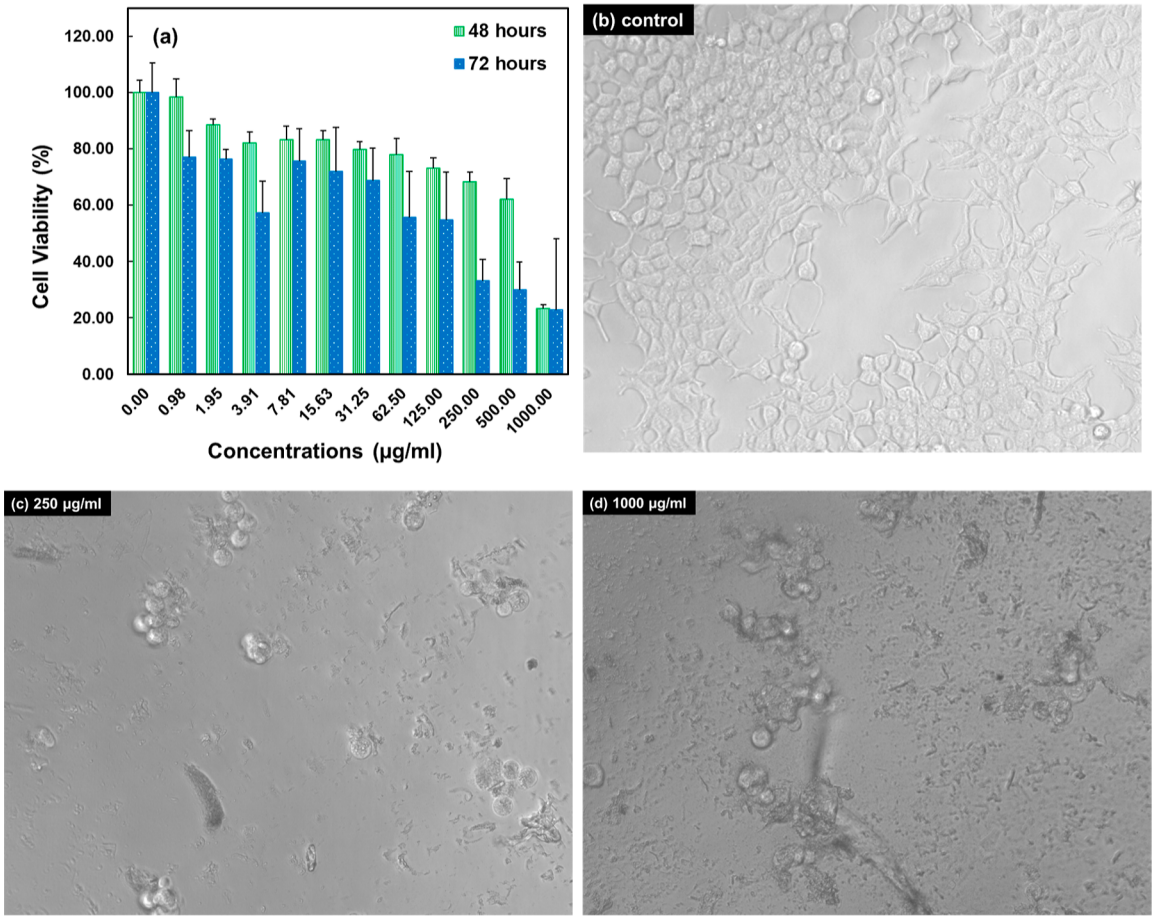

Figure 5. Cell viability and cell morphology of HEK293T cells: (a) viability of the cells treated with different concentrations of microplastics and nanoplastics for 48 and 72 h, respectively. Histograms represent the percentage, with respect to control cells (Ctrl, 100%), of viable cells after the exposure to microplastics and nanoplastics (0−1000 μg/mL). (Data show the mean ± SE (n = 3).) (b) Phase contrast image of the untreated cell. (c) Phase contrast image of the cell treated with 250 μg/mL microplastics and nanoplastics for 72 h. (d) Phase contrast image of the cell treated with 1000 μg/mL microplastics and nanoplastics for 72 h.

plastics in the release experiments (see Figures S4 and S5 in the Supporting Information). Overall, the findings suggest that microwave heating may result in higher EDIs of plastic particles compared to room temperature for both infants and toddlers.

For infants, the highest EDI was 20.3 ng/kg·day from drinking microwaved water stored in container 2, likely due to the higher release of plastic particles under microwave heating in contact with aqueous food and the relatively higher water intake compared to other food types. For toddlers, the highest EDI was 22.1 ng/kg·day from consuming microwaved dairy products stored in container 2, likely due to the high release of plastic particles under acidic conditions and the higher intake of dairy products by the toddler group.

The lowest EDIs were associated with vegetable intake for both the toddler and infant groups. For example, the lowest EDI for infants was 0.001 ng/kg·day for eating vegetables stored in container 1 or the food pouch under refrigeration storage. The lowest EDI for toddlers was 0.005 ng/kg·day from consuming vegetables stored in the food pouch under refrigeration storage. These lower EDIs are attributed to the relatively lower intake of vegetables for toddlers and infants compared to water and dairy products, as well as the relatively lower amount of plastic particles released under refrigeration conditions.

The present study found that, despite releasing the highest number of micro- and nanoplastics, the pouch had the lowest EDI values for all food types, compared to the other two containers. This is due to the low molecular weight of the polyethylene material used in the pouch, which resulted in low mass-based EDI values compared to the higher molecular weight polypropylene used in container 1 and container 2. However, it is important to note that infants and toddlers can still be exposed to microplastics and nanoplastics through the consumption of these products. At this time, it is unclear whether the mass or the number of particles is more directly linked to potential health risks associated with exposure to these materials. Further research is needed to better understand the potential health effects of microplastic and nanoplastic exposure in infants and toddlers.

In Vitro Cell Viability Study. Figure 5a illustrates the cell viability after 48 and 72 h of treatment with microplastics and nanoplastics. MTT assay results showed that cell viability was higher for the 48 h of treatment compared to the 72 h, except for the highest concentration (Figure 5d). At the highest concentration (i.e., 1000 μg/mL), about 23% cell viability was observed for both 48 and 72 h of treatment. Cell viability for both treatment periods gradually increased as the concentration of released plastic particles decreased. At the lowest concentration (i.e., 0.98 μg/mL), about 98 and 77% of the cell

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viability were observed after 48 and 72 h of treatment, respectively. The IC_{50} was calculated to be 3755.43 and 151.42 μg/mL, for 48 h and 72 h of treatment, respectively. The large difference in the IC_{50} between the two treatment periods refers to the impact of the contact time on the cytotoxicity of the microplastics and nanoplastics.

Figure 5b,c,d illustrates the cell morphology of culture in the control wells (Figure 5b) and the wells treated with microplastics and nanoplastics (Figure 5c,d). The cells in the control wells (untreated cells) grew nicely as a monolayer (Figure 5b). In contrast, the cells treated with microplastics and nanoplastics were dead primarily except for a few clusters of cells (Figure 5c,d).

Contradictory to our finding, a study[48] reported that the polypropylene microplastics, with 67.1 μm mean diameter, would not cause cytotoxicity for human intestinal cells, such as Caco-2, HepG2, and HepaRG, at a concentration as high as 50 mg/mL concentration and after 24 h of incubation. Another study[17] found an approximately 20% decrease in the viability of human dermal fibroblast (HDF) cells under the treatment of polypropylene microplastics with about 20 μm nominal size at a concentration of 1000 μg/mL for 48 h, but no toxicity for HDF cells was found if the diameters of polypropylene microplastics were in the range of 25−200 μm. The same study found that both smaller (i.e., ∼20 μm) and larger (i.e., 25−200 μm) microplastics caused a similar decrease of 20% cell viability for Murine macrophage (Raw 264.7) cells. Therefore, the cytotoxicity of microplastics depends on the cell type and the size of the microplastics used in the treatment. Sivagami et al.[49] reported the death of about 45% of human embryonic kidney cells (HEK293) under the treatment of the mixture of microplastics of different sizes and kinds extracted from the salt at 100 μg/mL concentration for 24 h.

However, the cytotoxicity observed in our study is higher than the cytotoxicity reported in the literature,[17,48] which could be due to two reasons. First, human embryonic kidney cells (HEK 293T) are probably more sensitive to the cytotoxicity of polypropylene than human intestinal cells, such as Caco-2, HepG2, and HepaRG or HDF cells. Second, the polypropylene particles used in these reported studies were larger (i.e., 20−200 μm) and either commercially purchased or artificially synthesized in the lab. On the contrary, the particles used in this study are polypropylene particles released during the microwave heating of polypropylene containers and highly polydisperse in size, comprising particles from 1 nm to 5 μm diameter (Figures 1d and 2d). Although the reason behind the cytotoxicity of polypropylene microplastics is not yet clearly known, it is robustly reported in the literature that the microparticle's and the nanoparticle's cytotoxicity depend on the particle's size.[25,50−53] The smaller particle generally results in more cytotoxicity compared to its larger counterpart.[17,50−52] The surface roughness and irregular shape were also reported to have more cytotoxicity due to its capacity to penetrate the cell by hurting the cell membrane.[23] The particles used in this study are irregular in shape, representing the particles that the human body would encounter from using plastics in food preparation or storage.

IMPLICATIONS

Our research has revealed that a significant quantity of micro- and nanoplastic particles are released from plastic baby food containers and reusable food pouches into the food, which has the potential to impact children's exposure to these particles. This release is influenced by various factors, including temperature (such as refrigeration, microwaving, and room temperature), plastic type (polyethylene or polypropylene), and food type (aqueous or acidic). Notably, microwaving food resulted in a higher release compared to other usage scenarios. Unfortunately, this exposure cannot be avoided for babies and toddlers. The highest EDI of these particles for infants occurred when they drank microwaved water stored in a plastic container, while for toddlers, it was when they consumed microwaved dairy products in a container. These findings emphasize the necessity of collaborating with manufacturers to establish guidelines for the appropriate usage of plastic containers. Additionally, it is crucial to work with caregivers in order to raise awareness about the potential impact of these particles.

Moreover, our laboratory study has provided evidence of the potential toxicity of these plastic particles on cells. It is important to note that our study is the first to use actual released microplastic and nanoplastic particles for in vitro toxicity testing, whereas previous studies utilized commercially available or laboratory-synthesized plastic particles. It is worth mentioning that the concentration used in our study was significantly higher than the concentration released. Nonetheless, it is crucial not to disregard the potential health risks associated with exposure to micro- and nanoplastics. Additionally, it is crucial to take into account that infants and toddlers regularly come into contact with multiple plastic products and consume a variety of foods prepared using plastics. The extent of plastic particle accumulation resulting from food ingestion, as well as the potential for exposure through inhalation and dermal absorption, are still unknown. This study underscores the urgent need for further research to investigate the health impacts of micro- and nanoplastic particles present in food.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c01942.

Gold-coated membrane filter before and after filtration, size distribution of microplastics and nanoplastics, and concentrations of microplastics and nanoplastics released from container 1, container 2, and reusable food pouch (PDF)

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Notes

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■ REFERENCES

(1) Gupta, J.; Hurley, F.; Grobicki, A.; Keating, T.; Stoett, P.; Baker, E.; Guhl, A.; Davies, J.; Ekins, P. Communicating the health of the planet and its links to human health. *Lancet Planet. Health* **2019**, *3*, e204−e206.
(2) Wright, S. L.; Kelly, F. J. Plastic and human health: a micro issue? *Environ. Sci. Technol.* **2017**, *51*, 6634−6647.
(3) Karami, A.; Golieskardi, A.; Keong Choo, C.; Larat, V.; Galloway, T. S.; Salamatinia, B. The presence of microplastics in commercial salts from different countries. *Sci. Rep.* **2017**, *7*, 46173.
(4) Neves, D.; Sobral, P.; Ferreira, J. L.; Pereira, T. Ingestion of microplastics by commercial fish off the Portuguese coast. *Mar. Pollut. Bull.* **2015**, *101*, 119−126.
(5) Van Cauwenberghe, L.; Janssen, C. R. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* **2014**, *193*, 65−70.
(6) Pivokonsky, M.; Cermakova, L.; Novotna, K.; Peer, P.; Cajthaml, T.; Janda, V. Occurrence of microplastics in raw and treated drinking water. *Sci. Total Environ.* **2018**, *643*, 1644−1651.
(7) Mason, S. A.; Welch, V. G.; Neratko, J. Synthetic polymer contamination in bottled water. *Front. Chem.* **2018**, *6*, 407.
(8) Hernandez, L. M.; Xu, E. G.; Larsson, H. C. E.; Tahara, R.; Maisuria, V. B.; Tufenkji, N. Plastic teabags release billions of microparticles and nanoparticles into tea. *Environ. Sci. Technol.* **2019**, *53*, 12300−12310.
(9) Du, F.; Cai, H.; Zhang, Q.; Chen, Q.; Shi, H. Microplastics in take-out food containers. *J. Hazard. Mater.* **2020**, *399*, 122969.
(10) Cox, K. D.; Covernton, G. A.; Davies, H. L.; Dower, J. F.; Juanes, F.; Dudas, S. E. Human consumption of microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068−7074.
(11) Wu, M.; Tu, C.; Liu, G.; Zhong, H. Time to safeguard the future generations from the omnipresent microplastics. *Bull. Environ. Contam. Toxicol.* **2021**, *107*, 793−799.
(12) Li, D.; Shi, Y.; Yang, L.; Xiao, L.; Kehoe, D. K.; Gun'ko, Y. K.; Boland, J. J.; Wang, J. J. Microplastic release from the degradation of polypropylene feeding bottles during infant formula preparation. *Nat. Food* **2020**, *1*, 746−754.
(13) Su, Y.; Hu, X.; Tang, H.; Lu, K.; Li, H.; Liu, S.; Xing, B.; Ji, R. Steam disinfection releases micro (nano) plastics from silicone-rubber baby teats as examined by optical photothermal infrared microspectroscopy. *Nat. Nanotechnol.* **2022**, *17*, 76−85.
(14) Braun, T.; Ehrlich, L.; Henrich, W.; Koeppel, S.; Lomako, I.; Schwabl, P.; Liebmann, B. Detection of microplastic in human placenta and meconium in a clinical setting. *Pharmaceutics* **2021**, *13*, 921.
(15) Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rongioletti, M. C. A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274.
(16) Dong, C.-D.; Chen, C.-W.; Chen, Y.-C.; Chen, H.-H.; Lee, J.-S.; Lin, C.-H. Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. *J. Hazard. Mater.* **2020**, *385*, 121575.
(17) Hwang, J.; Choi, D.; Han, S.; Choi, J.; Hong, J. An assessment of the toxicity of polypropylene microplastics in human derived cells. *Sci. Total Environ.* **2019**, *684*, 657−669.
(18) Poma, A.; Vecchiotti, G.; Colafarina, S.; Zarivi, O.; Aloisi, M.; Arrizza, L.; Chichiriccò, G.; Di Carlo, P. In vitro genotoxicity of polystyrene nanoparticles on the human fibroblast Hs27 cell line. *Nanomaterials* **2019**, *9*, 1299.
(19) Prietl, B.; Meindl, C.; Roblegg, E.; Pieber, T. R.; Lanzer, G.; Fröhlich, E. Nano-sized and micro-sized polystyrene particles affect phagocyte function. *Cell Biol. Toxicol.* **2014**, *30*, 1−16.
(20) Wu, B.; Wu, X.; Liu, S.; Wang, Z.; Chen, L. Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. *Chemosphere* **2019**, *221*, 333−341.
(21) Goodman, K. E.; Hua, T.; Sang, Q.-X. A. Effects of Polystyrene Microplastics on Human Kidney and Liver Cell Morphology, Cellular Proliferation, and Metabolism. *ACS Omega* **2022**, *7*, 34136−34153.
(22) Danopoulos, E.; Twiddy, M.; West, R.; Rotchell, J. M. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. *J. Hazard. Mater.* **2022**, *427*, 127861.
(23) Choi, D.; Hwang, J.; Bang, J.; Han, S.; Kim, T.; Oh, Y.; Hwang, Y.; Choi, J.; Hong, J. In vitro toxicity from a physical perspective of polyethylene microplastics based on statistical curvature change analysis. *Sci. Total Environ.* **2021**, *752*, 142242.
(24) Zhang, Y.; Wang, S.; Olga, V.; Xue, Y.; Lv, S.; Diao, X.; Zhang, Y.; Han, Q.; Zhou, H. The potential effects of microplastic pollution on human digestive tract cells. *Chemosphere* **2022**, *291*, 132714.
(25) Shi, Q.; Tang, J.; Liu, R.; Wang, L. Toxicity in vitro reveals potential impacts of microplastics and nanoplastics on human health: A review. *Crit. Rev. Environ. Sci. Technol.* **2022**, *52*, 3863−3895.
(26) Food and Drug Administration. *Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations*, 2007.
(27) Shopova, S.; Sieg, H.; Braeuning, A.; German Federal Institute for Risk Assessment (BfR), Department of Food Safety, Unit Effect-based Analytics and Toxicogenomics Unit and Nanotoxicology Junior

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(28) Jin, Y.; Xia, J.; Pan, Z.; Yang, J.; Wang, W.; Fu, Z. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. *Environ. Pollut.* **2018**, *235*, 322−329.
(29) Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M. E. J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; et al. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, 2430−2435.
(30) Lei, L.; Wu, S.; Lu, S.; Liu, M.; Song, Y.; Fu, Z.; Shi, H.; Raley-Susman, K. M.; He, D. Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Sci. Total Environ.* **2018**, *619−620*, 1−8.
(31) Lian, J.; Wu, J.; Xiong, H.; Zeb, A.; Yang, T.; Su, X.; Su, L.; Liu, W. Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (Triticum aestivum L.). *J. Hazard. Mater.* **2020**, *385*, 121620.
(32) Magni, S.; Gagné, F.; André, C.; Della Torre, C.; Auclair, J.; Hanana, H.; Parenti, C. C.; Bonasoro, F.; Binelli, A. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel Dreissena polymorpha (Mollusca: Bivalvia). *Sci. Total Environ.* **2018**, *631−632*, 778−788.
(33) Qu, M.; Xu, K.; Li, Y.; Wong, G.; Wang, D. Using acs-22 mutant Caenorhabditis elegans to detect the toxicity of nano-polystyrene particles. *Sci. Total Environ.* **2018**, *643*, 119−126.
(34) Yu, P.; Liu, Z.; Wu, D.; Chen, M.; Lv, W.; Zhao, Y. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. *Aquat. Toxicol.* **2018**, *200*, 28−36.
(35) EPA. *U. S. Exposure Factors Handbook 2011 Edition (Final Report)*; U.S. Environmental Protection Agency: Washington, DC, 2011; pp 1−1466.
(36) Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377−8391.
(37) Kniggendorf, A.-K.; Wetzel, C.; Roth, B. Microplastics detection in streaming tap water with Raman spectroscopy. *Sensors* **2019**, *19*, 1839.
(38) Liu, L.; Xu, M.; Ye, Y.; Zhang, B. On the degradation of (micro) plastics: Degradation methods, influencing factors, environmental impacts. *Sci. Total Environ.* **2022**, *806*, 151312.
(39) Lin, Z.; Jin, T.; Zou, T.; Xu, L.; Xi, B.; Xu, D.; He, J.; Xiong, L.; Tang, C.; Peng, J.; et al. Current progress on plastic/microplastic degradation: Fact influences and mechanism. *Environ. Pollut.* **2022**, *304*, 119159.
(40) Ariza-Tarazona, M. C.; Villarreal-Chiu, J. F.; Hernández-López, J. M.; Rivera De la Rosa, J.; Barbieri, V.; Siligardi, C.; Cedillo-González, E. I. Microplastic pollution reduction by a carbon and nitrogen-doped TiO2: Effect of pH and temperature in the photocatalytic degradation process. *J. Hazard. Mater.* **2020**, *395*, 122632.
(41) Zhang, K.; Hamidian, A. H.; Tubić, A.; Zhang, Y.; Fang, J. K. H.; Wu, C.; Lam, P. K. S. Understanding plastic degradation and microplastic formation in the environment: A review. *Environ. Pollut.* **2021**, *274*, 116554.
(42) Putra, P. H. M.; Rozali, S.; Patah, M. F. A.; Idris, A. A review of microwave pyrolysis as a sustainable plastic waste management technique. *J. Environ. Manage.* **2022**, *303*, 114240.
(43) Andrady, A. L.; Koongolla, B. Degradation and Fragmentation of Microplastics. *Plastics and the Ocean: Origin, Characterization, Fate, and Impacts*; John Wiley & Sons, 2022; pp 227−268.
(44) Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J. H.; Abu-Omar, M.; Scott, S. L.; Suh, S. Degradation rates of plastics in the environment. *ACS Sustainable Chem. Eng.* **2020**, *8*, 3494−3511.
(45) Rosmaninho, M. G.; Jardim, E.; Moura, F. C. C.; Ferreira, G. L.; Thom, V.; Yoshida, M. I.; Araujo, M. H.; Lago, R. M. Surface hydrolysis of postconsumer polyethylene terephthalate to produce adsorbents for cationic contaminants. *J. Appl. Polym. Sci.* **2006**, *102*, 5284−5291.
(46) Nakamura, K.; Shimizu, S.; Umemoto, S.; Thierry, A.; Lotz, B.; Okui, N. Temperature dependence of crystal growth rate for α and β forms of isotactic polypropylene. *Polym. J.* **2008**, *40*, 915−922.
(47) Lafrance, C. P.; Pezolet, M.; Prud'Homme, R. E. Study of the distribution of molecular orientation in highly oriented polyethylene by x-ray diffraction. *Macromolecules* **1991**, *24*, 4948−4956.
(48) Stock, V.; Laurisch, C.; Franke, J.; Dönmez, M. H.; Voss, L.; Böhmert, L.; Braeuning, A.; Sieg, H. Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. *Toxicol. in Vitro* **2021**, *70*, 105021.
(49) Sivagami, M.; Selvambigai, M.; Devan, U.; Velangani, A. A. J.; Karmegam, N.; Biruntha, M.; Arun, A.; Kim, W.; Govarthanan, M.; Kumar, P. Extraction of microplastics from commonly used sea salts in India and their toxicological evaluation. *Chemosphere* **2021**, *263*, 128181.
(50) Johnston, H. J.; Semmler-Behnke, M.; Brown, D. M.; Kreyling, W.; Tran, L.; Stone, V. Evaluating the uptake and intracellular fate of polystyrene nanoparticles by primary and hepatocyte cell lines in vitro. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 66−78.
(51) Wang, Q.; Bai, J.; Ning, B.; Fan, L.; Sun, T.; Fang, Y.; Wu, J.; Li, S.; Duan, C.; Zhang, Y.; et al. Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human Caco-2 cells. *Chemosphere* **2020**, *254*, 126788.
(52) Forte, M.; Iachetta, G.; Tussellino, M.; Carotenuto, R.; Prisco, M.; De Falco, M.; Laforgia, V.; Valiante, S. Polystyrene nanoparticles internalization in human gastric adenocarcinoma cells. *Toxicol. in Vitro* **2016**, *31*, 126−136.
(53) Varela, J. A.; Bexiga, M. G.; Åberg, C.; Simpson, J. C.; Dawson, K. A. Quantifying size-dependent interactions between fluorescently labeled polystyrene nanoparticles and mammalian cells. *J. Nanobiotechnol.* **2012**, *10*, 39−46.

https://doi.org/10.1021/acs.est.3c01942
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