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Review



Unveiling the hidden chronic health risks of nano- and microplastics in single-use plastic water bottles: A review

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HIGHLIGHTS

- Nano and microplastics in bottled water pose risks to human health and ecosystems.
- NMPs can infiltrate the body, raising concerns over long-term health effects.
- Exposure to NMPs may cause respiratory, reproductive, neurological, and cancer risks.
- Lack of standardized testing hinders accurate assessment of health impacts.
- Focused regulations to reduce single-use water bottles are essential.

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ABSTRACT

Single-use plastic products, such as water bottles, have become ubiquitous in modern society, contributing significantly to the growing problem of plastic waste in landfills, rivers, oceans, and natural habitats. This poses severe threats to biodiversity and ecosystem stability. The emergence of microplastics (1 µm to 5 mm) and nanoplastics (less than 1 µm) has raised alarms about their harmful effects on human health. Nanoplastics are especially hazardous due to their smaller size and enhanced ability to infiltrate the human body. There are critical gaps in the literature regarding the contamination of nano- and microplastics from single-use plastic water bottles, emphasizing the urgent need for further research. Here we review, we examine the global impact of nano- and microplastics from single-use plastic water bottles on human health, drawing insights from over 141 scientific articles. Key findings include the annual ingestion of 39,000-52,000 microplastic particles by individuals, with bottled water consumers ingesting up to 90,000 more particles than tap water consumers. The literature reveals variations in the number of nano- and microplastics particles, their sizes, and a lack of information on their physical properties. Moreover, the review highlights the chronic health issues linked to exposure to nano- and microplastics, including respiratory diseases, reproductive issues, neurotoxicity, and carcinogenicity. We highlight the challenges of standardized testing methods and the need for comprehensive regulations targeting nano- and microplastics in water bottles. This review article underscores the pressing need for expanding research, increasing public awareness, and implementing robust regulatory measures to address the adverse effects of nano- and microplastics from single-use plastic water bottles.

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1. Introduction

The widespread presence of single-use plastic products, such as water bottles, has become a pressing global issue in modern society in the need for alternative solutions, as emphasized by the United Nations Environment Program [1]. The production, consumption, and disposal of these bottles contribute significantly to the accumulation of plastic wastes in landfills, in water bodies such as rivers and oceans, and in natural habitats, posing severe threats to biodiversity and ecosystem stability [2–4].

The emergence of microplastics, ranging from 1 μm to 5 mm in size, and the potential existence of even smaller nanoplastics less than 1 μm have raised widespread concerns regarding their detrimental effects on human health. Notably, nanoplastics are considered more hazardous than microplastics due to their smaller size, which enhances their ability to infiltrate the human body [5]. Mason et al. [6] analyzed the annual

consumption rates of microplastics and reported that bottled water consumers faced a greater burden in terms of plastic consumption than tap water consumers. Moreover, Gigault et al. [7] and Mintenig [8], underscored the critical gaps in the literature regarding nano- and microplastics contamination from single-use plastic water bottles, emphasizing the need for further investigation [7,8].

The growing scientific interest in this topic is evident from the substantial increase in the number of articles related to nano- and microplastics and human health rose from seven in 2016–399 in 2023, reaching 133 in the first quarter of 2024. Similarly, the number of articles related to nano- and microplastics, and water bottles increased from two in 2016–86 in 2023, with over 24 articles published in the first quarter of 2024 [9]. Nanoplastics are likely more hazardous than microplastics because they are more abundant, reactive, and capable of penetrating living cells [10]. Nano- and microplastics from single-use plastic water bottles have profound implications for human health.

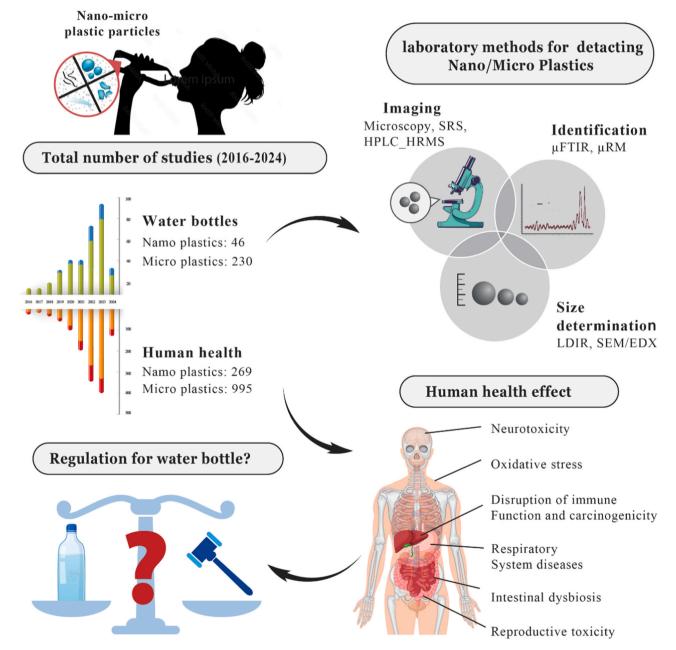


Fig. 1. Visual abstract summarizing the current state of research on nano- and microplastics in single-use plastic water bottles. This includes an extensive review of literature on the relationship between nano and micro plastics present in water bottles and their chronic human health impacts, existing methods for detecting nano- and microplastics, and the current regulatory landscape for single-use plastic water bottles.

Studies have linked nano- and microplastics exposure to various chronic health issues in humans, including respiratory diseases, reproductive issues, neurotoxicity, and carcinogenicity [11].

This review provides a comprehensive synthesis of current research on nanoplastic contamination specifically related to single-use plastic water bottles, offering a novel approach by integrating experimental findings with emerging analytical techniques. While prior studies often isolate aspects like source identification or particle characterization, this review introduces an interdisciplinary framework linking nanoplastic behavior, degradation, and chronic health risks. It critically evaluates methodological limitations such as inconsistent detection thresholds, inadequate blank controls, and variability across sampling protocols highlighting the challenges in reproducibility and reliability. By identifying these gaps and proposing future research directions, this review advances a more unified understanding of nanoplastics and their implications, laying the groundwork for more effective and evidence-based mitigation strategies (Fig. 1).

2. Multifaceted review of nano- and microplastics in single-use water bottles: studies, stressors, and detection

2.1. Overview of number of studies, sample sizes, and brand analysis

A major challenge in the field of nano- and microplastics is the limited number of comprehensive studies. Among the latest available data from January 2024, sixteen relevant studies were identified; these studies were conducted between 2018 and January 2024 which specifically addressed the presence of nano- and microplastics in single-use plastic water bottles [5,6,12-25]. These existing studies have undoubtedly provided valuable insights into the presence and types of nano- and microplastics found in single-use plastic water bottles. However, the

number of samples tested in these studies was somewhat limited, ranging from a minimum of eight to a maximum of 280 samples [19,23]. With such sample sizes, it is difficult to draw definitive conclusions that people can confidently rely on for regulatory decision-making. A previous study that included samples from 16 countries namely Chile, Australia, Iran, Malaysia, Thailand, Indonesia, the United States of America, India, Mexico, the United Kingdom, France, Germany, Brazil, Lebanon, Italy, and China undoubtedly contributed valuable data to the current research [6]. Additionally, the study by Mason et al. [6] stands out as one of the few studies in which researchers explored the correlation between water bottle brands and the water microplastics content (Fig. 2) [6]. Their study highlights the significant impact that different brands can have on microplastics contamination levels. For example, Nestle Pure Life (Amazon.com) and Bisleri (India) presented the highest average microplastics particle concentrations, ranging from 826 and 2277 microplastics particles per liter, respectively. Incorporating brand-specific data into future studies on nano- and microplastics contamination will significantly increase our understanding of this issue and facilitate the development of targeted solutions to address this problem effectively [6].

2.2. Varieties of water samples and impacts of stressors

Various factors significantly influence the prevalence of nano- and microplastics in water bottles. Notably, some studies have underscored the critical impact of water type i.e., drinking water, natural minerals, purified, distilled, ocean-purified, and spring and its source on the presence of microplastics in water bottles [15,18,19,26]. In addition to manufacturing processes and water sources, environmental stress can also affect the release of nano- and microplastics into water. Table 1 presents studies that have considered the effects of physical or

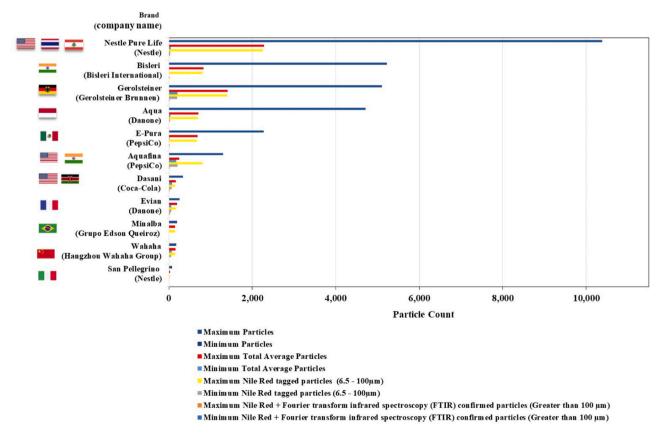


Fig. 2. Average microplastic particle concentrations (particles per liter) across various water bottle brands, broken down by size fractions and total, and averaged within each lot by country and brand. The study by Mason et al. [6] highlights significant differences in microplastics content, with Nestle Pure Life (Amazon.com) and Bisleri (India) showing the highest concentrations, ranging from 826 to 2277 particles per liter.

Table 1
Summary of previous studies on nano- and microplastics in single-use plastic water bottles, including the number of brands examined, sample sizes, and geographical coverage. The table also highlights studies that consider the effects of physical or environmental stressors on the release of nano- and microplastics, with a focus on potential sources such as polyethylene terephthalate (PET) bottlenecks and high-density polyethylene (HDPE) caps.

| Reference | Types of water | Types of bottles | Physical stress/ Types of environmental stress factors | Country | No of Brands/ Samples |
|-----------|---|---|---|---|-----------------------------|
| [22] | Drinking water | Single-use bottles made of polyethylene terephthalate | | Chile | 12/36 |
| [20] | Drinking water | Single-use and reusable water bottles made of polyethylene terephthalate, glass, and metal | | Australia | 16/48 |
| [21] | Drinking water | Single-use bottles made of polyethylene terephthalate | Mechanical stress from squeezing/ Freezing, sunlight exposure | Iran | Not available /23 |
| [19] | Mineral, drinking water | Single-use bottles made of polyethylene terephthalate | | Malaysian | 8/8 |
| [17] | Drinking water | Single-use bottles made of polyethylene terephthalate | Opening and closing of the bottle cap | USA | 1/48 |
| [18] | Nature mineral, purified, distilled, ocean purified, spring, drinking water | Single-use bottles made of polyethylene terephthalate | | China | 23/69 |
| [13] | Mineral water | Single-use bottles made of polyethylene terephthalate | Opening and closing of the bottle cap, squeezing water of bottle, and filling | Iran | 11/11 |
| [16] | Drinking water | Single-use bottles made of polyethylene terephthalate and glass | · · | Thailand | 10/95 |
| [15] | Mineral water sparkling and still | Single-use bottles made of polyethylene terephthalate | | Italy | 10/30 |
| [26] | Sparkling, natural drinking | Single-use bottles made of polyethylene terephthalate with screw caps made of high-density polyethylene | Opening and closing of the bottle cap, squeezing of the water bottle | Italy | 3/18 |
| [14] | Still Mineral, medium sparkling, sparkling | Single-use and reusable water bottles made of polyethylene terephthalate, beverage cartons, and glass | | Germany | Not available /30 |
| [12] | Mineral water | Single-use bottles made of polyethylene terephthalate, reusable polyethylene terephthalate, and glass | | Germany | Not available /32 |
| [6] | Natural drinking | 1.5 L single-use bottles made of polyethylene terephthalate | | Indonesia, USA, India, Mexico, United Kingdom, France, Germany, Brazil, Lebanon, Italy, China | 11/259 |
| [5] | Unknown | 1.0 L single-use bottles made of polyethylene terephthalate | | USA | 3/15 |
| [23] | Unknown | Single-use bottles made of polyethylene terephthalate | | Spain | 20/280 |
| [24] | Drinking water | Single-use bottles made of polyethylene terephthalate | | Norway | 4/Not available |

environmental stressors on nano- and microplastics release in single-use plastic water bottles. Particular attention is given to potential sources of nano- and microplastics, such as polyethylene terephthalate (commonly referred to as PET) bottlenecks and high-density polyethylene caps (commonly referred to as HDPE), which have been found to be significant microplastics contributors, especially when bottles are subjected to extended mechanical stress during opening and closing procedures [13, 17,21,27]. During everyday use, water bottles are often subjected to repeated opening and closing, and even the simple act of squeezing a bottle to drink can cause abrasion and friction between the bottle material and water. These actions may result in the shedding of nano- and microplastics particles into the water, contributing to the overall contamination level. Furthermore, environmental stress can also play a significant role in the release of nano- and microplastics from water bottles. Prolonged exposure to sunlight can exacerbate the degradation of plastic materials, leading to the release of nano and microplastic particles. In a study, Taheri et al. [21], demonstrated the substantial impact of sunlight exposure on the release of microplastics. Additional studies are necessary to further confirm the impact of various environmental stresses on the release of nano- and microplastics from single-use water bottles.

2.3. Importance of particle size in nano and microplastic research

The reported levels of nano- and microplastics levels in water bottles vary widely across previous studies. The microplastic concentrations in water can range from as low as 2 microplastics/liter to as high as 6626.7 microplastics/liter, with an even wider range of $(2.4\pm1.3)\times10^5$ for nanoplastics/liter [5,18,21]. The variability in these results can be attributed to the different analytical methods, sampling methodologies, and types of water bottles used by different researchers to quantify nano- and microplastics in water samples. Given the wide range of results, standardizing the methods across studies becomes crucial to ensure the comparability and reliability of the findings. Some studies have also considered the differences between single-use and reusable plastic products, which can lead to varying overall nano and microplastic counts [12,14].

The findings presented in Table 2 provide an overview of the size and number of nano- and microplastics particles found in single-use plastic water bottles. Notably, results from various studies have revealed a wide range of nano- and microplastics particle sizes, varying from 0.5 μm [15] to greater than 100 nm [5]. Despite this wide range, in most of these articles, the authors have emphasized the importance of focusing on particles smaller than 1.5 μm since small particles, as they have a greater risk of translocating into body tissues and causing harm [12,16,19,28]. Studies have revealed that microplastic particles less than 1.5 μm in size

Table 2 Overview of nano and microplastic particle sizes and analytical methods used in various studies on single-use plastic water bottles. The table highlights the range of particle sizes $(0.5 \, \mu m)$ to greater than $100 \, nm$ and the focus on particles smaller than $1.5 \, \mu m$ due to their higher potential for absorption into body tissues and associated health risks.

| Reference | Size of nano and microplastic particles | Total pieces of nano- and microplastics/liter | Nano and microplastic mass (µgrams/liter) | Analytical method |
|-----------|---|--|--|---|
| [22] | 5 and 20 μm | Avg 391 \pm 125,highest 633 \pm 33 | | Fluorescence microscopy with Nile Red |
| [20] | $77\pm22~\mu m$ | 13 ± 19 (standard deviation) | | Laser direct infrared imaging (LDIR) |
| [21] | Greater than 1 μm | Mean 1496.7 ± 1452.2max 6626.7 | | Scanning electron microscopy (SEM) |
| [19] | 100–300 μm | 11.7 ± 4.6 | | Fourier transform infrared spectroscopy (FTIR) |
| [17] | Greater than 4.7 μm | 553 ± 202 (standard error) | | Nile Red analysis with a trinocular optical microscope |
| [18] | 100–300 μm | 2 ± 23 | | Scanning electron microscopy (SEM) and Micro-Fourier transform infrared spectroscopy (μ-FTIR) |
| [13] | 1280-4200 μm | 8.5 ± 10.2 | | Fourier transform infrared spectroscopy (FTIR) and Raman stereoscopy |
| [16] | Average count for 6.5–20 μm, 20–50 μm, and | 140 ± 19 | | 1. Fluorescent tagging with Nile Red (greater than or equal 6.5 µm) 2. Optical microscopy (greater than or equal 50 µm) 3. Attenuated total |
| | greater than or equal 50 μm | | | reflectance - Fourier transform infrared spectroscopy (ATR-FTIR) (greater than or equal 50 μm) 4. Confocal Raman spectroscopy (1–50 μm) |
| [15] | 0.5–10 μm | $5.42E+07$ (standard deviation = \pm 1.95E + 07) | Total 656.8 (standard deviation = \pm 632.9) | Scanning electron microscopy (SEM) and Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDX) |
| [26] | Greater than or equal 3 μm | 148 ± 253 | 1.71 | Scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS) |
| [14] | Small (50–500 μ m) and very small (1–50 μ m) | 14 \pm 14 single-use118 \pm 88 reusable | | Micro-Raman (μ-Raman) |
| [12] | Greater than or equal 1.5 μm | 2649 ± 2857 single-use4889 \pm 5432 reusable | 0.1 | Micro-Raman (μ-Raman) |
| [6] | Greater than or equal 6.5–100 μm , greater than or equal 100 | 23.5, 325 | | Nile Red and Fourier transform infrared spectroscopy (FTIR) |
| rea. | μm Greater than 100 nm | $2.4\pm1.3\times10^5$ | | Humanan astrol stimulated Roman scattering (CRC) imaging |
| [5] | | $2.4 \pm 1.3 \times 10^{-6}$ | 250 (Modian) 4700 | Hyperspectral stimulated Raman scattering (SRS) imaging |
| [23] | 700 nm–20 μm | | 359 (Median)–4700 (max) nano-grams/liter | High-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) |
| [24] | Average mean size of approximately 8.2 nm | 10 ⁸ particles/milliliter | | Surface-enhanced Raman spectroscopy (SERS) imaging |

can be absorbed through the stomach lining and enter the bloodstream, ultimately reaching vital organs. The implications of such absorption on human health from food are of particular concern. Compared to large particles, smaller particles may be more likely to be absorbed by the digestive system [29], potentially resulting in increased accumulation within the human body. Given the potential health implications, it is essential to better understand and quantify the presence of smaller nanoand microplastics particles.

2.4. Analytical methods for nano- and microplastics: pros and cons

Since the early 2000s, researchers have increasingly focused their attention on this topic of nano- and microplastics. Ever since their emergence in scientific literature around 2004, thousands of studies have examined these tiny plastic particles from various perspectives. However, there is still a lack of standardization in the methods, which can vary widely [30]. The measurement techniques employed may have different sensitivities, accuracies, and limitations. Notably, techniques such as Fourier transform infrared (FTIR) or Raman microscopy have been utilized for single-particle chemical imaging; however, these methods have limitations in terms of instrumental resolution and detection sensitivity, which can impede their effectiveness in analyzing nanoplastics [31,32]. Electron microscopy and atomic force microscopy offer nano level sensitivity but lack chemical specificity [15,30]. More recently, single-particle chemical imaging techniques, such as photothermal infrared microscopy (AFM-IR) and total-field X-ray microscopy (TXM), have shown promise, but their throughput remains insufficient for comprehensive quantification of environmental nano- and microplastics [33-35]. Qian et al.[5], developed a novel hyperspectral stimulated Raman scattering (SRS) microscopy platform, leveraging the power of stimulated Raman spectroscopy as the imaging contrast

mechanism. Since analytical methods significantly affect the outcome of any study related to nano- and microplastics in single-use plastic water bottles, it is essential to determine the advantages and disadvantages of each method and select the technique that yields the most reproducible and reliable outcomes.

Nile red fluorescence spectroscopy (FS) can usually be used to evaluate microplastics greater than or equal to 3 μ m to less than or equal to 500 μ m in size [36]. The advantages of fluorescence spectroscopy include its low-cost, rapid analysis, and the potential for automated methods to identify microplastics with sample sizes down to the microscale range, which can then be visualized and counted [37,38]. However, the technique faces limitations, as the organic matter present in samples following treatment with acid, or alkaline, oxidizing chemicals is stained and fluoresces, perhaps leading to an overestimation of the number of microplastics present [36]. Additionally, both spectral data and visual data are required to fully analyze the results, which can be one of the significant challenges of fluorescence spectroscopy staining [36].

Fourier transform infrared (FTIR) spectroscopy can be used to usually detect particles down to a size of 10– $20\,\mu m$ [39]. One of the key advantages of this method is that the chemical imaging process is nondestructive and requires no contact with the sample, allowing the same samples to be used to conduct a range of analyses by employing various instruments. Another advantage of this technique is that chemical spectroscopic imaging can be used to acquire information on sample spatial features, enabling the characterization of sample morphology and chemical composition. Finally, an automated pipeline for spectroscopic analysis, which is more efficient and labor-saving than other analytical methods, can potentially be developed [39]. However, Fourier transform infrared (FTIR) spectroscopy also has some inherent limitations. Fourier transform infrared instruments typically have a

single beam design, whereas dispersive instruments generally have double beams. In addition, Fourier transform infrared spectra are affected by detector noise, fluctuations in the intensity of the radiation source used, and inconsistent sample thicknesses.

Micro-Fourier transform infrared (μ -FTIR) spectroscopy has several advantages over traditional dispersive infrared techniques. It offers increased speed, sensitivity, and the ability to perform nondestructive analysis. Unlike dispersive Infrared Spectroscopy, Micro-Fourier transform infrared can be used to simultaneously detect all wavelengths of light, significantly accelerating the analysis process. Additionally, the technique has enhanced sensitivity, allowing for more accurate measurements of weak signals, while preserving the integrity of the sample during analysis [40–42]. Disadvantages include limitations related to sample size and homogeneity, which impact the results, as well as the complexity of micro-Fourier transform infrared (μ -FTIR) instruments, which require careful maintenance [41].

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy offers several advantages. It enhances sensitivity relative to Fourier transform infrared, facilitating stronger sample absorbance and thus improving the detection of chemical constituents. Moreover, sample preparation is simple, labor-intensive pretreatment processes are eliminated, and chemical usage is reduced, thereby saving time and enabling real-time, in situ analysis. Additionally, due to the limited path length into samples, issues associated with the strong attenuation of the IR signal in highly absorbing media such as aqueous solutions are circumvented [43]. However, Attenuated total reflectance–Fourier transform infrared has drawbacks. Quantifying light absorbance can be challenging due to light scattering from multiple reflection elements. Furthermore, it is a surface-sensitive technique, and the bulk composition of samples cannot be analyzed as deeper layers remain inaccessible [44].

Laser direct infrared imaging (LDIR) offers the advantage of rapidly analyzing microplastics greater than 10 μm in clean matrices, such as bottled drinking water [45]. This technique involves sample scanning before actual imaging, and eventually, ensuring that only the areas with actual particles are analyzed [46]. However, one of the disadvantages of this technique is that if two particles are close to one another, they are considered a single particle, and only one spectrum is recorded [47]. Additionally, the infrared band recorded with Laser direct infrared imaging instruments is narrow approximately $1800-900~\text{cm}^{-1}$ [47]. As less information is collected, Laser direct infrared imaging is more prone to misidentification in the analysis of weathered particles than Micro-Fourier transform infrared [48].

Raman spectroscopy and micro-Raman (µ-Raman) spectroscopy advantages include broad spectral coverage, high sensitivity to nonpolar functional groups, and narrow spectral bands [37]. Microscopy coupled with Raman spectroscopy allows for the analysis of small particles between 1 and 20 µm in size with high spatial resolution, and the technique has relatively low sensitivity toward water. Nontransparent and dark particles can be analyzed, and fast chemical mapping can be performed via the Raman spectroscopy method, enabling fast and automatic data collection and processing [37]. Additionally, the spatial resolution of μ-Raman spectroscopy can be as high as 0.5–1.0 μm [21]. However, like the other techniques, Raman spectroscopy also has some limitations. Raman spectra need to be corrected for cosmic ray events, which can be generated by high-energy particles passing through the charge coupled device (CCD) camera, generate many electrons and can be interpreted by the charge coupled device camera as a signal. Additionally, Raman spectroscopy is susceptible to strong background fluorescence signal, which can be many times more intense than the weak Raman signals. Various processing methods, such as polynomial fitting, first- and second-order differentiation, and frequency domain filtering, have been used for baseline correction [39]. Raman spectra is also affected by detector noise and intensity fluctuations in the radiation source used, and the significant interference from fluorescence signals of biological, organic, and inorganic impurities can hamper the

identification of microplastics [49]. Sample purification and appropriate Raman acquisition parameters i.e., wavelength, laser power, and photobleaching are important, and the analysis via micro-Raman spectroscopy can be time-consuming [37].

Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDX and EDS) yields high-resolution images, which enables the quantification and detection of small particles on a surface [26]. Scanning electron microscopy (SEM) provides images i.e., with morphological information or surface features on a magnified scale from X10 to X100,000, although the usual range is typically X50 to X5000. Energy dispersive X-ray (EDX) can be used to determine the elemental composition of an area, with a sensitivity of 0.1–1 % and a spatial resolution of 1 μm [50]. However, while scanning electron microscopy and energy dispersive X-ray spectroscopy analysis is suitable for detecting polyethylene terephthalate particles and allowing for microplastic release studies for this type of plastic, it cannot be used to distinguish among other plastic polymers [26].

Hyperspectral stimulated Raman scattering (SRS) imaging platform, which leverages stimulated Raman spectroscopy as its imaging contrast mechanism in detecting nano plastic particles, offers several advantages. This platform leverages stimulated Raman spectroscopy as its imaging contrast mechanism, resulting in significantly improved imaging speeds compared to conventional Raman microscopy, which was originally utilized in biomedical imaging. By precisely focusing the stimulating beam's energy at specific vibrational modes, the platform achieves exceptional sensitivity at the nanoscale level, enabling the detection of nanoplastics as small as 100 nm. This provides invaluable insights into the complex world of nanoscale plastic pollution. However, the spectral features of hyperspectral stimulated Raman scattering imaging are constrained to the strongest vibrational signatures above the detection threshold, which poses challenges for automated spectrum identification, a crucial aspect for high-throughput analysis of plastic particles. The limited spectral features obtained may hinder the accurate identification and characterization of plastic particles, potentially impacting the efficiency and reliability of particle analysis processes [5].

High-performance liquid chromatography coupled with highresolution mass spectrometry (HPLC-HRMS) is a powerful analytical technique that combines liquid chromatography is combined with highresolution mass spectrometry. This approach offers several advantages in diverse applications, such as the identification of unknown compounds from complex matrices like food, environmental samples, or biological fluids. Due to its high resolution and accurate mass measurements, it can assist in pinpointing specific analytes, addressing challenging identification scenarios [51]. Coelution, a phenomenon in which compounds overlap in a chromatogram, is effectively managed via high-performance liquid chromatography coupled high-resolution mass spectrometry. This technique provides two identification parameters retention time and mass charge ratio (m/z) of fragments - allowing for the effective separation and identification of coeluted compounds [52]. The integration of liquid chromatography with mass spectrometry also ensures the precise quantification of analytes, enabling researchers to confidently measure compound concentrations in various samples and enhance the reliability of quantitative analyses [53]. However, despite its strengths, high-performance liquid chromatography coupled with high-resolution mass spectrometry presents certain challenges, such as cost and complexity. Establishing and maintaining a high-performance liquid chromatography coupled with high-resolution mass spectrometry system can be financially burdensome due to the high cost of instrumentation, consumables, and the need for skilled personnel. Sample preparation for High-performance liquid chromatography coupled with high-resolution mass spectrometry can also be time-consuming and labor-intensive, requiring meticulous extraction, cleanup, and concentration steps to ensure accurate results. Additionally, variations in ionization efficiency across different compounds can affect sensitivity and detection limits, posing challenges in achieving consistent analytical performance [51,54].

Surface-enhanced Raman spectroscopy (SERS) is another powerful vibrational spectroscopic technique that offers several advantages, such as high sensitivity, even at the single-molecule level, allowing for the detection of trace amounts of analytes, making it valuable for applications in chemistry, materials science, and biomedicine. It generates unique molecular fingerprints, enabling the direct identification of target molecules, which aids in accurate analysis and characterization [55]. However, the choice of substrate significantly impacts surface-enhanced Raman spectroscopy performance. To achieve reproducible and reliable results, careful substrate design and preparation must be carried out [56]. Fluorescence signals from impurities or background materials can also interfere with surface-enhanced Raman spectroscopy signals, and researchers must address this issue to increase specificity [57]. Despite its potential, surface-enhanced Raman spectroscopy has not become a routinely used analytical technique due to challenges in scalability, reproducibility, and practical implementation [55]. In Table 3, we present a summary of the analytical detection methods for nano and micro plastics, including their respective detec-

In this review, we explored various analytical techniques for the detection and characterization of nano- and microplastics. The

Table 3
Summary of analytical detection methods for nanoplastic and microplastic particles and their respective descriptions, detection limits and references. Methods include tagging and visual observation, vibrational spectroscopy, electron microscopy, and liquid chromatography-mass spectrometry.

| Analytical method name | Analytical method description | Detection limits | Reference | Methods of detection |
|------------------------------|---|---|-------------------|--|
| FS | Fluorescence spectroscopy | Greater than or equal 3 µm – less than or equal 5 mm | [36,38, 58,59] | Tagging + visual observation |
| LDIR imaging | Laser Direct Infrared imaging | Greater than 10 µm | [45–48] | Vibrational spectroscopic |
| FTIR | Fourier transform infrared | 10–20 μm | [39] | Vibrational spectroscopic |
| μ-FTIR | Micro-Fourier transform infrared spectroscopy | Less than 20 μm | [14] | Vibrational spectroscopic |
| ATR-FTIR | Attenuated total reflectance Fourier transform infrared spectroscopy | Greater than 500 μm | [59,60] | Vibrational spectroscopic |
| Raman | Raman Spectroscopy | 1–20 μm | [31,39, 49,59] | Vibrational spectroscopic |
| μ-Raman | Micro - Raman Spectroscopy | 0.5–1.0 μm | [14] | Vibrational spectroscopic |
| SEM/EDX or EDS | Scanning Electron Microscopy and Energy dispersive X-ray spectroscopy | Greater than 1 μm | [26,50] | Electron Microscopy |
| SRS | Hyperspectral stimulated Raman scattering (SRS) imaging | Greater than 100 nm | [5] | Vibrational spectroscopic |
| HPLC- HRMS | High- performance liquid chromatography coupled with high-resolution mass spectrometry | Greater than 700 nm | [23] | Liquid chromatography + mass spectrometry |
| SERS | Surface-enhanced Raman spectroscopy | Greater than 50 nm | [24] | Vibrational spectroscopic |

inconsistencies observed across existing studies underscore the need for methods that provide consistent and reproducible quantification results for nano- and microplastics. Additionally, there is a pressing need for effective characterization techniques that are capable of handling large sample quantities in a cost-effective manner. The development and application of robust analytical methods for nano and microplastic detection and characterization are pivotal for environmental monitoring, human health assessment, and regulatory compliance, as well as policy making. These data are essential for understanding the scope of nano and microplastic pollution, evaluating related risks, and implementing effective regulatory measures to safeguard the environment and public health. Continued advancements in analytical techniques are vital for overcoming the challenges inherent in nano and microplastic analysis and regulation. As of now, there is no universally accepted or standardized method for quantifying nanoplastics, which presents a significant challenge in this area of research. Unlike more established contaminants such as bacteria or lead, for which reliable testing methods and protocols have been developed and approved by governmental agencies e.g., Environmental Protection Agency (EPA) or the World Health Organization (WHO), the detection and measurement of nanoplastics remains in a state of development. While numerous analytical techniques have been proposed, they often suffer from limitations. Many of these methods are not easily reproducible, require highly specialized equipment, or are prohibitively expensive for widespread use.

3. Examining the chronic health effects of nano/microplastic consumption

Nano- and microplastics can enter the human body through ingestion and inhalation, accumulating in various organs and leading to a range of chronic health effects. The gastrointestinal system is particularly vulnerable, as these particles can cause inflammation and disrupt normal digestive processes. Once absorbed, NMPs can enter the bloodstream and affect multiple systems. The immune system may be compromised due to the triggering of chronic inflammatory responses. The endocrine system is also impacted, with additives such as phthalates functioning as endocrine disruptors, leading to hormonal imbalances and reproductive health issues [61,62]. Neurotoxic effects on the brain and nervous system may impair cognitive function, while the accumulation of microplastics in vital organs can contribute to chromosomal abnormalities and an increased cancer risk [62,63].

In addition to carcinogenic risks demonstrated in both *in vitro* and *in vivo* studies, NMP exposure has been associated with metabolic disorders such as obesity, diabetes, and non-alcoholic fatty liver disease [61]. Immediate symptoms may include pain, inflammation, and hormonal imbalances, while long-term exposure has been linked to conditions such as infertility, chronic obstructive pulmonary disease (COPD), and various cancers [63]. Despite growing evidence of these health risks, significant gaps remain in the scientific understanding of NMP toxicity. Most existing studies have focused on monodisperse particles, particularly polystyrene, with sizes ranging from 10 nm to 200 μ m. There is a need for more comprehensive studies on the long-term and cumulative effects of diverse NMP types [64].

3.1. Size matters: the fate of nano- and microplastics in the human body

Plastic pollution has emerged as a pressing and evolving problem, which significantly impacts the climate, environment, and human health. The rising production and demand for single-use plastics have contributed to the growing challenge of managing plastic waste effectively. This issue requires concerted attention and collective efforts to mitigate the detrimental effects of plastics on the planet and all living beings [65–67]. Nano- and microplastics, in particular, pose a significant threat, as they can enter the human body in various ways. The annual microplastic consumption varies depending on age, sex, and

method of intake. For individuals' annual ingestion, consumption ranges from 39,000 to 52,000 particles. Moreover, individuals who meet their recommended water intake solely from single-use plastic water bottles may ingest an additional 90,000 microplastics per year, whereas individuals who consume only tap water may ingest approximately 4000 microplastics annually [68]. Lusher et al. [69], reported that the fate of microplastics within the mammalian body is strongly influenced by the size of the particle. Notably, microplastics larger than 150 µm are not likely to be absorbed by the body. Conversely, microplastics smaller than 150 μm have been observed to pass from the intestinal cavity into the lymphatic and circulatory systems, with an absorption rate of less than or equal to 0.3 %, leading to systemic exposure [70]. Additionally, microplastics approximately 100 µm in size can enter the portal vein, which transports them from the stomach to the liver. Furthermore, microplastics smaller than or equal to 20 µm have the potential to enter various organs. Notably, nanoplastics smaller than or equal to 100 nm can reach all organs, with 7 % translocation across the blood—brain and placental barriers [69]. Fig. 3 illustrates the fate of microplastics within the mammalian body, which is contingent upon the size of the nano and microplastic particles [69].

3.2. Health implications of increased nano/microplastic exposure

Increased exposure to nano- and microplastics poses significant chronic health risks and can lead to a range of health problems. This includes metabolic disruption, and reproductive harm, as they interact with other environmental pollutants and release harmful additives like endocrine-disrupting phthalates [71]. Respiratory diseases [72,73], oxidative stress [73–77], reproductive issues [78,79], neurotoxicity i.e.,

toxic effects on the nervous system [80–82], disruptions to immune function, and cancer [73,79,83–86], intestinal dysbiosis [75,87–89] are among the major chronic health concerns associated with nano and microplastic exposure. Recognizing the risks of nano and microplastic is crucial, and appropriate precautions should be taken to minimize human exposure [11]. At the nanoscale level, nano- and microplastics have significant biological impacts, which include inducing reactive oxygen species (ROS) generation, triggering inflammatory responses to activate the immune system, and potentially causing genotoxic deoxyribonucleic acid (DNA) damage [90] and may be linked to colorectal cancer in human [91]. Fig. 4 depicts the potential chronic health risks of nano- and microplastics on humans.

Studies on nano and microplastic exposure have been conducted using polystyrene due to its high toxicity characteristics in mammals. [75,79,92–94]. Nanoplastics are believed to be able to breach biological barriers, making them toxicologically relevant and potentially reaching various organs and tissues postexposure [95]. While specific studies on the cellular uptake of polyethylene terephthalate nanoplastics are lacking, their presence in Caco-2 cell lysosomes suggests potential interactions and internalization within cells [96]. Their uptake may occur through endocytosis, similar to the uptake of polystyrene nanoplastics, which was identified as the primary cellular absorption pathway in prior research [96]. Interestingly, the oral bioavailability of 50-nm polystyrene nanoparticles was estimated to be ten to one hundred times greater than that of microplastics (2–7 %) [97,98].

In an article, Yee et al. [99], suggested that the absorption rates of nanoplastics within the gastrointestinal tract lumen are difficult to measure but likely occur. Following ingestion, nanoplastics undergo transformations that can affect their absorption rates [99]. Interactions

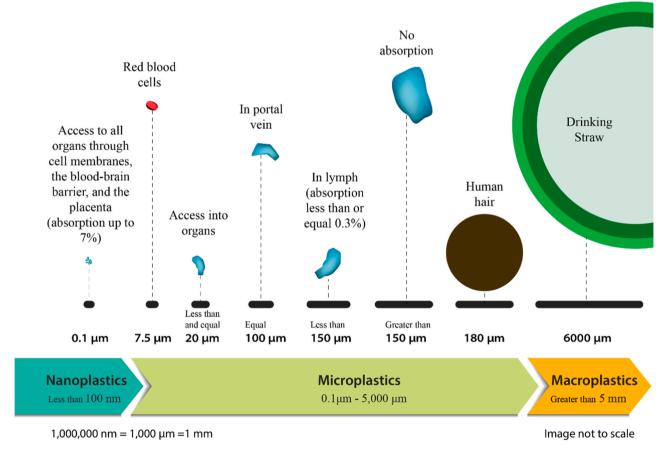


Fig. 3. The fate of microplastics within the mammalian body is contingent upon nano and microplastic particle size. Microplastics larger than 150 μm are unlikely to be absorbed, while smaller particles can enter the lymphatic and circulatory systems. Nanoplastics smaller than 100 nm can reach all organs, crossing the blood-brain and placental barriers.

Health effects of nano-microplastics

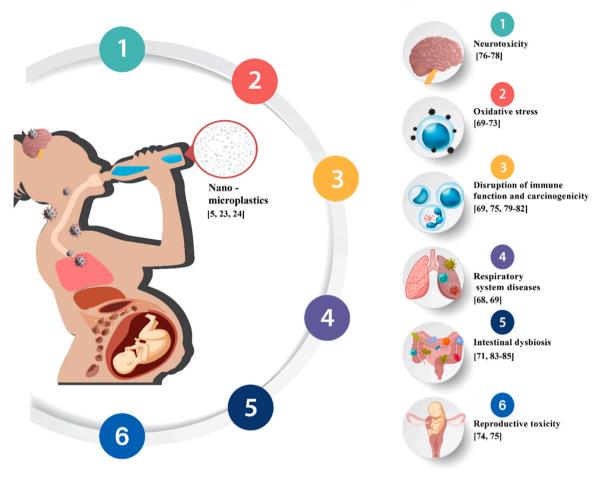


Fig. 4. Potential chronic health risks of nano- and microplastics on human health, including metabolic disruption, reproductive harm, respiratory diseases, oxidative stress, neurotoxicity, immune function disruptions, and cancer. The figure highlights the findings from various studies on these health impacts and the importance of minimizing human exposure.

with various molecules in the gastrointestinal tract, including proteins, lipids, carbohydrates, nucleic acids, ions, and water, can influence this process [100,101]. Specifically, a group of proteins can envelop nanoplastics, forming a 'corona' [102]. Studies suggest that the protein corona undergoes changes in an in vitro model that mimics human digestion, leading to increased nanoparticle translocation across the gut lining and facilitating nano plastic entry into the bloodstream [98]. Fig. 5a illustrates the pathway through which nanoplastic particles enter the human body via ingestion. It shows how changes in the protein corona, observed in an in vitro model simulating human digestion, may enhance nanoparticle translocation across the gut lining and facilitate the entry of nanoplastics into the bloodstream.

Rodríguez-Hernández et al. [103], developed a method to produce nanosized polyethylene terephthalate particles for toxicological and environmental investigations. Preliminary cell internalization experiments were conducted using fluorescent-labeled nano-polyethylene terephthalate particles. Confocal microscopy revealed that macrophages surrounded the nano-polyethylene terephthalate agglomerates, and internalized nano-polyethylene terephthalate particles. Further observation revealed that actin spaces merged with internalized nano-polyethylene terephthalate fluorescence regions. Transmission electron microscopy (TEM) observations (Figs. 5b-1) confirmed the cell internalization of nano-polyethylene terephthalate particles, with small nano-polyethylene terephthalate particles aggregating in the nucleus (Figs. 5b-2) and dispersed nanoparticles appearing in small vesicles (Figs. 5b-3). The control cultures lacking nano-polyethylene

terephthalate showed no red fluorescence. These findings may help in understanding the potential ingestion-mediated uptake of nanoplastics into cells [103].

Goodman et al. [104], investigated the toxicological effects of 1 µm polystyrene- microplastics on human embryonic kidney (HEK293) cells and human hepatocellular (Hep G2) liver cells. When both cell lines were exposed to polystyrene-microplastics, there was a significant reduction in cell proliferation but no significant decrease in cell viability. Cell viability remained high (at least 94 %), even at the highest polystyrene -microplastics concentration of 100 μg/mL. Phase-contrast imaging and confocal fluorescence microscopy confirmed that both kidney and liver cells took up the 1 µm polystyrene-microplastic particles after 72 h of exposure, with more than 70 % of the cells internalizing the particles within 48 h. Overall, the adverse effects of polystyrene-microplastics on human kidney and liver cells indicate that the ingestion of microplastics may have toxicological implications for cell metabolism and cell—cell interactions. These findings highlight the potential undesirable effects of microplastics on human health, leading to morphological, metabolic, and proliferative changes, as well as cellular stress in human cells [104]. Fig. 4c illustrates a graphical representation of the chronic health risks of microplastics on human liver and kidney cells.

Despite the limitations in nano plastic detection methodologies, numerous relevant studies have demonstrated that microplastics are associated with a range of adverse chronic human health. These effects include oxidative stress, which can lead to cellular damage, disruption of

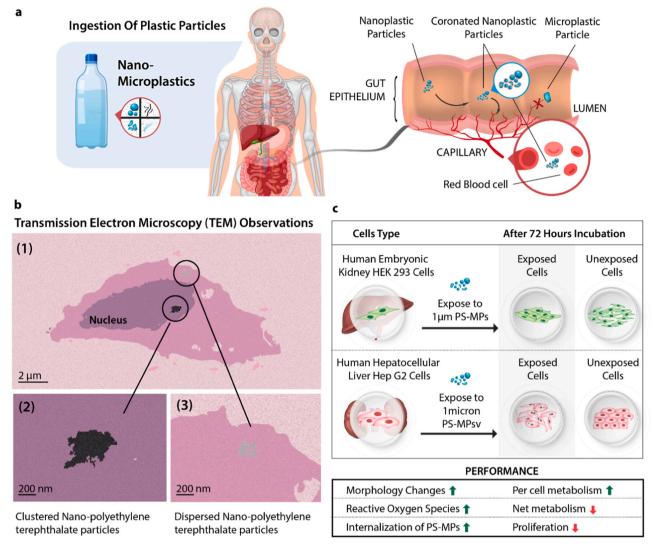


Fig. 5. Chronic health risks via (a) Ingestion is one route through which plastic particles enter the human body. Nanoplastics may interact with proteins, lipids, carbohydrates, nucleic acids, ions, and water in the human body, leading to the formation of coronated nanoplastic particles for absorption. (b) The nanopolyethylene terephthalate particles were analyzed by transmission electron microscopy (TEM) observations (1) Cross-sections of macrophages in which internalized nano-polyethylene terephthalate particles were detected. Agglomerated (2) and dispersed (3) nanoparticles observed inside cells. (c) Impacts of microplastics on human liver and kidney.

immune functions, and potential carcinogenicity [74,76,77,88]. Furthermore, microplastics have also been linked to neurotoxicity, causing harm to the nervous system [80,81]. They can also contribute to intestinal dysbiosis, disrupt the balance of gut microbiota [75], and may lead to respiratory system diseases when inhaled [72,86]. Additionally, there are concerns about the reproductive toxicity of microplastics, as they can potentially interfere with reproductive processes [78,79]. These wide-ranging chronic health risks highlight the importance of recognizing and addressing the impact of nano- and microplastics to safeguard human health.

4. Regulatory landscape of single-use plastics: efforts and gaps

4.1. Worldwide regulations

The global imperative to address plastic pollution has driven the international community to forge a transformative path toward a comprehensive global treaty to eradicate plastic pollution [105]. Gaps and synergies within multilevel governance and regulatory frameworks are being examined, and efforts are underway to bolster the efficacy of these endeavors. These efforts include global conventions focused on

ocean protection, without a plastic-specific agreement, as well as regional protocols and collaborations to address plastic pollution, international plans and campaigns to provide strategies for global action with a focus on education and awareness, and finally, plastic waste alliances that focus on coordinating the efforts of the public to promote education and public awareness [105,106]. The timeline illustrating the significant global initiatives and actions addressing plastic waste in marine environments is depicted in Fig. 6. This timeline highlights key milestones, international agreements, policy implementations, and conservation efforts aimed at reducing plastic pollution and protecting marine ecosystems.

The growing global response to plastic waste is evident in the significant rise in the number of conferences addressing plastic pollution since 1960, reflecting increasing awareness and collaborative efforts to tackle this urgent issue. Notably, a sharp surge in attention began in 2009, coinciding with a rise in scholarly articles examining the effects of nano- and microplastics on human health and the environment, as shown in Fig. 7.

TIMELINE OF GLOBAL PLASTIC WASTE ACTION

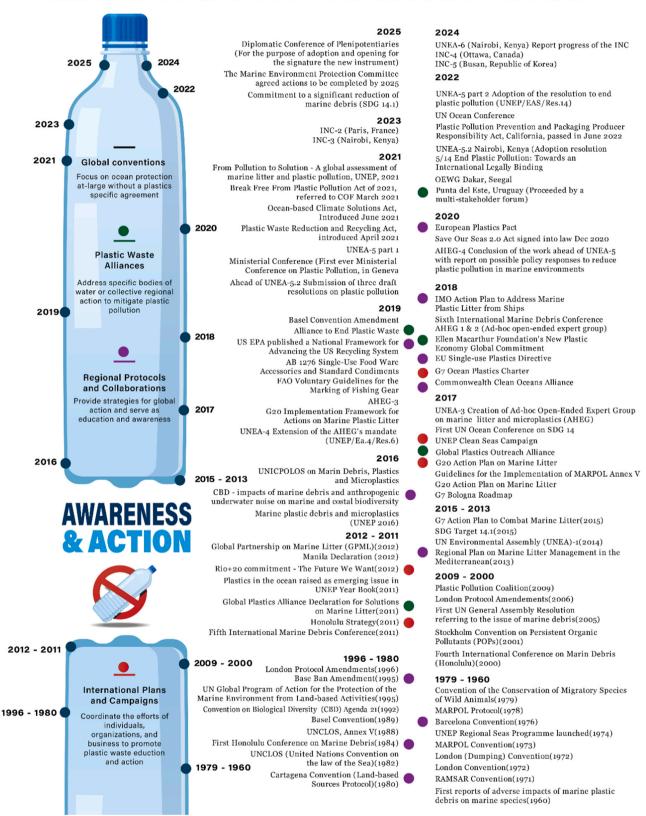


Fig. 6. Timeline of key global initiatives and actions addressing plastic waste in marine environments. Highlights include significant milestones, international agreements, policy implementations, and conservation efforts aimed at reducing plastic pollution and protecting marine ecosystems.

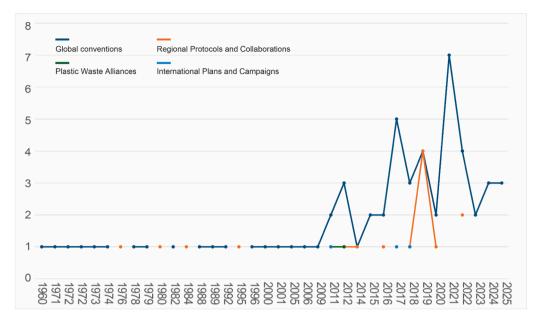


Fig. 7. Increase in global conferences on plastic pollution since 1960, reflecting heightened awareness and collaborative efforts. Notably, a rise in scholarly articles on nano- and microplastics since 2009 correlates with these efforts, emphasizing the implications for human health and the environment.

4.2. United nations environmental protection agency

The United Nations Environment Assembly (UNEA-5) passed a landmark resolution to address the full life cycle of plastics, including plastic production, design and disposal on March 2nd, 2022. The UN assembly, held in Nairobi, passed a historic resolution to forge an international, legally binding agreement to end plastic pollution by the end of 2024. This resolution was endorsed by heads of state, environment ministers and other representatives from 175 nations. The United Nations Environment Program issued a draft of their own legally binding resolution to address pollution [1,107]. Alongside the UN's ground-breaking efforts, many countries have also sought to implement improved plastic regulations and initiatives.

4.3. Asia and the European Union

In January 2020, China's National Development and Reform Commission (NDRC) and the Ministry of Ecology and Environment issued a policy document outlining a five-year roadmap to restrict the use of certain single-use plastic products. This included bans on non-degradable plastic bags, straws, and utensils in major cities by the end of 2020, with further restrictions extending to other cities and counties by 2022. By 2025, the use of non-degradable single-use plastic tableware for takeout in cities must be reduced by 30 %. The revised Law on the Prevention and Control of Environmental Pollution Caused by Solid Waste, effective from September 2020, imposes fines for non-compliance [108].

In August 2021, India's Ministry of Environment, Forest and Climate Change notified the Plastic Waste Management Amendment Rules, 2021, which prohibit identified single-use plastic items from July 1, 2022. Items banned include plastic earbuds, balloon sticks, plastic flags, candy sticks, ice cream sticks, polystyrene for decoration, plates, cups, glasses, cutlery, straws, trays, and wrapping films [109]. The rules also increased the thickness of plastic carry bags to promote reuse [110]. These measures aim to mitigate pollution caused by littered single-use plastics [111].

Thailand's roadmap for plastic waste management includes a ban on foam food containers, plastic straws, plastic cups, and plastic bags, with further reductions or bans on additional single-use plastic items planned until 2026 [112]. The Draft Sustainable Packaging Management Act,

currently under consultation, aims to introduce extended producer responsibility (EPR) for packaging, which would legally mandate producers to ensure the recovery and recycling of packaging waste [113].

Indonesia's Ministry of Environment and Forestry announced in June 2023 that it would extend its bans on certain single-use plastic items to the end of 2029. These bans cover single-use plastic shopping bags, plastic straws and cutlery, and Styrofoam food packaging [114]. Local regulations have been adopted by various provincial and city governments since 2018, focusing on reducing the use of plastic bags, straws, and Styrofoam [115].

The Plastic Resource Circulation Act, effective from April 2022, addresses the entire lifecycle of plastics, from product design to waste disposal in Japan. It sets criteria for retailers and service providers to reduce single-use plastics, such as by mandating charges for plastic bags and promoting the use of eco-friendly alternatives [116]. The act involves all stakeholders in promoting the principles of reduce, reuse, recycle, and recover [117].

The European Union Directive 2019/904 of the European Parliament and of the Council, issued on 5 June 2019, is focused on reducing the impact of certain plastic products, especially on aquatic ecosystems and human health. The Directive introduces restrictions on the provision of single-use plastic items detailed in the Annex, products made from oxodegradable plastic, and fishing gear incorporating plastic, and promotes a circular economy through innovative and sustainable practices. The Annex includes cups for beverages, food containers for immediate consumption, cutlery, plates, straws, stirrers, balloon sticks, and so on. The bill restricts their provision unless requested, and items cannot be bundled. The measure extends to items such as cotton bud sticks, tobacco filters, wet wipes, and so on, promoting a circular economy. Separate collection and awareness strategies are also outlined for specific items, all aimed at reducing the impact of plastic waste on the environment and human health [118].

In Germany, a five-point plan was introduced to drastically reduce plastic waste. First, the use of unnecessary packaging should be avoided; for example, bananas need not be packaged as they are protected by a natural "skin." Second, eco-friendly packaging should be promoted through new licensing rules. Third, the plan aims to significantly increase plastic recycling targets, from the current 36 % to an ambitious 63 % by 2022. Fourth, plastics should be prevented from entering organic waste to enhance compost quality. Finally, the plan emphasizes

the importance of supporting international efforts to combat oceanic plastic pollution, including increased aid for cleaning up the most impacted rivers [119].

Meanwhile, Sweden has implemented plastic bottle return systems, where users pay a deposit for plastic bottles and return the bottles to retailers after use. The collected bottles are then transported by beverage companies to recyclers. The act on certain beverage containers mandates the refilling of polyethylene terephthalate bottles, rather than solely recycling. Small producers protested this act because it favors large producers. A 1993 resolution allowed plastic recycling instead of refilling, with a deposit system in place to increase the rate of returns. Refillable polyethylene terephthalate bottles are cleaned and refilled, while single-use bottles are sorted, with 50 % recycled into new bottles and the other 50 % recycled into other products. The return pack-polyethylene terephthalate guidelines aim to regulate the refillable plastic system, which maintains a remarkable 98 % reuse rate. The popularity of reusable polyethylene terephthalate bottles is decreasing, while that of recycled bottles is on the rise [120].

4.4. Canada and the United States

In 2020 the Government of Canada undertook an analysis of available data by government representatives to identify single-use plastic items for a proposed ban. Six items, including checkout bags, cutlery, problematic plastic foodservice ware, ring carriers, stirring sticks, and straws, were considered for prohibition. In June 2022, the single-use plastic Prohibition Regulations SOR/2022–138 i.e., Canada Gazette, Part 2, Volume 156, Number 13 became a law [121] was promulgated.

Two California, USA regulations are noteworthy, the first being California AB-793 recycling plastic beverage containers, minimum recycled content in September 2020. The California beverage container recycling and litter reduction act mandates a minimum refund value for all beverage containers sold in the state. The department of resource recycling and recovery calculates a processing fee for these containers, with a scrap value paid by manufacturers to distributors. Effective January 1, 2022, this bill requires a specified amount of average postconsumer recycled plastic content per year for all plastic beverage containers sold by manufacturers. The plan establishes tiers, with the goal of a minimum of 50 % postconsumer recycled plastic content per year for all plastic beverage containers by January 1, 2030, except under special circumstances [122]. The second notable regulation is Bill AB-1276: Single-use food ware accessories and standard condiments, October 2021. This bill restricts food facilities from providing single-use food ware accessories or standard condiments to customers unless specifically requested. Additionally, it prohibits the bundling of these items to prevent customer selection [123].

In addition to California, USA regulations, several other statespecific regulations have been introduced to address plastic waste and improve recycling efforts. For example, in 2022, Colorado introduced the plastic pollution reduction act (Colorado House Bill 21-1162, 25-17-501, 2021) to address the growing concern over plastic waste. The Colorado General Assembly acknowledged the importance of limiting single-use plastic carryout bags and reducing the use of polystyrene products to protect the state's environment and natural resources. This legislation includes a ban on single-use plastic bags in most stores and prohibits the use of expanded polystyrene (EPS) foam takeout containers in most restaurants [124]. The Connecticut general assembly introduced S.B. No. 928, an act addressing the minimum recycled plastic content in products sold within the state. This legislation directs the department of energy and environmental protection to develop a plan for implementing a minimum recycled content policy. Additionally, it includes provisions related to solid waste management, replacing raised S.B. No. 1037 on the bottle bill. Key changes under this bill include doubling the bottle deposit value to 10 cents and expanding the range of containers accepted by collection facilities [125,126]. In 2021, the state of Maine introduced H.P. 1146 - L.D. 1541, an act designed to support

and enhance municipal recycling programs while reducing costs for taxpayers. The legislation covers most types of consumer packaging and requires producers to contribute to a stewardship organization, which funds local governments' packaging management expenses [127]. The Rhode Island's senate bill S 0155, part of title 21-food and drugs, seeks to reduce plastic pollution by banning single-use plastic straws in food service establishments. By restricting the use of these items, the legislation aims to minimize plastic waste and its environmental impact [128]. The Washington state's senate bill 5219 focuses on plastic packaging management to mitigate environmental impact. The bill holds producers accountable for the entire lifecycle of plastic packaging, sets ambitious recycling targets, and mandates that a portion of packaging be made from recycled materials. Additionally, it includes public education initiatives on recycling and establishes a system to monitor progress [129]. The New Jersey's senate bill No. 2515, introduced in the 219th legislature, establishes recycled content requirements for plastic containers, glass containers, paper carryout bags, reusable plastic film carryout bags, and plastic trash bags. The bill also prohibits the sale of loose polystyrene foam packaging [130].

This extensive review of worldwide regulations demonstrates the need for targeted legislation to address the issue of single-use plastic water bottles. Legislation has been effective in regulating the problem of single-use plastic bags, and a similar approach should be adopted for plastic water bottles. Early recognition of single-use plastic bags problem began in the early 2000s, with countries like Bangladesh leading the way by implementing bans due to the bags' role in flooding and pollution. In 2013, the European Union took action with Directive 2015/720, mandating member states to reduce plastic bag consumption through charges or bans and set targets to decrease per capita use significantly by 2025. This effort was further expanded in 2021 with the Single-Use Plastics Directive, which introduced stricter measures on a broader range of plastic items.

The movement to ban single-use plastic bags in North America began with San Francisco's landmark ban in 2007, followed by California's statewide ban in 2014, which came into effect in 2016. By 2020, a patchwork of local and state regulations had emerged across the U.S. addressing plastic bag use. In Canada, Vancouver's 2016 ban on plastic bags was an early step, with other cities and provinces following suit. In 2021, the Canadian government announced a nationwide ban on single-use plastic bags, marking a significant stride towards reducing plastic waste and promoting sustainable practices. This global trend highlights a shift from initial awareness to comprehensive regulations aimed at mitigating plastic pollution and fostering environmental sustainability.

By focusing legislative measures on specific items, such as single-use plastic bags, we can now target single-use plastic water bottles, which are among the most common sources of plastic pollution. The power of legislation lies not only in its ability to enforce change but also in its capacity to raise awareness among the masses. It provides a tangible target for collective action and promotes a shift in consumer behavior toward more sustainable choices. By spotlighting single-use plastic water bottles through legislation, we can encourage individuals, businesses, and governments to consider alternative options, such as promoting the use of reusable water containers, supporting the expansion of water-refilling stations, and fostering a culture of mindful consumption.

5. Gaps and opportunities for future research

5.1. Human behavior studies

Human behavior studies should delve into consumer behavioral patterns surrounding the usage of single-use plastic water bottles. This could include surveys, interviews, and observational studies to explore factors influencing consumer decisions, such as convenience, affordability, and perceived water quality. Understanding consumer preferences and habits can provide valuable insights for designing targeted interventions and behavioral change campaigns aimed at reducing the

consumption of single-use plastic water bottles. Country-specific studies play a vital role in advancing our understanding of the environmental and health impacts of single-use plastic water bottles within localized contexts. By tailoring research efforts to the unique socioeconomic, environmental, and cultural conditions influencing plastic consumption and pollution in specific countries, researchers can develop targeted interventions and policy recommendations to mitigate the adverse effects of single-use plastic water bottle usage and promote more sustainable alternatives.

5.2. Environmental and physical stressors evaluating exposure

The research on single-use plastic water bottles must consider the various conditions during transport, storage and consumption. Investigating the effects of environmental and physical stressors on nano and microplastic release from single-use plastic water bottles is essential for understanding the dynamics of plastic pollution and its potential impacts on the environment and human health. Controlled experiments can simulate real-world scenarios to assess the release of nano- and microplastics under different conditions. One aspect of this research involves studying the effects of environmental stressors, such as sunlight and heat, on single-use plastic water bottles. Researchers can design experiments to expose single-use plastic water bottles to varying levels of sunlight and temperatures over time, monitoring the changes in nanoand microplastics release. Understanding how environmental factors influence nano and microplastic release can provide valuable insights into the risk of plastic exposure to human health. Furthermore, investigating the impact of physical stressors, such as squeezing and bottle cap opening and closing, is crucial for comprehending how human interactions with single-use plastic water bottles contribute to nano and microplastic release. Additionally, exposure assessment studies can be conducted to evaluate the scope of human exposure to nano- and microplastics from single-use plastic water bottles under various scenarios. This could involve measuring the concentration of nano- and microplastics in bottled water samples and estimating the potential intake of nano- and microplastics through drinking water consumption. By conducting controlled experiments and exposure assessments, researchers can generate valuable data to support evidence-based decision-making and promote sustainable practices for plastic use and disposal.

5.3. Fate of nano- and microplastics according to size

Exploring the influence of nano- and microplastics in single-use plastic water bottles on their fate of nano within different human organs is crucial for understanding the potential health impacts of plastic pollution. Research in this area can elucidate transport, deposition, and potential uptake of nano- and microplastics by various organs in the human body. A key element of this research should involve studying the transport of nano- and microplastics in the human body after ingestion. Investigating the size-dependent transport mechanisms of nano- and microplastics can provide insights into their distribution in different organs and tissues. Smaller particles, such as nanoplastics, can penetrate cell membranes more easily and disrupt normal cellular processes, potentially leading to cytotoxicity, inflammation, and oxidative stress. Studies have indicated that particles smaller than 1 µm can be internalized by nonphagocytic cells via clathrin- and caveolin-mediated endocytosis pathways [61]. Understanding these mechanisms is essential for assessing the health risks associated with nano- and microplastics.

Understanding the deposition of nano- and microplastics in human organs is essential for assessing their potential health effects. Nano- and microplastics may accumulate in organs such as the liver, kidneys, and lungs through processes such as filtration and phagocytosis. Research has shown that smaller particles exhibit higher transendothelial transport and permeability, leading to increased cellular damage[131]. This

translocation can result in the accumulation of nano- and microplastics in sensitive organs like the brain, potentially impacting neurological health. Through targeted studies, researchers can investigate how the size of nano- and microplastics influences their deposition patterns and whether nano- and microplastics of certain sizes are more likely to accumulate in specific organs. Nano- and microplastics can translocate cross biological barriers such as the blood-brain barrier (BBB) and the placental barrier. Size-dependent studies have demonstrated that smaller particles exhibit higher transendothelial transport and permeability, leading to increased cellular damage [131].

The long-term health effects of nano- and microplastics are still being explored. Chronic exposure to these particles may lead to persistent inflammation and contribute to the development of diseases such as cancer, cardiovascular diseases, and metabolic disorders [61]. Research into the genotoxicity and mutagenicity of nano- and microplastics is essential for understanding their potential role in disease progression. Through targeted studies, researchers can investigate how the size of nano- and microplastics influences their deposition patterns and whether nano- and microplastics of certain sizes are more likely to accumulate in specific organs.

5.4. Regulatory oversight deficiency and policy vacuum

The lack of comprehensive regulations and policies regarding nano and microplastic pollution from single-use plastic water bottles is a critical issue that requires thorough examination and targeted intervention. To address this challenge effectively, a holistic approach is necessary, involving a comprehensive study of existing regulations pertaining to single-use plastic items, including bags, utensils, and water bottles, both at the national and global levels. The first step in this process is to conduct an in-depth analysis of the current regulatory landscape surrounding single-use plastics. This includes identifying and reviewing relevant laws, policies, and initiatives aimed at regulating production, distribution, use, and disposal of single-use plastic products. By examining the strengths and limitations of existing regulations, researchers can gain insight into the gaps and shortcomings that need to be addressed to effectively mitigate the problems related to nano- and microplastics from single-use plastic water bottles. It is crucial to assess the effectiveness of current regulations in addressing the specific risks associated with nano and microplastic contamination from single-use plastic water bottles.

It is important to note that while there is mounting evidence of the potential chronic health and environmental risks posed by nano- and microplastics, particularly in relation to water and food contamination, the scientific community is still working to fully characterize these risks. Therefore, this section does not present these risks as definitively proven, but instead emphasizes the regulatory gap surrounding them, particularly concerning single-use plastic water bottles.

Based on the findings of this regulatory analysis, researchers can propose more targeted and stringent regulations that specifically address problems related to nano- and microplastics from single-use plastic water bottles. The key components of these proposed regulations could include the following:

1.Mandatory labeling requirements: Manufacturers should be required to label single-use plastic water bottles with clear and prominent information about the presence of nano- and microplastics and their potential health and environmental impacts. This labeling can help consumers make more informed choices and incentivize companies to reduce nano and microplastic contamination in their products.

2.Extended producer responsibility (EPR) programs: EPR programs that hold manufacturers accountable for the entire lifecycle of their products, including the issues related to nano and microplastic, should be implemented. This may involve imposing fees or levies on producers to fund cleanup and mitigation efforts.

The reliability of the proposed regulation is based on extensive research and analysis of existing successful models such as single-use plastic bags and best practices. The regulation aims to address critical environmental concerns while providing clear guidelines and standards for compliance. By leveraging proven methodologies and technologies, we believe the proposed regulation will be reliable in achieving its intended outcomes. This confidence is further supported by the worldwide success of single-use plastic bag regulations. For instance, as of 2018, at least 127 countries have adopted some form of legislation to regulate plastic bags [132]. These regulations have shown significant progress in reducing plastic pollution and promoting sustainable alternatives [133,134]

The regulatory oversight deficiency and policy vacuum surrounding nano and microplastic pollution from single-use plastic water bottles is a pressing issue that demands immediate attention and action. While scientific research continues to explore the full scope of health and environmental risks, the lack of targeted policies leaves a critical gap that must be addressed. By conducting a thorough analysis of current regulations and identifying their limitations, we can begin to craft more effective, targeted measures that not only mitigate the pollution caused by single-use plastics but also protect human health and the environment. The implementation of the suggested actions is estimated to take approximately three to five years. This timeline accounts for the necessary legislative processes, stakeholder engagement, infrastructure development, and public awareness campaigns. The phased approach outlined in the regulation allows for gradual adaptation and compliance, ensuring that all parties involved have sufficient time to transition and meet the new standards. Continuous monitoring and evaluation will be conducted to assess progress and make any necessary adjustments to ensure successful implementation.

Access to safe drinking water is a fundamental human right, and while single-use plastic water bottles provide an immediate solution in areas with inadequate infrastructure, they should not be relied upon long-term. This paper advocates for a shift towards sustainable water access solutions through infrastructure development, innovative water sourcing methods, and enhanced recycling initiatives. Research into nano- and microplastics, particularly in bottled water, highlights the potential health risks, urging a cultural transformation towards more sustainable water consumption practices. The lack of regulatory frameworks to address the environmental impacts of single-use plastic water bottles is a critical concern. Implementing regulations that reduce production, promote reusable alternatives, and boost recycling initiatives can drive industry and consumer behavior changes. Furthermore, improving public access to safe drinking water and educating the public are essential steps in reducing reliance on single-use plastics. While the understanding of nano- and microplastics' impact is advancing, further research is necessary. Regulatory measures must be implemented to address environmental and health risks, ensuring a more sustainable and resilient future for water access.

6. Conclusion

This paper provides a comprehensive review of nano- and microplastics in single-use plastic water bottles, covering various study types, environmental stressors, detection methods, and the chronic health implications of their consumption. While prior reviews have examined plastic pollution more broadly including microplastics in food, beverages, and environmental matrices, as well as their removal [135–139], this review uniquely focuses on single-use plastic water bottles as a distinct and underexplored vector of nano- and microplastics exposure. It critically evaluates the limitations in current research, such as small sample sizes, inconsistent lab conditions, and a lack of standardized detection protocols.

Despite the growing concern, there are limited studies specifically focused on single-use plastic water bottles and the different laboratory conditions under which they should be tested. Additionally, the number of samples tested in existing studies is often very limited, which hampers the ability to draw definitive conclusions. The findings highlight

significant gaps in the instrumentation used in detection methods. However, there have been major strides in detection methodologies to quantify nano- and microplastics in 2023 and 2024. The chronic health implications of consuming nano- and microplastics, particularly those related to Polyethylene Terephthalate (PET), are also explored, though studies in this area remain scarce.

The paper examines the current regulatory landscape of single-use plastic products, highlighting major gaps in regulations specifically focused on single-use plastic water bottles. It identifies opportunities for future research, including studies on human behavior, the evaluation of environmental and physical stressors affecting exposure, the fate of nano- and microplastics according to size, and deficiencies in regulatory oversight and policies. By addressing these issues, the paper aims to provide a clearer understanding of the broader consequences of plastic pollution and the necessary steps to mitigate its impact on both human health and the environment.

Access to safe drinking water is a fundamental human right, and while single-use plastic water bottles may offer an immediate solution in regions with limited access to clean water, they should not be viewed as a long-term answer. This paper emphasizes the need to transition from reliance on single-use plastics to sustainable, long-term water access solutions. Prioritizing infrastructure development, and innovative water sourcing methods, initiatives will help reduce dependence on single-use plastics and create more resilient, sustainable water systems. The research into nano- and microplastics and their potential chronic health impacts, especially related to single-use plastic water bottles, is crucial in understanding the broader consequences of plastic pollution. As water consumption habits shift, with increased reliance on bottled water due to the decline in public water fountain accessibility, it is essential to raise awareness about the chronic health risks associated with nano- and microplastics. Effective regulations, promoting reusable alternatives, and reducing production can drive changes in both industry practices and consumer behavior. Addressing public access to safe drinking water and improving water infrastructure are vital in reducing reliance on single-use plastics.

By integrating toxicological evidence, regulatory perspectives, and material degradation research within a focused context, this review addresses a critical gap in the literature. Unlike broader plastic pollution reviews, this work provides a detailed, interdisciplinary framework specifically centered on single-use bottled water. Its novel contribution lies in bridging analytical, environmental, and public health domains to support a more comprehensive understanding of plastic contamination and its consequences. This positions the review as a foundation for developing targeted mitigation strategies and regulatory reforms tailored to single-use plastic water bottles, a key but often overlooked source of micro- and nanoplastic exposure. Comprehensive research is still needed to fully understand the health and environmental impacts of nano- and microplastics in bottled water, and implementing regulatory measures to manage these impacts will be key to promoting both public health and sustainability. By addressing these issues holistically, we can build a more sustainable future.

Environmental Implication

The environmental implications of nano and microplastics from single-use plastic water bottles, as explored in this review study, have direct and severe consequences for human health. These plastic particles infiltrate drinking water, leading to chronic exposure that poses risks such as respiratory diseases, reproductive issues, neurotoxicity, and potential carcinogenic effects. The study highlights the lack of standardized testing methods, making it difficult to assess long-term health impacts accurately. Without regulatory action, continued plastic contamination may exacerbate public health crises, emphasizing the urgent need for stricter policies, improved monitoring, and sustainable alternatives to mitigate the risks of plastic exposure.

CRediT authorship contribution statement

Zhi Chen: Writing – review & editing. **Sarah Sajedi:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chunjiang An:** Writing – review & editing, Supervision.

Authors' Contributions

SS conceived the Article conducted the literature analysis and wrote the manuscript. CA revised the manuscript. ZC revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Code Availability

Not applicable

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The data supporting this article have been included in the article.

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