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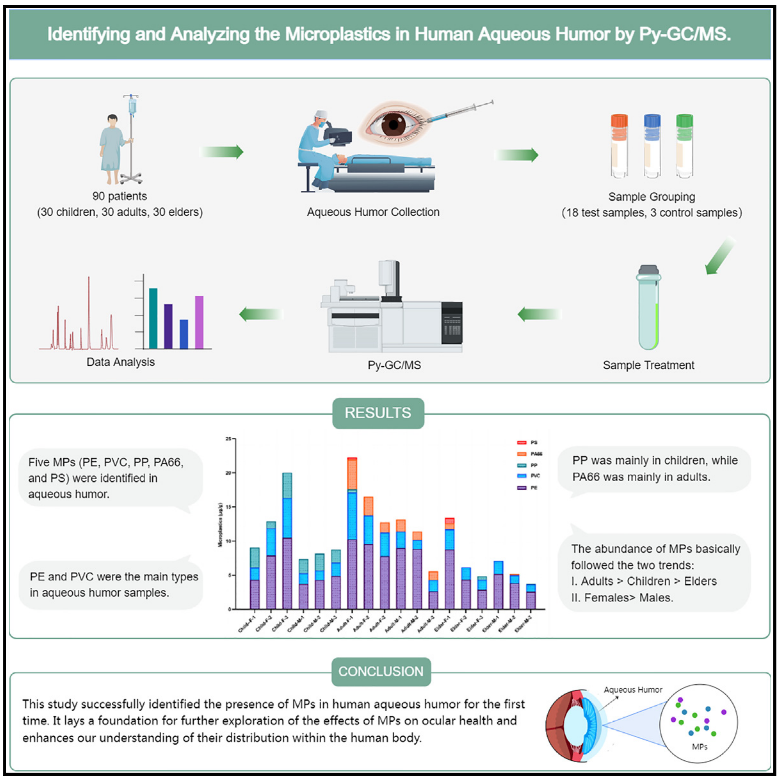
Graphical abstract



Highlights

- MPs were detected in human aqueous humor for the first time
- PE and PVC were identified as the predominant polymers in the aqueous humor
- PP was more prevalent in children, while PA66 was more common in adults
- A higher abundance of MPs was observed in the aqueous humor of adult and female individuals

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In brief

Environmental chemistry; Environmental health; Pollution

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Identifying and analyzing the microplastics in human aqueous humor by pyrolysis-gas chromatography/mass spectrometry

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SUMMARY

Microplastics (MPs), an emerging global pollutant, pose potential risks to human health and have garnered increasing attention. Previous research has identified MPs in various human tissues and organs, but not in the aqueous humor of the eyes. This study used pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) to explore MPs in aqueous humor. Five types of MPs—polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polyamide 66 (PA66), and polystyrene (PS)—were found, with PE and PVC being most common. PP was more prevalent in children, while PA66 was more common in adults. MPs abundance generally followed the trend: adults > children > elders among age groups, and females > males between gender groups. Notably, this study is the first to confirm MPs in human aqueous humor, providing a foundation for future research on their impact on intraocular health and enhancing our understanding of the MPs' body distribution.

INTRODUCTION

With the advancement of modern industry, plastic products have become integral to human life, owing to their durability, practicality, and versatility. However, plastics can gradually deteriorate in the natural environment through an "aging" process influenced by various factors, including ultraviolet radiation, temperature fluctuations, photooxidation, mechanical wear, and biological effects.[1,2] When plastic decomposes into particles smaller than 5 mm, they are termed microplastics (MPs), and those smaller than 1 μm are called nanoplastics (NPs).[3] Currently, MPs are extensively found in water resources, the atmosphere, land, and biological populations.[4] MPs have been identified along coastlines globally, including polar regions and the Mariana Trench.[5] MPs have been found in the water of the Yellow River Liujiaxia Reservoir and Qinghai Lake in China.[6,7] Differences in the absorption of microplastic components have been observed in rice and maize.[8,9] Additionally, atmospheric MPs have been discovered in the lungs of Japan's wild migratory birds.[10] Accordingly, MPs have emerged as a recognized new global pollutant with potentially significant adverse effects on Earth's ecology.

Humans are not exempt from environmental impacts as integral components of Earth's ecology. Research demonstrates that humans are invariably exposed to MPs through gastrointestinal exposure, inhalation, and dermal contact with mucous membranes.[11] Among these routes, gastrointestinal exposure to MPs has two primary sources: direct contact and food chain transfer.[12] Consequently, the concentration of MPs in the human body and their potential health risks has garnered significant attention in the medical community.[13] Previous studies have confirmed the presence of MPs in various human tissues and organs. Austrian researchers identified a range of MPs in human feces in 2019.[14] In 2020, the first evidence of MPs in the human placenta was published.[15] Malaysian researchers discovered 11 microplastic components in human colon tissue for the first time in the same year.[16] In 2022, a Dutch study revealed microplastic particles in human blood.[17] Subsequently, Chinese researchers reported MPs in human blood clots.[18] Additionally, German scientists detected MPs in liver tissues from cirrhosis patients.[19] A UK team identified the accumulation of MPs in human lung tissue,[20] supported by findings of MPs in human sputum and alveolar lavage fluid.[21,22] In 2024, researchers analyzed variations in MPs abundance in urine between urban and rural areas in Chongqing, China.[23] Recent studies have also documented microplastic components in human heart and arteries,[24,25] testis,[26] and uterus.[27] Alarmingly, two studies have detected microplastic components in human breast milk,[28,29] indicating that microplastic particles are ingested, transferred, and resecreted by cells. In summary, extensive microplastic deposits are present in human tissues and organs.

However, the question remains: can microplastic particles infiltrate the aqueous humor? To date, no relevant studies have been published. The concern arises because the eyeball, a component organ of the human body, may allow MPs to enter through previously mentioned pathways. Still, its exposed nature

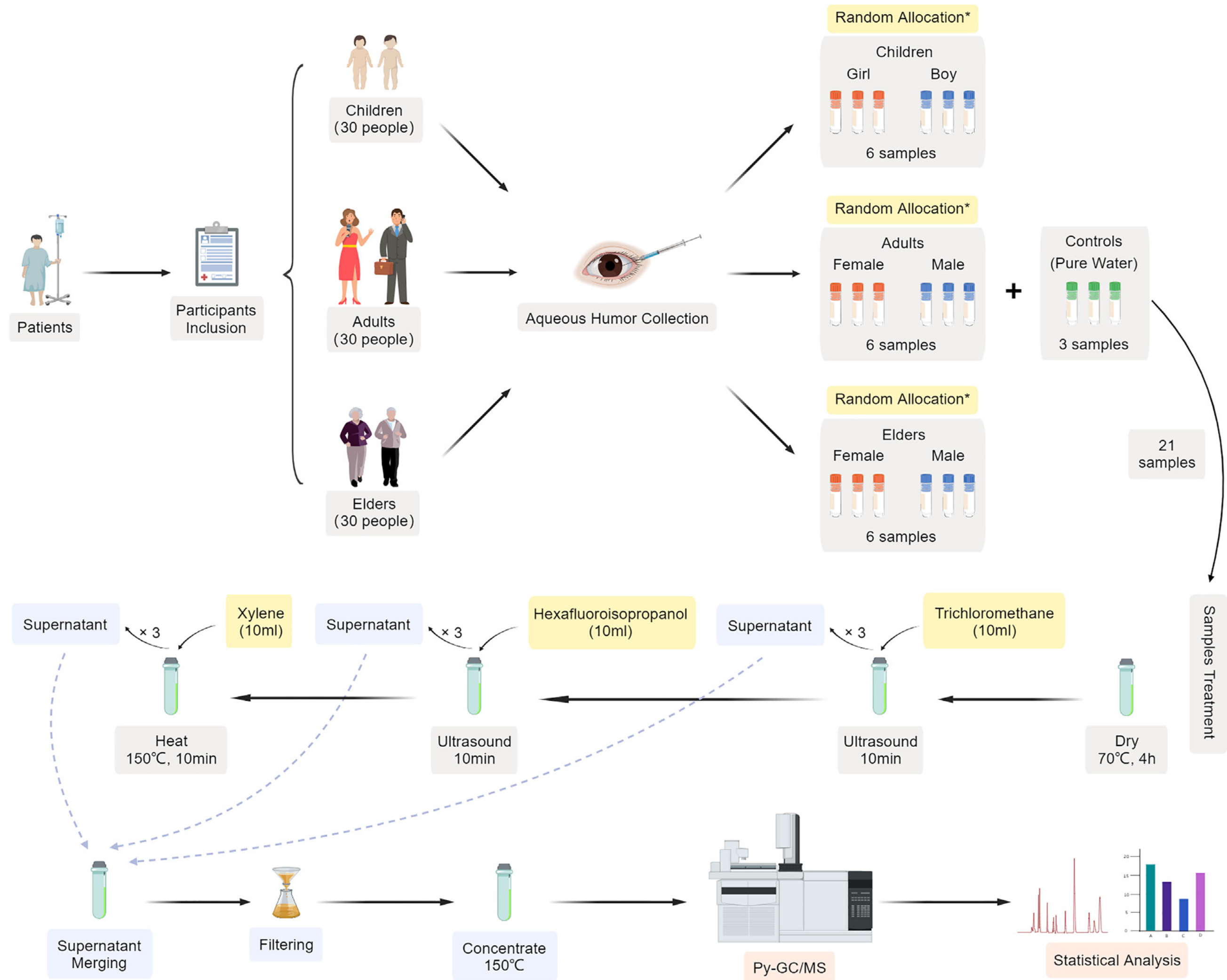


Figure 1. The flow chart of the study process

*: Random allocation means each testing sample (approximately 500 μL) was a composite of five randomly selected aqueous humor samples, labeled by gender and age group, resulting in six testing samples per age group (three from males and three from females).

potentially heightens its risk of MPs exposure compared to internal organs. Notably, a study indicated that contact lenses significantly contribute to microplastic release, potentially directly introducing approximately 90,000 microplastic particles to the eye annually.[30] Furthermore, aqueous humor—the clear fluid filling the eye's anterior and posterior chambers—is critical in maintaining physiological homeostasis by nourishing the eye and regulating ocular pressure. Alterations in its composition can impair eye health and lead to disease. Consequently, this study seeks to explore the presence of MPs in the aqueous humor of human eyes using the pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) technique. Py-GC/MS is an established method for separating and identifying volatile and non-volatile compounds, extensively applied in analyzing various synthetic polymers.[31] Given its capability to measure the pyrolysis products of specific polymers and to quantify the composition and content of MPs by mass concentration irrespective of particle size, this technique enables the comprehensive detection of MPs in the aqueous humor.[32]

This study encompassed 90 participants: 30 children, 30 adults, and 30 elders. Eighteen mixed aqueous humor samples were collected, and the Py-GC/MS method was used to detect the target eleven types of MPs. Subsequently, the study examined the compositional characteristics of MPs in aqueous humor and their correlation with age and gender. Figure 1 shows the process of this study. These polymers are prevalent in various plastic products that humans frequently encounter. The study confirmed MPs in the aqueous humor of human eyes and provided crucial foundational data for further investigation into the relationship between MPs and ocular health. Additionally, it offered unique insights into the distribution of MPs throughout the human body.

Table 1. Sample information and MNPs detection results in aqueous humor

Sample information					MNPs Detection results						
					Concentration (μg/g)						Quality[a] (ng)
NO.	Population	Age (mean)	Gender	Disease	Total	PE	PVC	PP	PA66	PS	Total
1	Child	3.3	F	CC	9.0638	4.3311	1.7881	2.9446	–	–	9.0638
2	Child	4.3	F	CC	12.8891	7.8780	3.9863	1.0248	–	–	12.8891
3	Child	5.1	F	CC	20.0213	10.4918	5.8117	3.7178	–	–	20.0213
4	Child	2.6	M	CC	7.3568	3.7174	1.5702	2.0692	–	–	7.3568
5	Child	5.7	M	CC	8.1770	4.2981	1.4010	2.4779	–	–	8.1770
6	Child	4.5	M	CC	8.7830	4.9005	1.9544	1.9281	–	–	8.7830
7	Adult	28.3	F	Myopia	22.2361	10.2642	6.8518	0.4642	4.3575	0.2984	22.2361
8	Adult	29.5	F	Myopia	16.4767	9.5620	4.1938	–	2.7209	–	16.4767
9	Adult	35.2	F	Myopia	12.7176	7.7894	3.4705	–	1.4577	–	12.7176
10	Adult	23.0	M	Myopia	13.1544	8.9905	2.4202	–	1.7437	–	13.1544
11	Adult	27.4	M	Myopia	11.4037	8.8799	1.2706	–	1.2532	–	11.4037
12	Adult	31.9	M	Myopia	5.5771	2.6218	1.6739	–	1.2814	–	5.5771
13	Elder	67.6	F	ARC	13.3843	8.7768	2.9295	–	0.9012	0.7768	13.3843
14	Elder	74.3	F	ARC	6.1680	4.3635	1.8045	–	–	–	6.1680
15	Elder	61.8	F	ARC	4.8616	2.8574	1.4818	0.5224	–	–	4.8616
16	Elder	73.5	M	ARC	7.1030	5.2142	1.8888	–	–	–	7.1030
17	Elder	67.1	M	ARC	5.2163	3.8366	1.1632	–	0.2165	–	5.2163
18	Elder	72.0	M	ARC	3.7713	2.5869	1.0667	0.1177	–	–	3.7713

F: female; M: male; CC: congenital cataract; ARC: age-related cataract; PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; PA66: polyamide 66; PS: polystyrene.
[a]Quality of MNPs per 1mg aqueous humor.

RESULTS

Recovery experiment

A spiking experiment was conducted to evaluate the recovery rates of MPs in aqueous humor using this method. The mixture of eleven microplastic standards was added to aqueous humor samples to create the spiked samples. Unspiked aqueous humor samples were also included as controls. All samples in the recovery experiment were analyzed following the same Py-GC/MS procedures described previously. The calculation equation and results for the recovery rates are detailed in Table S1. The recovery rate for each type of MPs exceeded 80%, demonstrating the applicability of the Py-GC/MS method for identifying and quantifying MPs in aqueous humor.

Py-GC/MS results of MPs in aqueous humor

The specific information and MPs detection results are summarized in Table 1. This study enrolled 90 patients (45 males and 45 females) whose aqueous humor was combined and divided into 18 samples (six children, six adults, and six elders with a gender ratio of 1:1) according to the aforementioned methods. The average age of the three groups was 4.25 ± 1.14 years (Child), 29.22 ± 4.14 years (Adult), and 69.38 ± 4.77 years (Elder). We analyzed 21 samples, including three control samples, using the Py-GC/MS technique. No MPs were detected in the control samples by matching the characteristics of fragments from standard substances. In contrast, five different MPs (PE, PVC, PP, PA66, and PS) were identified in the aqueous humor, with variations in composition across different ages and genders. To illustrate the experimental results more intuitively, the concentrations of MPs in each sample are plotted in Figure 2, revealing the following characteristics: (1) PE and PVC were the predominant microplastic components, present in all samples with relatively high proportions, ranging from 2.5869 to 10.4918 μg/g and 1.0667–6.8518 μg/g, respectively. (2) PP (1.0248–3.7178 μg/g) was predominantly found in children, whereas PA66 (1.2532–4.3575 μg/g) was primarily detected in adults. (3) PS was seldom detected; it was found in trace amounts in one adult sample (0.2984 μg/g) and another elder sample (0.7768 μg/g), both sourced from female donors. Additionally, MPs abundances in the aqueous humor samples varied significantly, with the No. 18 sample (a male elder) showing the lowest abundance (3.7713 μg/g) and the No.7 sample (a female adult) displaying the highest concentration (3.7713 μg/g). Lastly, six other target MPs (PMAA, PET, PC, PA6, PLA, and PBAT) were not detected in any samples.

Analysis of total MPs in aqueous humor across ages

Further analysis explored the relationship between MPs in aqueous humor and age groups. The average concentration of total MPs was quantified as 11.0485 ± 4.7926 μg/g in children, 13.5943 ± 5.5322 μg/g in adults, and 6.7508 ± 3.4429 μg/g in older people. Notably, the adult group exhibited the highest abundance, significantly differing from the elderly group (p = 0.0278), as shown in Figure 3A. However, no statistical differences were observed between adults and children (p = 0.4142) or older

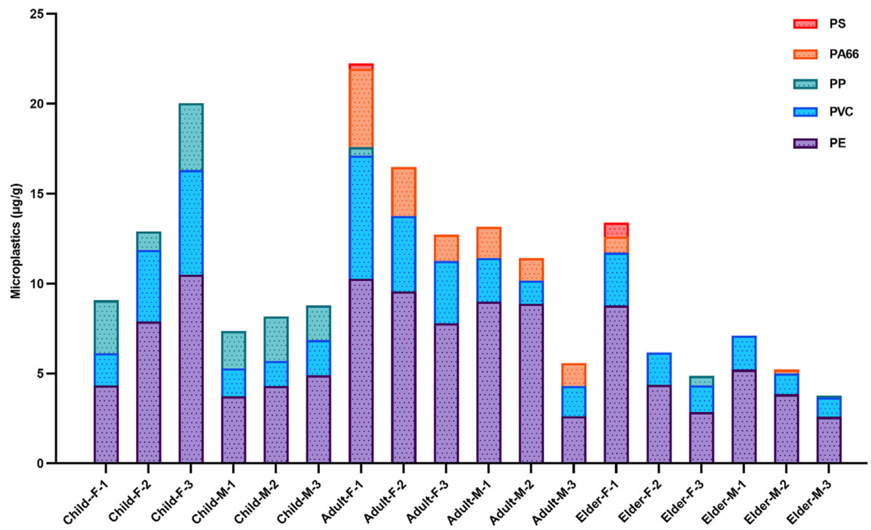


Figure 2. Concentrations of MPs in aqueous humor samples

Abbreviations: PE, polyethylene; PVC, polyvinyl chloride; PP, polypropylene; PA66, polyamide 66; PS, polystyrene; F, female; M, male.

people and children (p = 0.1047), also illustrated in Figure 3A. Pie charts indicate variations in the average proportions of MPs components among the groups. An apparent upward trend in the abundance of polyethylene (PE) with age is evident (Figures 3B–3D); polypropylene (PP) was predominantly found in children (Figure 3B); polyamide 66 (PA66) was a significant component in adults but was almost absent in the other groups (Figure 3C).

Analysis of total MPs in aqueous humor across genders

The correlation between MPs abundance and gender was also analyzed in this study. The content of MPs in the aqueous humor of the female population was significantly higher than that of the male population (p = 0.0292), with an average level of 13.0909 ± 5.8658 μg/g in female and 7.8381 ± 2.9850 μg/g in male (Figure 4A). In terms of the proportion of each microplastic component, there was not much difference between men and women (Figures 4B and 4C). It differed from the phenomenon that the composition of MPs changes among ages.

Analysis of major MPs detected in aqueous humor

Based on the previous results, PE, PVC, PP, and PA66 emerged as the most significant microplastic constituents in the aqueous humor. Consequently, we conducted distinct statistical analyses for each type of MPs. The chromatograms and mass spectrograms for these MPs in representative samples from each group are presented in Figures 5, 6, and 7.

Polyethylene

Polyethylene (PE) was identified as the predominant microplastic component in the aqueous humor, representing the most substantial proportion across all samples. Initially, the study explored the variance in PE concentrations among different age groups. The findings demonstrated a significantly higher content of PE in the aqueous humor of adults (8.0180 ± 2.7673 μg/g) compared to elders (4.6059 ± 2.2606 μg/g), with a statistical significance (p = 0.0414). Conversely, there was no statistical difference in PE levels between children (5.9362 ± 2.6751 μg/g) and adults (p = 0.2147), nor between children and elders (p = 0.3741), despite the apparent higher abundance of PE in adults (Figure 5A). Additional analysis indicated no statistical differences in PE concentrations between genders across any age groups (Child-F vs. Child-M, p = 0.1472; Adult-F vs. Adult-M, p = 0.3469; Elder-F vs. Elder-M, p = 0.4936) (Figure 5B).

Polyvinyl chloride

Polyvinyl chloride (PVC) was the second principal microplastic component in aqueous humor, detected in every sample. First, from the perspective of age, the concentration of PVC had no statistical difference among the three age groups (Child vs. Adult, p = 0.6225; Adult vs. Elder, p = 0.1010: Child vs. Elder, p = 0.2129) (Figure 6A). Next, the analysis of the PVC differences between the genders within age group showed that only the PVC in female adults (9.2052 ± 1.2754 μg/g) was significantly higher than that in males adults (6.8307 ± 3.6455 μg/g, p = 0.0478), while other groups showed no differences in statistics (Child-F vs. Child-M, p = 0.1317; Elder-F vs. Elder-M, p = 0.2422) (Figure 6B).

Polypropylene and polyamide 66

Polypropylene (PP) and polyamide 66 (PA66) were predominantly detected in the aqueous humor of children and adults, respectively. Consequently, inter-gender analyses were conducted only within each age group. The results indicated no significant differences in the concentrations of PP and PA66 between genders (PP: Child-F vs. Child-M, p = 0.6470; PA66: Adult-F vs. Adult-M, p = 0.1720) (Figures 7A and 7B).

DISCUSSION

This study utilized Py-GC/MS to detect 11 MPs in human eyes, confirming their presence in human aqueous humor for the first time. Five types of MPs—PE, PVC, PP, PA66, and PS—were identified in the samples. PE and PVC were the predominant types, and MPs composition varied with age. MPs abundance was highest in adults, followed by children, and lowest in older people, with a significant statistical difference observed only between the adult and elderly groups. Moreover, the concentration of MPs was significantly higher in females than in males. A similar age and gender trend was observed when analyzing individual microplastic components (PE, PVC, PP, and PA66). However, statistical differences were only noted for PE between adults and older people and for PVC between males and females within the adult group. Based on this research, the following points are worth further discussion.

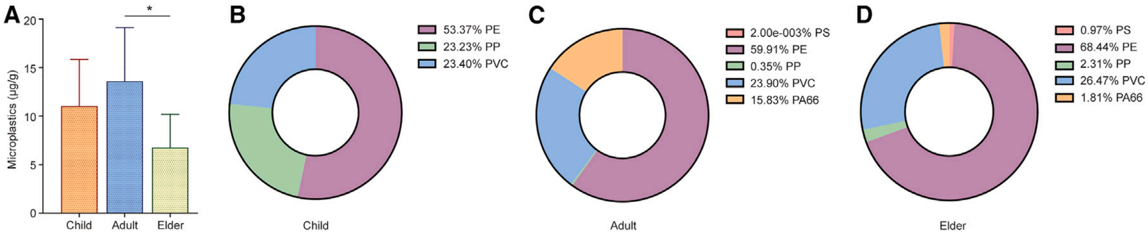


Figure 3. Analysis of total MPs in aqueous humor across ages

(A) Comparison of total MPs among age groups.

(B–D) Average proportion of MP components in each age group. All differences with $p < 0.05$ are indicated with an asterisk (*). Abbreviations: PE, polyethylene; PVC, polyvinyl chloride; PP, polypropylene; PA66, polyamide 66; PS, polystyrene.

Reasons for the existence of five types of MPs in aqueous humor

First, the five MPs (PE, PVC, PP, PA66, and PS) detected in the aqueous humor, predominately PE and PVC, are present in all samples. These substances are commonly encountered as plastic components in daily life. PE, a fundamental raw material in the chemical industry, constitutes a quarter of total plastic output and is widely employed in producing films, containers, pipes, cables, wires, and daily necessities.[33] PVC, known for its versatility, widespread production, and affordability, is extensively utilized in pipes, construction, packaging, clothing, and household products.[34] Statistics forecast global PVC production to reach 59.72 million tons annually by 2030.[34] Therefore, it is not surprising that these two components are the most prevalent MPs in the aqueous humor.

Secondly, PP has become one of the most extensively utilized thermoplastic polymers in recent decades, attributed to its outstanding properties, cost-effectiveness, and ease of processing.[35] However, our results indicated that PP predominantly occurred in children's samples rather than all. PP applications include food packaging, containers, toys, plastic furniture, clothing, diapers, carpeting, mats, and disposables. These consumer products frequently come into prolonged, close contact with infants and children. Additionally, due to the oral stage of a child's development, their exposure to PP is more direct. Therefore, this may explain why PP was mainly detected in the aqueous humor of children and not in other age groups.

Thirdly, another notable finding from the study is that PA66 predominantly exists in adult aqueous humor. PA66 is widely used in various applications, such as textiles, cooking utensils, carpets, fishing lines, electro-insulating elements, pipes, machine parts, zip ties, conveyor belts, and guitar nuts. The explanation for this phenomenon is currently unclear; however, research examining the migration of PA6 and PA66 from kitchenware to food offers some insights. This study revealed that PA66 oligomers were the primary migrants from the utensils, and their migration volumes significantly exceeded those of PA6.[36] This suggests that PA66 particles can migrate, leading to ingestion through food or contact with kitchen utensils. Additionally, the greater ease of PA66 particle migration compared to PA6 might explain another observation: the scarcity of PA6 in the aqueous humor.

Fourthly, PS is one of the most widely utilized plastic materials across various industries.[37] In this study, PS was only detected in two female samples, likely due to the abundance of PS in other samples not meeting the Py-GC/MS detection threshold. This might also explain the absence of other MPs (PMAA, PET, PC, PA6, PLA, and PBAT) in aqueous humor. Other reasons for the six undetected MPs could include their inability to permeate the blood-aqueous barrier or complete degradation before or within the body. For instance, PLA and PBAT are among the most extensively produced and utilized genuinely biodegradable bioplastics.[38]

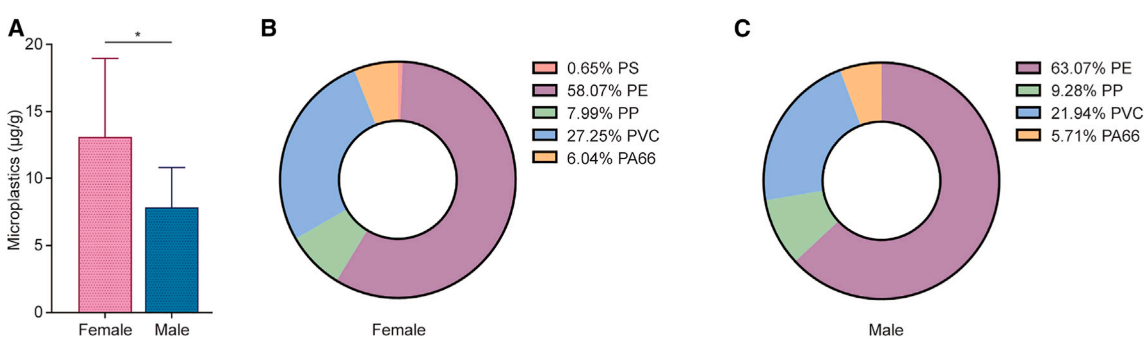
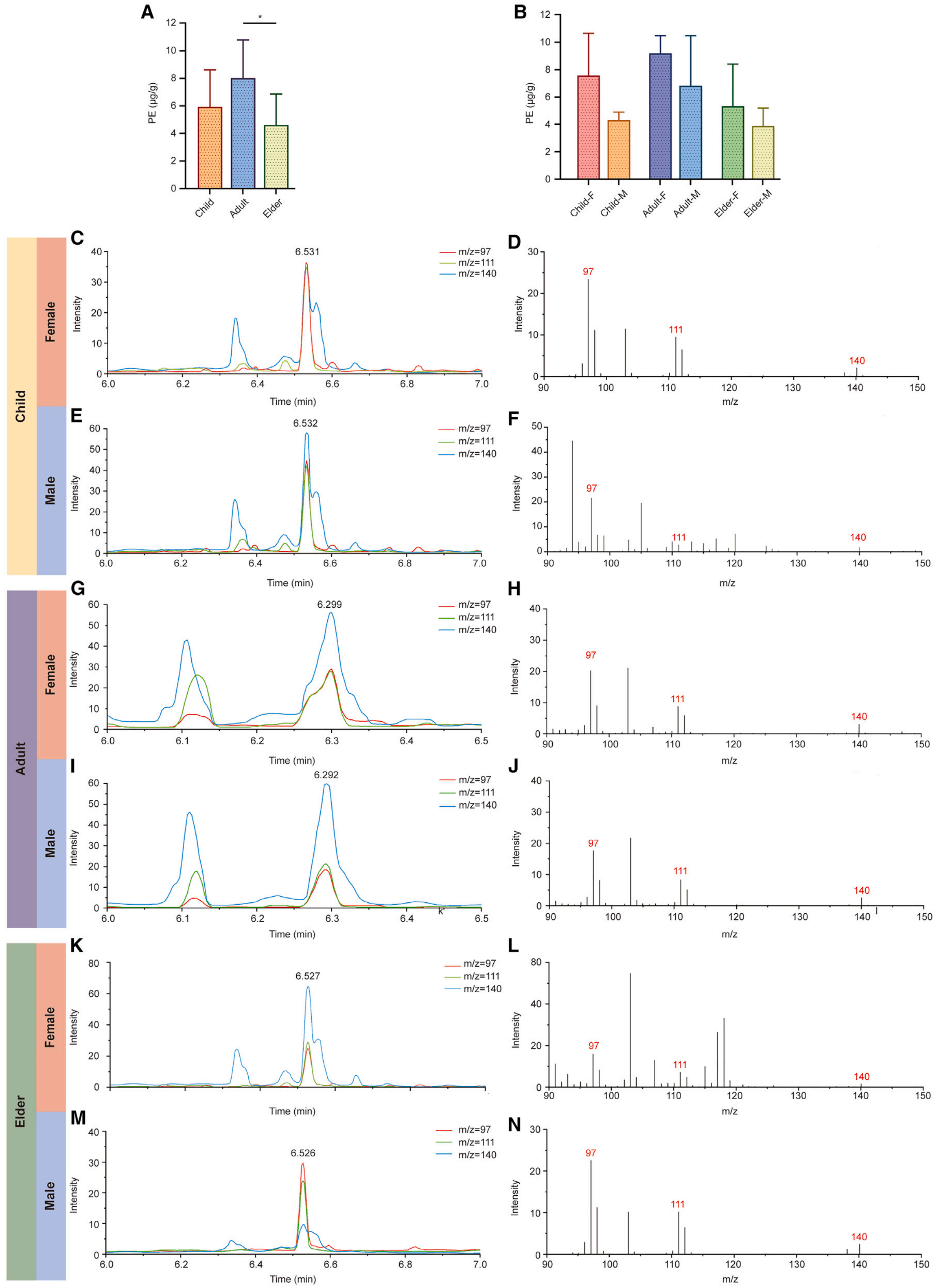


Figure 4. Analysis of total MPs in aqueous humor across genders

(A) Comparison of total MPs between gender groups.

(B and C) Average proportion of MP components in each gender group. Differences significant at $p < 0.05$ are indicated with an asterisk (*). Abbreviations: PE, polyethylene; PVC, polyvinyl chloride; PP, polypropylene; PA66, polyamide 66; PS, polystyrene.



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Implications of variations in MPs abundance across genders and ages

This study's abundance of total MPs and each component primarily exhibited two trends: adults > children > elders and females > males. While there were no statistically significant differences among most groups, this could be attributed to the limited sample size; larger samples might reveal more pronounced differences. These patterns suggest that the variations in MPs abundance in aqueous humor may be associated with differing daily exposures to MPs across genders and ages. A 2022 study conducted a risk assessment on MPs exposure from processed foods, citing that the estimated daily intake (EDI) for female adults (17.6 ng kg^{-1} d^{-1}) was slightly higher than that for male adults (12.6 ng kg^{-1} d^{-1}).[39] Another study assessed microplastic exposure from beverage consumption across various age groups, finding the highest daily and annual exposure rates among males aged 15–18 and females aged 19–64.[40] This increased exposure in females may be linked to their higher consumption of beverages. In contrast, males consume more sparkling soft drinks, cold tea, and other traditional beverages. These studies indirectly support our findings. Furthermore, the frequent occupational exposure of adults to MPs at work compared to different age groups could also influence microplastic levels. Research involving workers in industrial settings indicated that MPs could be absorbed through the skin, hair, and saliva, serving as conduits for MPs to enter the body and impact levels found in occupational settings.[41] Hence, the effects of lifestyle and occupational environments on microplastic exposure merit further investigation.

Potential pathway of MPs transfer into the aqueous humor of the eye

To date, research provides only a partial answer to this question; however, insights can be garnered from producing aqueous humor and related literature. It is well established that aqueous humor is secreted by the non-pigmented epithelium of the ciliary body (pars plicata), originating from the highly vascular stroma beneath the ciliary muscle and formed through processes, such as diffusion, ultrafiltration, and active secretion.[42] Aqueous humor comprises water (98%), protein, lactic acid, glucose, ascorbic acid, and various electrolytes.[43] Given that small molecules can transfer from the blood to the aqueous humor, microplastic particles might follow a similar pathway. This assumption is supported by detecting microplastic particles in breast milk, urine, and seminal fluid, as found in studies conducted in 2022, 2023, and 2024.[28,29,44] The presence of MPs in these fluids suggests that MPs may integrate into and become components of these body fluids once internalized. Moreover, the occurrence of MPs in aqueous humor implies that the blood-aqueous barrier may be permeable to MPs, a notion also supported by studies showing MPs traversing the blood-brain barrier. For instance, one study detected MPs in rat brain tissue post-ingestion.[45] In contrast, another study indicated that once MPs enter the bloodstream, they could be disguised by biomolecules, facilitating their entry into cells or crossing the blood-brain barrier.[46] Therefore, it is possible that MPs could enter the epithelial cells of the ciliary body from the bloodstream and subsequently be transferred into the aqueous humor. This hypothesis, however, requires further verification through additional studies.

The comparison of MPs in aqueous humor with other body fluids

Several prior studies have documented the composition and concentration of MPs in other human body fluids, with findings indicating variability in microplastic characteristics across different fluid types. The presence of MPs in blood was first documented in a 2022 study, which revealed that blood samples primarily contained three plastic particles, including PE, PS, and PET.[17] However, a 2024 study identified 24 distinct microplastic particles in blood samples, including ordinary MPs like PE, PS, PET, and PP.[47] Two independent studies investigated the MPs in urine and semen, revealing that PE, PS, and PA66 were the predominant types in urine,[23] while PE, PS, PVC, PC, PP, PET, ABS, and PTFE were identified in semen,[48] In addition, two additional studies have covered the characteristics of MPs in breast milk; however, their outcomes present marked discrepancies. One study revealed that the predominant MPs in breast milk mainly consist of PE, PVC, and PP.[47] In contrast, the main components detected in another study were polyamide (PA) and polyurethane (PU).[28] With reference to the aforementioned detection data, the conclusion of the first study is potentially more reliable. Since there is currently no evidence suggesting that the MPs in the blood are mainly PA and PU, the possibility that these two components are the predominant particles in breast milk cannot be high. Furthermore, a recent study points out that PE is the most dominant microplastic in sputum, bronchoalveolar lavage fluid, and pleural fluid, with a proportion of over 60% in each.[49]

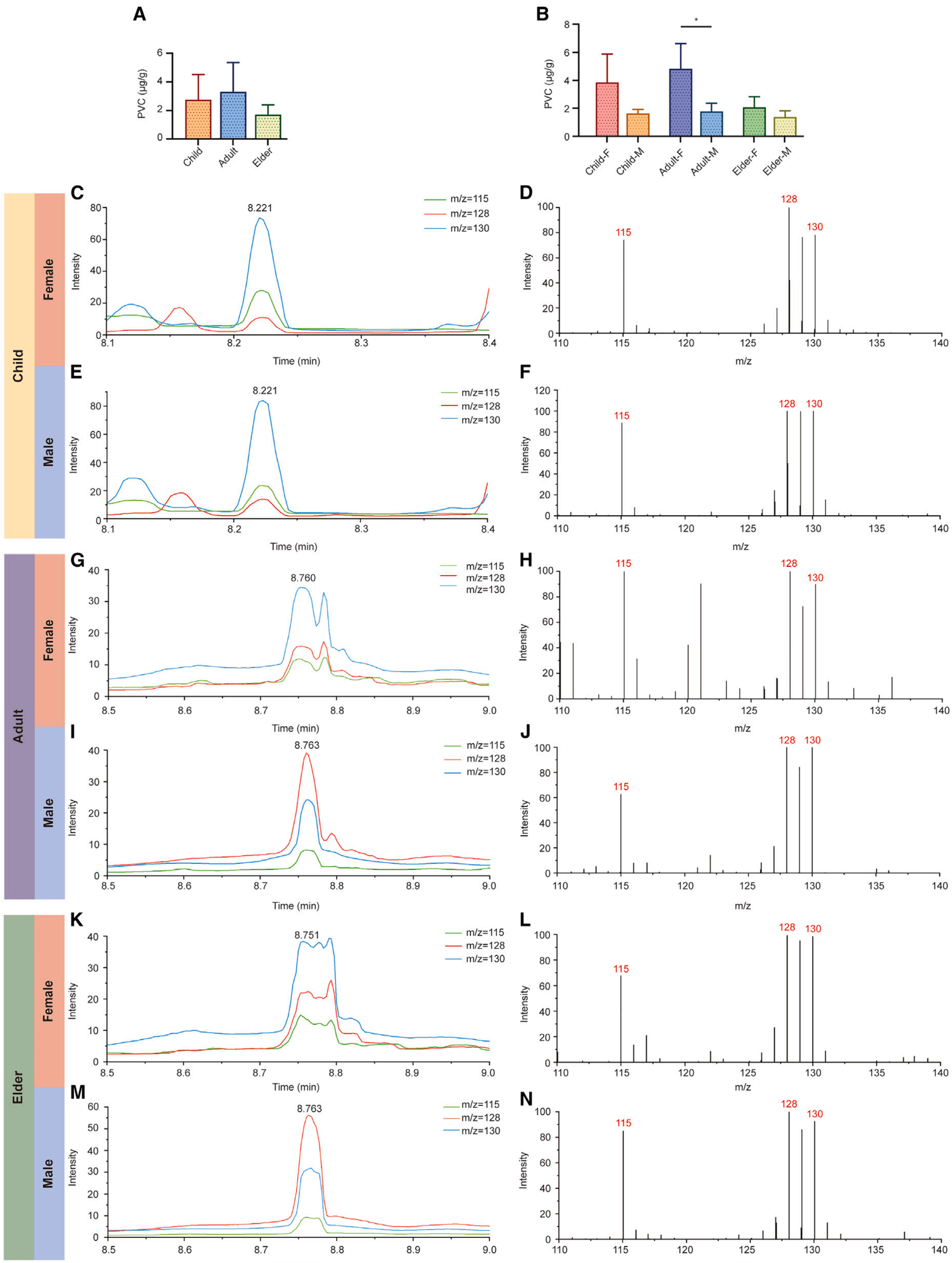
In summary, blood contained the most considerable quantity of MPs in body fluids, while other body fluids exhibited relatively few. The findings in this study's aqueous humor followed a similar trend. Second, the MPs identified in all body fluids were predominantly composed of PE, PS, PP, and PVC, which were also the prominent particles in the aqueous humor. Notably, PE constituted the most significant proportion across all body fluids. This observation underscores the necessity for increased attention to the potential impacts of PE on the human body. Regrettably, it is challenging to directly compare the MPs' abundance between aqueous humor and other body fluids, mainly

Figure 5. The analysis of PE in aqueous humor

(A) Comparison of PE among age groups.

(B) Comparison of PE between genders in each age group.

(C–N) The chromatograms (on the left side) and mass spectrograms (on the right side) of PE in representative samples are shown. The chromatograms show overlapped peaks of three ions at the same retention time in each sample. The mass spectrograms display characteristic quantitative ions for PE at m/z 97, 111, and 140, respectively. Differences significant at $p < 0.05$ are indicated with an asterisk (*). Abbreviations: PE, polyethylene; F, female; M, male; m/z, mass-to-charge ratio.



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due to distinct detection approaches leading to inconsistent concentration units.

The impact of MPs on ocular health: A negative influence?

Although no study has directly explored the effects of MPs on intraocular tissues, existing research provides some indications. A recent study identified the presence of MPs, specifically PS, PA66, and PVC, in the vitreous humor for the first time. This study also assessed the correlation between MPs levels and ocular health parameters using rigorous partial least squares path modeling (PLS-PM). The findings revealed a positive direct correlation between MPs in the vitreous and adverse ocular health impacts,[50] suggesting that increased MPs levels in the vitreous are significantly associated with ocular damage. However, this association does not directly address MPs' damage to intraocular tissues. Another study examined the effects of PS on the neuromuscular, retinal, and reproductive phenotypes of fruit flies, discovering that PS reduced the frequency of spontaneous junction currents at synapses and altered the receptor potential amplitude in the retina.[51] However, this subject was not human-based. These findings imply that MPs could negatively affect or even damage intraocular tissues. The impact of MPs on ocular health can be categorized into intraocular and ocular surface health effects. While experimental data on MPs damage to intraocular tissues is lacking, one study explored the toxic effects of PS on ocular surface tissues both *in vivo* and *in vitro*.[52] The study demonstrated that PS decreased cell viability, induced oxidative stress, and triggered apoptosis in human corneal epithelial cells, as well as reduced goblet cell numbers in the conjunctival sacs of mice's lower eyelids. Additionally, dry eye-like damage on the ocular surface and inflammation in mice's conjunctiva and lacrimal glands were noted. Moreover, extensive research in other organs confirms that MPs can cause adverse effects through inflammation, oxidative damage, microbial dysbiosis, and toxic effects.[53] Based on the aforementioned studies, MPs are likely to affect ocular tissue adversely. However, direct evidence remains insufficient, and further research is warranted.

The potential biotoxicity of MPs

To date, the presence of MPs has been documented in various human tissues, including feces, blood, placenta, lower airway, lungs, etc. Although relevant studies on the eyes are scarce, biotoxicological investigations of MPs have gradually increased. Firstly, many investigations have pointed out the crucial role of oxidative stress in toxicity elicited by MPs. Exposure to PS can increase intracellular reactive oxygen species (ROS) levels, promote the generation of superoxide anions, upregulate superoxide dismutase (SOD) expression, and ultimately induce oxidative stress.[54,55] The induction effect of oxidative stress from PS can cause cardiotoxicity in carp hearts.[56] Another study also found that MPs could promote aortic inflammation and elevate the levels of adhesion molecules in the aortic tissue.[57] Secondly, multiple studies have confirmed that MPs can induce an inflammatory response by promoting the release of cytokines like IL-6, IL-8, IL-10, and IL-1β, leading to tissue impairment.[58,59] For example, Meng et al. demonstrated that MPs could activate the NF-κB p65 signaling pathway, upregulate the expression of pro-inflammatory factors (TNF-α, iNOS, IL-1β, and IL-6), leading to inflammatory infiltration and tissue damage in the kidney.[60] Also, MPs can exert toxic effects on the urinary system in multiple other respects, such as oxidative stress, apoptosis, ferroptosis, and cell-cycle arrest.[61] Thirdly, there is evidence that MPs exposure may lead to disturbances of metabolic homeostasis. For example, Shi et al. found that oral exposure to MPs led to insulin instances and even diabetes through the crosstalk between the gut and liver[62]; PS can affect the metabolism by disturbing the amount of proteobacteria and Fusobacteria in the zebrafish model[63]; a study even suggested that maternal exposure to MPs during gestation can cause metabolic disorders in their offspring.[64] Fourthly, speaking of the transgenerational harm, the reproductive toxicity caused by MPs cannot be ignored.[65] Such as PS exposure has been reported to cause gonadal damage in Zebrafish[66]; MPs were also observed to induce fibrosis in ovarian granulosa cells and decrease sperm viability in rat model.[67,68] Fifthly, the genotoxic potential of MPs also raised people's concerns. PS-MPs led to DNA damage in both the nucleus and mitochondria,[69] which can even occur at a relatively low MPs concentration.[70] Noticeably, the biotoxicity effects of MPs are not merely limited to this, and more efforts are required from scholars for research and verification.

Conclusion

Our study successfully identified the presence of MPs in human aqueous humor for the first time. We detected five microplastic components—PE, PVC, PP, PA66, and polystyrene (PS)—distributed across ages and genders. PE and PVC were the predominant types, while PP and PA66 were primarily found in children and adults separately. The abundance of MPs in the aqueous humor generally followed the trend of adults > children > elders among age groups and the trend of females > males between gender groups. Notably, our study lays a foundation for further exploration of the effects of MPs on ocular health and enhances our understanding of their distribution and transfer within the human body. MPs have emerged so abruptly throughout human evolution that our bodies have not yet evolved mechanisms to recognize or cope with them. In our eyes, as uninvited visitors, MPs may have potential adverse effects far more significant than currently recognized.

Figure 6. The analysis of PVC in aqueous humor

(A) Comparison of PVC among age groups.

(B) Comparison of PVC between genders in each age group.

(C–N) The chromatograms (on the left side) and mass spectrograms (on the right side) of PVC in representative samples are shown. The chromatograms show overlapped peaks of three ions at the same retention time in each sample. The mass spectrograms display characteristic quantitative ions for PVC at m/z 115, 128, and 130, respectively. Differences significant at $p < 0.05$ are indicated with an asterisk (*). Abbreviations: PVC, polyvinyl chloride; F, female; M, male; m/z, mass-to-charge ratio.

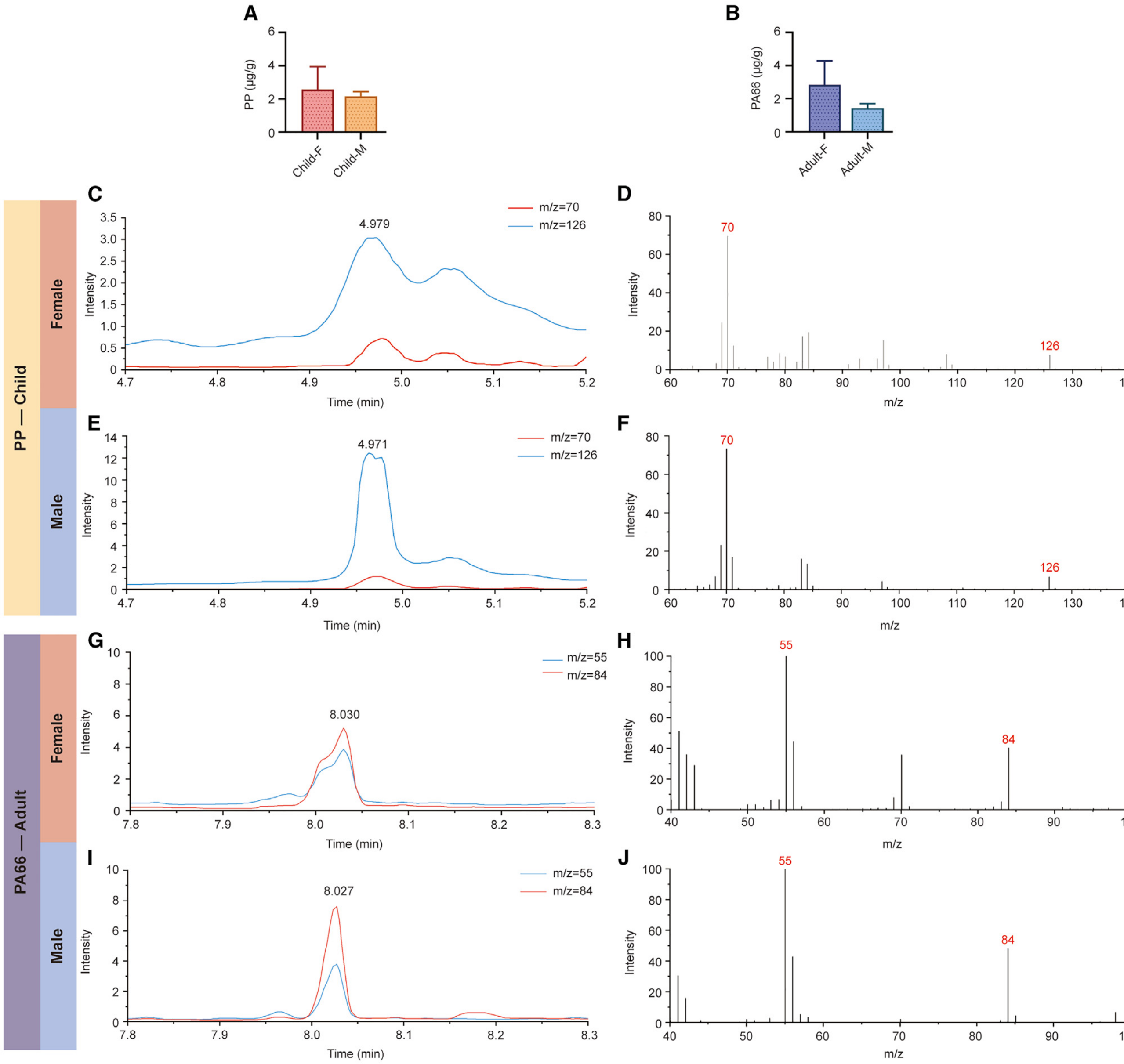

Figure 7. The analysis of PP and PA66 in aqueous humor

(A) Comparison of PP between genders in child group.

(B) Comparison of PA66 between genders in the adult group.

(C–F) The chromatograms (on the left side) and mass spectrograms (on the right side) of PP in representative samples are shown.

(G–J) The chromatograms (on the left side) and mass spectrograms (on the right side) of PA66 in representative samples are shown. The chromatograms show overlapped peaks of three ions at the same retention time in each sample. The mass spectrograms display characteristic quantitative ions for PP at m/z 70 and 126, respectively. The mass spectrograms display characteristic quantitative ions for PA66 at m/z 55 and 85, respectively. Differences significant at $p < 0.05$ are indicated with an asterisk (*). Abbreviations: PP, polypropylene; PA66, polyamide 66; F, female; M, male; m/z, mass-to-charge ratio.

However, our understanding remains in its infancy, and much exploration is still required.

Limitations of the study

This study assessed MPs in aqueous humor using standardized detection and data analysis methods but exhibited several limitations. Firstly, instead of analyzing individual aqueous humor samples, the study pooled five samples into a single test sample. This approach was adopted due to uncertainties about MPs in human aqueous humor, as this research was exploratory. Pooling samples was deemed a reasonable strategy to maximize the detection of MPs. However, this method precluded further analysis of potential correlations between MPs' abundance of aqueous humor and participants' lifestyle habits, an aspect that warrants future investigation. Additionally, the pooling sample approach also brings some potential limitations, but the impact of these limitations on this study was not significant. The detailed contents are in the Table S2. Secondly, the inability to detect certain MPs does not necessarily indicate their absence. Still, it may instead be attributable to their concentrations falling below the Py-GC/MS detection threshold or due to the current technological limitations of Py-GC/MS, which can only identify 11 types of MPs. Hence, further verification using alternative techniques is essential. Thirdly, while the study aimed to include a diverse age range of participants, the actual age distribution was discontinuous due to the typical ages of patients seeking treatment, alongside ethical and clinical constraints. Finally, although the study incorporated 18 test samples from 90 participants, participant biases due to the single-center study might limit the applicability of the findings to broader populations and regions. More precisely, since the participants enrolled in this study at our hospital were mainly from the five northwestern provinces of China, the findings of this study primarily reflect the MPs' characteristics in the aqueous humor of the population in the northwest region of China. Future studies are necessary to enhance the research on MPs in ocular contexts and broaden our comprehension of this issue. Nonetheless, this study reveals the importance of addressing human exposure to MPs. Specifically, the novel detection of MPs in intraocular fluids highlights the urgent need for more stringent measures to reduce such exposure. The implications of this research for reducing MPs exposure and informing public health policy are detailed in Table S3.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hong Yan (yan2128ts@hotmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The published article includes all datasets generated or analyzed during this study.
- This study did not generate any code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

K.Z.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing—original draft, visualization, and funding acquisition. L.Y.: methodology, investigation, resources. L.Q. and N.H.: validation and resources. L.C. and J.W.: data curation. H.Y.: conceptualization, methodology, investigation, writing—review and editing, supervision, project administration and funding acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
polystyrene (PS)	CHIMEI, China	PG-33
polyethylene (PE)	Sinopec, China	PEM-1850A
polypropylene (PP)	Sinopec, China	F401
polymethyl methacrylate (PMAA)	CHIMEI, China	CM-211
polyvinyl chloride (PVC)	Shang Hai Chlor-Alkali Chemical, China	SG5
polyethylene terephthalate (PET)	CR CHEM-MAT, China	CR-8863
polycarbonate (PC)	TEIJIN, Japan	PC94
polyamide 6 (PA6)	Ube Industries, Ltd., Japan	1013B
polyamide 66 (PA66)	Hua Fon, China	EP-158
polylactic acid (PLA)	HISUN, China	REVODE110
polybutylene adipate terephthalate (PBAT)	Blue Ridge Tunhe Sci. & Tech., China	TH801T
Ethanol $\geq$95%	TITAN, China	64-17-5
trichloromethane	HUSHI, China	YH-10006818
hexafluoroisopropanol	Aladdin, China	920-66-1
xylene	TITAN, China	1330-20-7
PTFE Membrane, Φ47mm*0.45μm	Anpel, China	SCEQ-CM0311
Software and algorithms		
OriginLab 2024	OriginLab Corporation	Version 10.1.0
GraphPad Prism 9	GraphPad Software	Version 9.0.0
Other		
Water Purification System	Milli-Q®, MERK, Germany	Elix® Essential
Pyrolysis System	Frontier, Japan	Py-3030D
Gas Chromatography System	Shimadzu, Japan	GC 2020
Mmass Spectrometry	Shimadzu, Japan	MS-QP 2020

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

The participants in this study were recruited from our hospital from October 2023 to April 2024, and were categorized into three groups: the child group (children with congenital cataracts (CC)), the adult group (myopia patients undergoing intraocular Implantable Collamer Lens (ICL) implantation), and the elder group (patients with age-related cataracts (ARC)). Researchers collected aqueous humor samples for each patient during surgeries. Inclusion criteria are detailed in Table S4. The rationale for selecting these populations includes 1) representation of different age groups for analyzing potential age-related differences in MPs composition; 2) feasibility and consistency in collecting aqueous humor samples, as all procedures involved anterior segment surgery, ensuring similar procedural times and sample collection methods. Approximately 100 μl of aqueous humor was extracted via corneal paracentesis using a 30-gauge needle from each patient. Each group consisted of 30 aqueous humor samples (15 males and 15 females), and each testing sample (approximately 500 μL) was a composite of five randomly selected aqueous humor samples labeled by gender and age group, resulting in six testing samples per age group (three from males and three from females). Additionally, three control samples of ultrapure water (approximately 500 μL each) were included. Therefore, 21 aqueous humor samples were analyzed for MPs detection.

Ethics

All the participants meeting the study's inclusion criteria and providing informed consent. Written informed consent was obtained from participants or their parents; written informed assent was obtained from competent older children. This study adhered to the Helsinki Declaration guidelines and received approval from the Ethics Committee of Xi People's Hospital (Xi'an Fourth Hospital) (No: KJLL-Z-H-2024011).

METHOD DETAILS

Collection of aqueous humor samples

Aqueous humor samples were collected during the phacoemulsification of pediatric congenital cataracts and adult age-related cataracts (ARC) and the intraocular lens (ICL) implantation in myopic adults. All procedures, including the collection of aqueous humor, were conducted by an experienced ophthalmologist (Hong Yan) in a sterile operating room environment. Collection occurred at the beginning of each surgery. Before injecting viscoelastic substances, approximately 100 μl of aqueous humor was extracted per eye using a 27-gauge syringe for corneal paracentesis, carefully avoiding contact with the corneal endothelium, iris, or lens. The samples were immediately placed into 2 ml glass bottles, sealed, and stored at -20°C. They were later transported to the research laboratory for MPs detection via Py-GC/MS. Ultra-pure water control samples were retrieved using the same syringe, stored under identical conditions, and obtained from Elix® Essential, Milli-Q® (MERK, Germany).

Sample pretreatment

We pretreated the samples using the following steps before analyzing MPs in aqueous humor using PyGC/MS. Initially, each sample was transferred into a 20 ml aseptic glass sample bottle and weighed (wet weight post-peeling), then dried at 70°C for 4 hours and reweighed (dry weight post-peeling). Subsequently, we added 10 ml of trichloromethane (for extracting PS, PVC, PMMA, PC, PLA, and PBAT) directly to the sample bottle and subjected it to ultrasonication for 10 minutes. We extracted the supernatant and repeated the step mentioned above three times. Next, the remaining samples were transferred to a different glass bottle, to which 10 ml of hexafluoroisopropanol (for extracting PET, PA, and PVC) was added. The samples were then ultrasonicated for 10 minutes, and the supernatant was retained. This step was repeated three times. Subsequently, we transferred the residual solution to a new glass bottle and replaced the solvent with 10 ml of xylene (to extract PP and PE). The samples were heated on a plate at 150°C for 10 minutes, and the supernatant was preserved. This step was also conducted three times. Finally, we combined all supernatants, filtered them using qualitative filter paper to collect the soluble substances, and heated them 150°C to the concentrated solution. The concentrated solution was then added into the crucible of Py-GC/MS using a glass pipette. Once the solvent in the crucible evaporated utterly, the sample was subjected to Py-GC/MS analysis.

Py-GC/MS detection of MPs

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is utilized to identify polymer types and quantify their mass concentrations with high sensitivity; however, it does not count particles. Currently, this technique can quantify 11 types of MPs, including polystyrene (PS), polyethylene (PE), polypropylene (PP), polymethyl methacrylate (PMAA), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polycarbonate (PC), polyamide 6 (PA6), polyamide 66 (PA66), polylactic acid (PLA), and polybutylene adipate terephthalate (PBAT). In our study, MP detection was conducted using a pyrolysis instrument (Py-3030D, Frontier), gas chromatography (GC 2020, Shimadzu), and mass spectrometry (MS-QP 2020, Shimadzu). The concentrated solution of aqueous humor samples was introduced into the pyrolysis crucible using a glass pipette, and the pyrolysis was performed under a temperature of 550°C. Once the solvent evaporated entirely, the detection commenced. Helium was utilized as the carrier gas with a 1 mL/min flow rate, a split ratio of 1:5, and a linear velocity of 36.1 cm/s. The compounds were captured on a Rtx-5MS® chromatographic column (Thermo Fisher TG-5 Silims), which was 30 cm long, with an inner diameter of 0.25 mm and a film thickness of 0.25 μm. Chromatographic separation involved a temperature program set at 40°C for 2 min, followed by an increase to 320°C at a rate of 20°C/min and held for 14 min, totaling a runtime of 30 min. Mass spectrometry was conducted coupled to the gas chromatography. The interface temperature was maintained at 320°C to avoid cooling the oven. The ion source temperature was kept to 230°C, and selected ion monitoring mode (SIM) was employed to identify and quantify the target MP polymers within a scan range of 29-600 m/z in mass-to-charge ratio. All samples were identified by Lab Solutions software 4.45. The detection process and details are listed in Table S5.

Quality assurance and quality control (QA/QC)

To ensure the reliability of our study, QA/QC measures were implemented at each stage, with a primary focus on minimizing contact with plastic products. Initially, the syringe was the only plastic item the aqueous humor samples contacted. Ultra-pure water, drawn using the same syringe, was the control to mitigate any interference. Moreover, all experimental reagents were filtered through PTFE membranes (0.45 μm), and all glassware was thoroughly washed with ultra-pure water and oven-dried before use. During sample collection, medical staff wore cotton surgical gowns, caps, masks, and single-use nitrile gloves in the operating room. Efforts were made to minimize the contact time between the aqueous humor and the syringe, typically transferring the humor into a glass bottle within 10 seconds. These protocols were also adhered to during the ultra-pure water extraction for the control group. Throughout the sample treatment and Py-GC/MS analysis stages, experiments were conducted in a controlled laboratory environment free from plastic contact, with researchers wearing cotton lab coats and single-use nitrile gloves. The pyrolysis crucible used in Py-GC/MS was preheated with a spirit lamp for at least 5 minutes before sample addition, and blank tests were performed before each analysis to ensure the absence of plastic contamination. Control samples were analyzed before the aqueous humor samples, and no trace of the target 11 MPs was detected, indicating that the brief exposure to the syringe did not result in plastic particle dissolution into the aqueous humor. Thus, the final aqueous humor sample results accurately reflect the microplastic composition in the aqueous humor.

Recovery experiment method

The recovery experiment samples were divided into two equal parts. One part consisted of the aqueous humor samples spiked with 11 microplastic standards, while the other part was unspiked aqueous humor samples serving as controls. The pretreatment and Py-GC/MS detection procedures for the recovery experiment samples followed the same protocols described above. Recovery rates were calculated using the following equation:

$$\text{Recovery}\ (\%) = (\text{M1} - \text{M2}) / \text{Ms} * 100\%$$

where M1 (μg) represents the mass of a specific type of MP in the spiked sample, M2 (μg) represents the mass of the same type of MP in the unspiked sample, and Ms (μg) denotes the added mass of the corresponding standard MP.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification

Each microplastic produces characteristic fragments upon pyrolysis, identifiable by m/z characteristic quantitative ions. Overlapping these ions' wave peaks indicates the microplastic's characteristic fragments. To quantify MPs, standard substances for the 11 MPs were first analyzed to construct standard curves and equations before the formal sample detection. Standard curves were generated by analyzing graded quantities of the 11 standard plastics; these equations elucidate the quantitative relationship between the area under the characteristic ion wave peak and the mass concentration of MPs. After Py-GC/MS detection was conducted on all the samples, the mass concentration of each detected microplastic in the samples was calculated by inputting the area under the wave peak of the characteristic quantitative ion into the corresponding quantitative equation. The details of microplastic characteristic fragments and calibration curves of microplastics were all documented in Table S6.

Statistical analysis

All chromatograms and mass spectrograms were generated using OriginLab 2024 version 10.1.0. GraphPad Prism version 9.0.0 was utilized for statistical analysis and illustration production. The concentrations of microplastics in the samples were measured in mass concentration (μg/g), classified as continuous variable data. For calculation of mean values, data adhering to a normal distribution were expressed as Mean ± SD, and an Unpaired t-test was applied to compare two groups; data that did not follow normal distribution were presented as Median (IQR), with the Mann-Whitney U test used for intergroup comparisons. A P-value of less than 0.05 was deemed statistically significant for all outcomes.