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Microplastics in human blood: Polymer types, concentrations and characterisation using μFTIR

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ABSTRACT

Microplastics (MPs) are an everyday part of life, and are now ubiquitous in the environment. Crucially, MPs have not just been found within the environment, but also within human bodies, including the blood. We aimed to provide novel information on the range of MP polymer types present, as well as their size and shape characteristics, in human whole blood from 20 healthy volunteers. Twenty-four polymer types were identified from 18 out of 20 (90 %) donors and quantified in blood, with the majority observed for the first time. Using an LOQ approach, five polymer types met the threshold with a lower mean ± SD of 2466 ± 4174 MP/L. The concentrations of plastics analysed in blood samples ranged from 1.84 − 4.65 μg/mL. Polyethylene (32 %), ethylene propylene diene (14 %), and ethylene–vinyl-acetate/alcohol (12 %) fragments were the most abundant. MP particles that were identified within the blood samples had a mean particle length of 127.99 ± 293.26 μm (7–3000 μm), and a mean particle width of 57.88 ± 88.89 μm (5–800 μm). The MPs were predominantly categorised as fragments (88 %) and were white/clear (79 %). A variety of plastic additive chemicals were identified including endocrine disrupting-classed phthalates. The procedural blank samples comprised 7 polymer types, that were distinct from those identified in blood, mainly resin (25 %), polyethylene terephthalate (17 %), and polystyrene (17 %) with a mean ± SD of 4.80 ± 5.59 MP/L. This study adds to the growing evidence that MPs are taken up into the human body and are transported via the bloodstream. The shape and sizes of the particles raise important questions with respect to their presence and associated hazards in terms of potential detrimental impacts such as vascular inflammation, build up within major organs, and changes to either immune cell response, or haemostasis and thrombosis.

1. Introduction

Microplastics (MPs) are defined as synthetic plastic particles that typically range between 1 μm and 5 mm in diameter (Hartmann et al., 2019), and can be categorised as primary or secondary (Cole et al., 2011). Primary MPs are intentionally manufactured for commercial use while secondary MPs are generated via the weathering and breakdown of larger plastics (Cole et al., 2011). MPs have been identified across multiple environments including the air (O'Brien et al., 2023), soil (Yang et al., 2021), the food chain (Mamun et al., 2023), and drinking water (Li et al., 2022). This means that the potential for human exposure to MPs is significant (Zhu et al., 2023). Indeed, MPs have already been found in an ever-increasing variety of human tissues from the initial detection in stool samples (Schwabl et al., 2019) and cadaver lung tissues (Amato-Lourenço et al., 2021), to more recently analysed patient samples from lung (Jenner et al., 2022), colon (Ibrahim et al., 2021), liver (Horvatits et al., 2022), placenta (Ragusa et al., 2021), breast milk (Ragusa et al., 2022), vein (Rotchell et al., 2023), and testis/sperm (Zhao Q et al., 2023). Given this expanding field of research one of the likely routes for MPs to travel through the body and accumulate in different human tissues and organs is via the bloodstream (Dong et al., 2023). The most likely route to enter the blood is via diet/gut but there are certainly two additional routes of exposure: inhaled MPs crossing the lung to the bloodstream (Jenner et al., 2022) or during surgery directly into the bloodstream as airborne fallout or from spallation of medical equipment (Field et al., 2022).

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Importantly, a subset of MP polymers has been previously identified within human blood (Leslie et al., 2022). Leslie et al. (2022) used a pyrolysis–gas chromatography/mass spectrometry (pyrolysis-GC/MS) methodological approach to identify the presence of five MP polymer types; polymethyl methacrylate (PMMA), polypropylene (PP), polymerized styrene (PS), polyethylene (PE) and polyethylene terephthalate (PET) in 17 out of 22 (77 %) of the human blood samples. Importantly, although the presence of MPs was identified there was no information on MP particle size, shape or the presence of diverse polymer types or additives due to the limitations of the analytical method used.

The clinical implications of the presence of MPs within the human body are not known. However, there is evidence to suggest that the shape and size of MPs are crucial characteristics in determining their potential toxicity measured as inflammation, oxidative stress, and barrier integrity (for review: Danopoulos et al., 2022). For example, nano-sized particles have been noted to induce or reduce platelet aggregation, thrombus formation, and coagulation dependent on their coating (Nemmar et al., 2002; Bihari et al., 2010; Griffin et al., 2018), MPs however, are less well characterised. Cell-based and animal studies to date have linked MP exposure to reproduction toxicity in mice (Liu et al., 2022), inflammation in mice and humans (Li et al., 2020; Yan et al., 2022), developmental and endocrine disorders in mice (Zhao T et al., 2023), genomic instability (Çobanoğlu et al., 2021) and increased cardiovascular risk (Tadic et al., 2018; Zhu et al., 2023). However, these various investigations did not have the benefit of knowing precisely which types of MP polymers and their size/shape characteristics to employ an environmentally-relevant exposure regime.

This study aimed to identify as large a range of MPs polymer types present within the blood as possible. Using an μFTIR microscopy technique, we also aimed to add important information regarding the size and shape of the MP particles identified, as well as the presence of any chemical additives commonly associated with the manufacture of plastics. This allows a deeper understanding of the characteristics of MPs present in the blood and therefore facilitates a better understanding of how our cells may respond to the presence of these MPs.

2. Methods

2.1. Blood sample acquisition

Blood samples were collected from healthy drug-free volunteers (aged over 18 years) attending the Centre of Biomedicine, University of Hull, and in accordance with relevant health and safety guidelines. Work was carried out under ethical permission granted NHS REC study 'Investigation of blood cells for research into cardiovascular disease' (21/SC0215). Volunteers were assigned a sample number to maintain anonymity. Blood samples were collected from healthy donors during summer 2023. Blood samples were collected using 8.5 mL ± 10 % vacutainers containing 1.5 mL acid citrate dextrose solution A (sodium citrate, dextrose, citric acid and antimycotic (K sorbate) reagent) (Becton Dickinson, Medisave, U.K.). Blood samples were collected in the same medical setting and processed within the same day as donation. From the moment a blood sample is obtained from a donor, there is distinct opportunity for the sample to be exposed to the indoor air environment and any background contaminants including airborne MPs. To address this, 10 procedural blanks were initiated throughout the blood collection dates, mimicking the production of a blood sample, opening the vacutainer, and transferring the sample into a clear, pre-cleaned Durham bottle for a similar length of time.

2.2. Blood sample digestion and filtration

Blood samples were decanted into a Durham bottle containing pre-filtered tris buffer (50 mL at pH 8), pig pancreatic enzyme (1.4 mg/mL) (Sigma-Aldrich, Dorset, UK) and porcine lipase (6 mg/mL) (Sigma-Aldrich, Dorset, UK) and incubated at 37 °C for 6 h with shaking every 30 min. After incubation, samples were heated in a water bath at 70 °C for 15 min and left on ice till cold. Blood samples (n = 20) and procedural blanks (n = 10) were poured into pre-cleaned glass flasks containing hydrogen peroxide (100 mL of 30 % H_2O_2) and placed into a shaking incubator at 65 °C at 65 rpm for 7 days. Adapted from Jenner et al. (2022), the digest step maintains MP integrity while encourages the removal of organic particles (Munno et al., 2018), the heating step denatured enzymes and prevented explosive reactions. Using a pre-cleaned glass filtration system, samples were filtered onto aluminium oxide filters (0.02 μm Anodisc, Watford, U.K.). Filters were stored in petri dishes before chemical composition and shape/size analysis.

2.3. Chemical characterisation of particles using μFTIR analysis

A Nicolet™ iN10 Infrared Microscope (ThermoFisher, Waltham MA, U.S.A) was used in liquid nitrogen cooled transmission mode to conduct μFTIR spectroscopy. All Anodsic filters were placed onto the μFTIR spectroscopy platform (ThermoScientific Nicolet iN10), and operators methodically navigated the anodisc surface using the motorised stage and live feed from the inbuilt colour CCD digital video camera, which is equipped with independent reflection and transmission illuminations. Particle analysis was performed by manually targeting particles allowing for a more rapid data collection at the levels of particle loading obtained compared to the automatic wizard functions to scan all locations over a specified grid size. Trained operators are also able to save time by distinguishing larger background undigested organic material from defined particles. Spectrum are simultaneously obtained while the operator observes individual particles using the microscope. Analysis of particles as small as 5 μm was facilitated by the collect mercury cadmium telluride (MCT) detector. The Nicolet iN10 microscope is equipped with 15 x 0.7 N.A. high efficiency objective and condenser and has a standardised 123x magnification with the aperture setting used. The length (largest side) and width (second largest size) of any particle identified was recorded using the aperture height, width and angle size selection tool (ThermoScientific Omnic Picta Nicolet iN10 microscopy software). Particles were classified by their shape (fibre, film, fragment, foam or sphere) (Free et al., 2014), fibrous particles were required to have a length to width ratio greater than 3 (Vianello et al., 2019). 'Irregular' was used when the particle shape could not clearly be defined as either fragment or film. 'Screen capture' images of particles were taken from the camera live feed as image capture is not a feature of the software.

Only a quarter of each filter (procedural blanks and digested blood samples) was analysed. Before analysis, a background reference spectrum was recorded from an area of the filter with no particles or background undigested organic debris present. μFTIR parameters were; spectral range of 4000–1250 cm^{-1}, high spectral resolution 8 cm^{-1}, scan number of 64. This scan range is truncated at 1250 cm^{-1} due to the aluminium oxide-composed anodisc filter masking the range below this value. Data transformation, smoothing and baseline corrections were not used. The resulting sample spectra was compared across multiple polymer and common plastic additive chemical libraries (Omnic Picta, Omnic Polymer Libraries), particles with a full spectral match of ≥ 70 % were recorded. Three attempts were made to collect a successful (≥70 %) match for particles that fell below the ≥ 70 % match threshold before moving onto the next particle. All particles (MP polymers, associated additive chemicals and other non-plastic polymers) that achieved the ≥ 70 % match were recorded and included in the results shown (dataset made available at the following https://doi.org/10.6084/m9.figshare.24268474). The total number of particles identified was 1713, of which 192 (11 %) were MPs or particles containing associated plastic additives. Only the MPs, the associated additive chemicals, and alternative plastic polymer data are presented in the results. The associated additive chemicals data was available using the OMNIC Picta Polymer Library of spectra as additional information on the composition of particles.

2.4. Quality assurance and control measures

Procedural blanks (n = 10) were collected alongside the blood samples to quantify, characterise, and adjust for any background contamination (Noonan et al., 2023). Having procedural blanks and other quality control measures achieves better sensitivity and reduces the possibility of false positives. The procedural blank mirrored the entire sample processing steps without the addition of blood. A procedural blank approach accepts that a small amount of contamination from the air or solutions used *may occur* and these quantify the levels and characteristics of any such background contamination. The procedural blanks contained triple distilled water (pre-filtered) and air from the room. All reagents were pre-filtered and prepared in bulk. MPs found within the procedural blanks represent contamination from indoor atmosphere during blood collection, enzyme digest, contamination from laboratory reagents, equipment, or fallout from the air during transfer of samples between glassware. The tris buffer and H_2O_2 was triple filtered across 47 mm glass fibre grade 6 filters using an all-glass vacuum filtration kit (GE Healthcare Life Sciences, Marlborough MA, U.S.A). All glassware was run through the dishwasher using distilled water before being rinsed five times using triple filtered MilliQ water. Small openings were made in the tinfoil lids that covered all equipment and reagents whenever pouring. To avoid sample particle loss when filtering digested samples, glassware and the sides of the filtration kit were rinse three times with triple filtered MilliQ water. Additionally, to avoid cross contamination, each sample was processed individually.

There is no standard protocol to account for background contamination within the MP research field at present. Instead, multiple contamination adjustments were applied in this study for comparison. Two adjustment approaches were used: a limit of detection (LOD) and limit of quantification (LOQ) approach (Horton et al., 2021) and a subtraction approach (most used in MP research). The LOQ is typically approximated by multiplying the LOD by 3.3 (Supplemental Material Table S1). The results using the LOQ technique is presented, while the raw data, LOD and subtraction adjusted values are presented in Supplemental Material Table S1 to allow comparisons to be made. To estimate recovery rates of MPs from the blood samples, a parallel spiking experiment was carried out using pre-filtered water and commercially supplied 200 μm MPs at two concentrations (Supplemental Material SM1).

2.5. Statistical analysis

Tests for homogeneity and statistical significance were performed on unadjusted MP values using GraphPad Prism 8.0.1 Software (GraphPad, SanDiego, USA). All data was determined non normally distributed using a Shapiro-Wilk test and a Mann-Whitney test conducted to establish significance. No standard method exists for the calculation of MP concentrations, therefore three are used: the unadjusted values, the adjusted values minus the mean of the procedural blank values (regardless of polymer type) subtracted, and an LOQ method taking procedural blank data into account (Horton et al., 2021). The LOQ derived values are used as the most robust values, and the unadjusted/subtracted values are contained in the Supplemental Material tables.

An ***estimation*** of the mass of each MP polymer with a donor's blood sample, where detected above the LOQ, was conducted using an adapted method of Leusch and Ziajahromi (2021) surrogating the 'fibre' calculation for fragments. There is currently no method available, within the MPs literature, for conversion of fragment-shaped particles/L to mass values. The mass was estimated using the assumption that the particles are solid volumes with constant density of shape and dimensions determined from the microscope measurements. The density of the particles was taken from the identification of the material by IR spectra simultaneously detected. The explicit formulas to convert to / from volume / concentration were taken from Leusch and Ziajahromi (2021).

3. Results

3.1. MP concentrations detected in human blood samples

In total, 736 MP particles were characterised from all the 8.5 mL blood samples collected (Fig. 1). Before applying the LOQ threshold approach, these initially comprised 24 polymer types and MPs were detected in 18 out of the 20 (90 %) of the donor blood samples (Fig. 2). Using only those MPs that met the LOQ criteria, MPs were detected in 8 out of the 20 (40 %) of the donor blood samples and the mean value detected was 2465.85 ± 4173.51 MP/L (Table 1). The raw data using the unadjusted and subtraction calculations are available in Table S1. Five of the 24 MP polymers identified, namely: PE (sample 1, 6, 8, 15, 17), ethylene propylene diene monomer (EPDM) (sample 6, 20), ethylene–vinyl acetate/ethylene vinyl alcohol (EVA/EVOH) (sample 1, 17, 20), polyamide (PA) (sample, 12, 13), ethylene–vinyl acetate (EVA) (sample 1) (Fig. 1; Table 1), were above the LOQ (Table S2) for blood samples from each donor where indicated.

MP concentration values detected in blood were generated for PE, EPDM, EVA/EVOH, PA, and EVA demonstrating that 40 % of donors (n = 8 out of 20) carried a quantifiable (>LOQ) mass of particles in their blood (Fig. 1 and Table 1). The polymer type and concentrations varied per sample, but up to three polymer types in a single sample (Table S2) could be identified, with PE the most widely encountered (>LOQ value in 25 % of all tested donors), followed by EVA/EVOH (15 %), EPDM (10 %), PA (10 %) and EVA (5 %). The ***estimated*** maximum concentration in a blood sample was 4.65 μg/mL for PE, 1.84 μg/mL for EVA/EVOH, 2.22 μg/mL for EPDM, 1.84 μg/mL for PA, and 0.61 μg/mL for EVA. To conduct a conservative estimate of the quantifiable sum total of the polymer concentrations in the blood donors, the sum of all polymer values > LOQ per sample were used. Where a donor had no polymer detected at the level of the LOQ, these were considered as zero. The ***estimated*** mean (±SD) sum concentration for each donor was 0.66 (±0.87) μg/mL total mass of plastic particles per blood sample.

The combined blanks contained 4.80 ± 5.59 per samples (range 0–48 MPs) with 7 MP types of different composition, with 2 polymers, polytetrafluoroethylene (PTFE) and polyacrylamide (PAM) only identified in the procedural blank samples. The number of MP particles identified in the blood samples was significantly ($p = 0.007$) greater than found in the procedural blanks.

3.2. MP particle characterisation from blood samples

Having established the presence of MPs within the blood, we next sought to determine their characteristics. Of the 24 polymers detected in human blood samples, the three most abundant polymers made up over 50 % of those identified, with PE (32 %), EPDM (13 %) and ethylene–vinyl acetate/ethylene vinyl alcohol (EVA/EVOH) (12 %) (Fig. 3; Fig S1). MPs particles that were identified within the blood samples had a mean particle length of 127.99 ± 293.26 μm (7 – 3000 μm), and a mean particle width of 57.88 ± 88.89 μm (5 – 800 μm) (Fig. 4). The MPs were also predominantly categorised as fragments (88 %) and were white/clear in appearance (41 % and 38 % respectively), although there were a sizeable number of fragments of different colours.

In comparison in the procedural blank samples only 7 MP polymers were identified. In this case however while PE (17 %) was still present, resin (25 %), PET (17 %) and PS (17 %) alongside PE made up 75 % of the MPs present (Fig S2). Interestingly although there were far fewer MPs present in the procedural blanks, the mean particle length of 81.83 ± 101.31 μm (27 – 350 μm) and width of 30.25 ± 8.32 μm (12 – 45 μm) (Fig. 4) was not significantly different ($p = 0.723$ and $p = 0.803$) to the MPs found in blood. Furthermore, the MPs in the procedural blanks were again, mainly found to be fragments, with only a small increase in the percentage of fibres identified. There was however fewer coloured MPs present, with only predominately white and clear MPs (42 % and 50 % respectively) with only a small number of black MPs identified in the

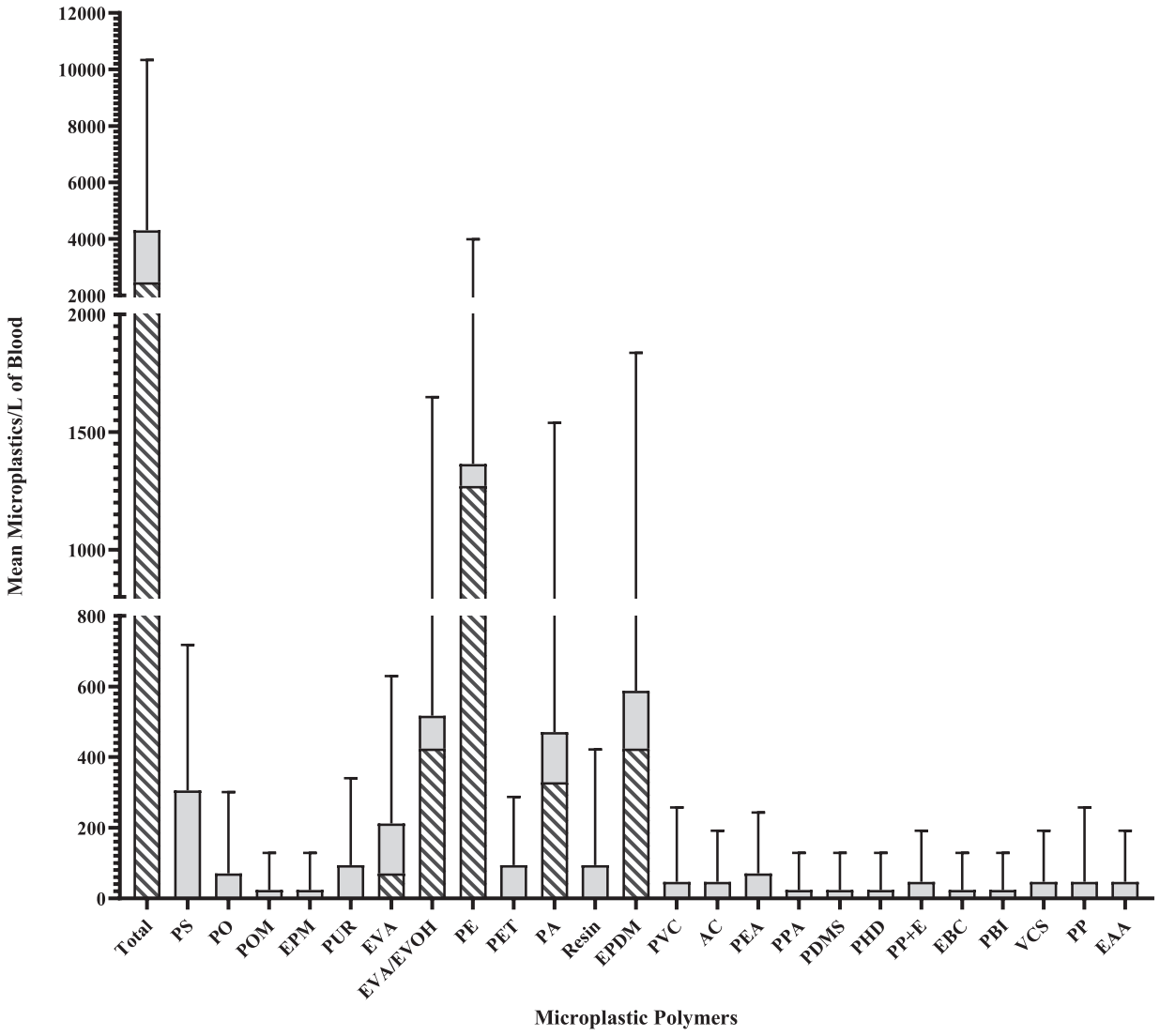

Fig. 1. Concentrations of MP particles in human blood samples per litre of blood (mean ± SD). Mean number of MP particles/L in blood samples. Using unadjusted values, based on number of particles detected in 8.5 mL of blood multiplied by 118 (for 1L approximation) and on one quarter of a filter multiplied by four (Table S2, **A**), error bars denote standard deviation. Mean number of MPs 4,306 and range (0–19,765). Abbreviations*:* AC, acrylic; EAA, ethylene acrylic acid copolymer; EBC, ethylene-butane copolymer; EPDM, ethylene propylene diene monomer; EPM, ethylene propylene; PBI, polybenzimidazole; PDMS, poly-dimethyl siloxane; PEA, polyethylene adipate diol; PET, polyethylene terephthalate; PHD, poly(1-hexadecene); PO, polyolefin; POM, polyoxymethylene; PP, polypropylene; PP + E, polypropylene and ethylene; PPA, polyphthalamide; PS, polystyrene; PUR, polyether urethane; PVC, polyvinyl chloride; resin, alkyd resins; VCS, vinylidene chloride-styrene copolymer.

procedural blanks. The spiking experiment using commercial PP and PVC MPs of ~ 200 μm size provided an indicative mean recovery rate of 66 % from pre-filtered water samples (Supplemental Material SM1).

3.3. MP additives found in human blood samples

Several MP polymer additive chemicals or plastic alternatives were observed in particles obtained from the blood samples. The most prevalent additive chemicals associated within particles were phthalates which were detected in 20 % (4/20) samples with 988 ± 2868 particles/L of blood. Tri(n-octyl,n-decyl)trimellitate was associated with particles in 25 % (5/20) blood samples with 518 ± 1023 particles/L while, reacted alpha-olefin, trilauryl trithiophosphite, phosphate ester polyolefin and 1,4-difluorobenzene-D4 were only present in a single blood sample. Another additive, 1-decanol was associated with particles in 60 % (12/20) of all blood samples whilst poly(3-hydroxybutyrate), a bacterial thermoplastic, biodegradable polyester-type plastic alternative, was detected in 20 % (4/20) of the blood samples.

4. Discussion

In a novel finding, the analysis of blood from 20 healthy human donors reveals MP particles with differing polymer types than previously detected. The MP size and shape characteristics represent the first such dataset for MP particles in human blood and provides important shape and size property details for determining potential biological implications of their presence in the blood. Specifically, the μFTIR methodological approach has provided novel information of the MP polymer types present as well as shape and size dimensions that challenge the current accepted paradigm for MP characteristics within human tissues. Of twenty-four plastic polymer types identified from 18 out of 20 (90 %) donors, five polymer types met the LOQ threshold with an average result of 2465.85 ± 4173.51 MP/L (range 0–86588 MPs). PE (32 %), EPDM (14 %), and EVA-EVOH (12 %) fragments were the most abundant.

Focussing only on those polymers that met the LOQ threshold, mass concentrations in blood demonstrated that 40 % of donors (n = 8 out of 20) were quantifiable which is less than the 77 % of donors reported in

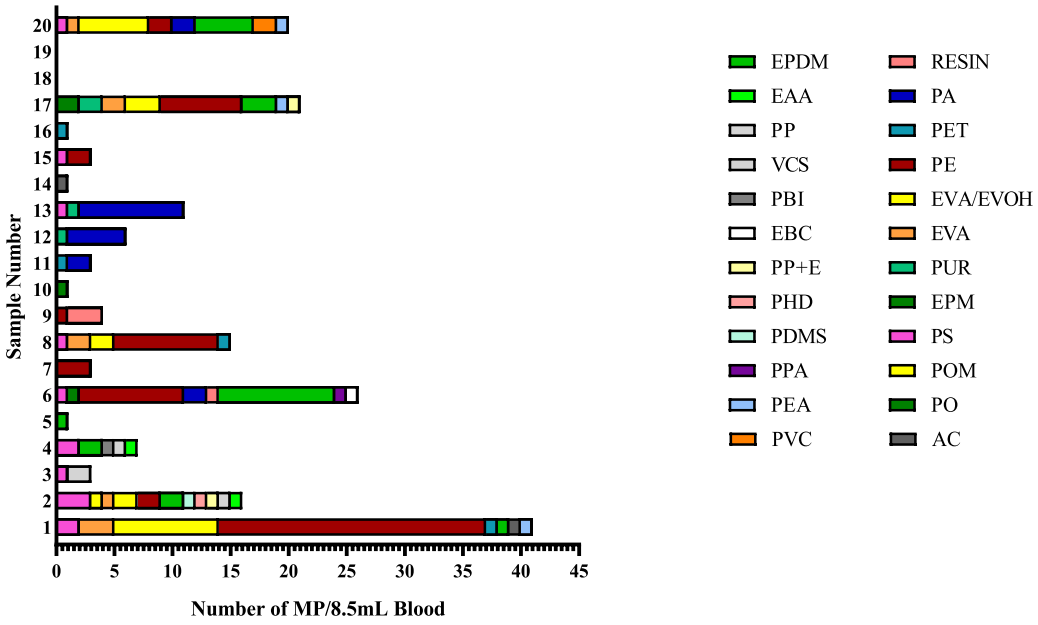

Fig. 2. Individual variation in the concentration and types of MP polymer particles detected in donor's ~ 8.5 mL blood samples. Unadjusted values, one quarter of the anodisc filter area analysed. Abbreviations: refer to Fig. 1.

Table 1
The number of MPs identified within the blood samples by μFTIR spectroscopy. Polymer types that met the LOQ criteria are displayed in units of MP/L of blood. Abbreviations; – Did not meet LOQ criteria. EPDM, ethylene propylene diene monomer; EVA, ethylene–vinyl acetate; EVA/EVOH, ethylene–vinyl acetate/ethylene vinyl alcohol; PA, polyamide/nylon; PE, polyethylene.

Blood sample number	PE	EVA/EVOH	EVA	EPDM	PA
1	10,729	4,235	1,412	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	4,141	–	–	5,129	–
7	–	–	–	–	–
8	4,141	–	–	–	–
9	–	–	–	–	–
10	–	–	–	–	–
11	–	–	–	–	–
12	–	–	–	–	2,353
13	–	–	–	–	4,235
14	–	–	–	–	–
15	3,200	–	–	–	–
16	–	–	–	–	–
17	3,200	1,412	–	–	–
18	–	–	–	–	–
19	–	–	–	–	–
20	–	2,824	–	2,306	–
Overall Mean	**2465.85**				
SD	**4173.51**				

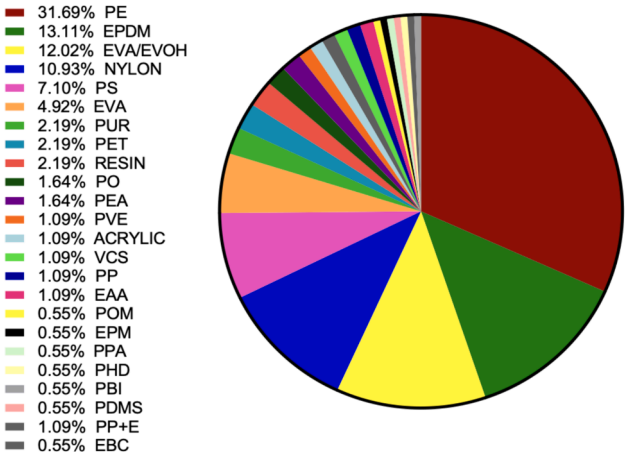

Fig. 3. Microplastic polymer types identified in blood samples. Abbreviations: see Fig. 1. Using all (unadjusted dataset) particles.

the Dutch blood study (Leslie et al., 2022). However, similarly to that first study, the polymer type and concentrations also varied per sample. In that previous study, which focussed on specific polymer types only, PE also featured. PE was the most widely encountered (using LOQ values in 25 % of all tested donors), with an estimated lower maximum of 4.65 μg/mL in this study relative to 7.1 μg/mL reported in the Dutch study. The ***estimated*** mean (±SD) sum concentration for each donor was 0.66 (±0.87) μg/mL total mass of plastic particles per blood sample in this study which is lower compared with 1.6 μg/mL reported in the Dutch study (Leslie et al., 2022). It is important to note that the latter mass is an actual pyrolysis-GCMS-derived value, while the μFTIR approach provides particle numbers/sizes, and the mass calculation has been estimated using an equation designed for fibres rather than fragments (Leusch & Ziajahromi, 2021), which may have resulted in an underestimation of mass. Another limitation of the μFTIR approach adopted herein is that only one quarter of a filter (representing one quarter of the actual blood sample) has been analysed. The values presented are the particles detected multiplied by four (to represent a whole filter). It is therefore possible that particles of other polymers were present but not detected. Also, calculations may contain rounding errors where the assumption has been made, potentially incorrectly, that particles of specific polymer types are evenly distributed upon a filter surface. The spiking experiment with commercially supplied 'virgin' PP and PVC MPs (of ~ 200 μm size) indicated a mean recovery rate of 66 % from pre-filtered water samples (Supplemental Material SM1), within the range reported in a recent *meta*-analysis of 71 microplastics studies (Way et al., 2022) and represents another consideration in favour of the values obtained from the blood sample analysis being an under-estimation. On balance, the mass estimation value relative to the previous blood study

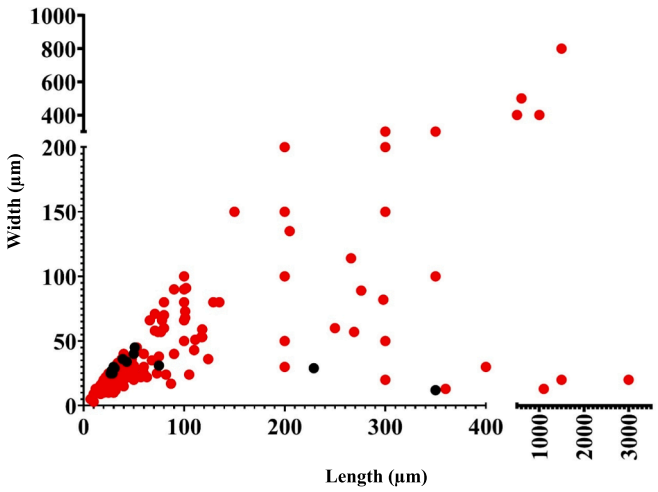

Fig. 4. Microplastics size dimension distribution observed in blood (red) and procedural blank (black) samples. Using all (unadjusted dataset) particles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the mean recovery rate of 66 % would suggest that the MP values reported herein are an underestimation. Other, general, limitations of the methodological approach used include incomplete organic material digestion, the use of the Anodisc filters necessitating a shorter scan range employed, and the 70 % match threshold used with the polymer library of spectra. While a match of > 70 % may be obtained, only further, additional chemical analysis can provide 100 % certainty of particle composition.

Taking each of the most abundant polymer types in turn, PE was identified in 5 out of 20 donor samples (using LOQ values) and has previously been isolated from 5 out of 22 human bloods using the pyrolysis approach, where it was also the second most abundant polymer detected (Leslie et al., 2022). PE is used in packaging film and products, bags, insulation for wires, bottles, many household items as well as a biomaterial for medical implants (Paxton et al., 2019). PE has previously been identified in human lung tissue (Jenner et al., 2022) and identified as the most abundant MP in human breastmilk samples (Ragusa et al., 2022). With respect to biological impacts, PE exposure (of beads sized 10–45 µm) increased the level of genomic instability in a blood cell study (Cobanoglu et al., 2021).

In contrast, EPDM has not been previously reported in any human tissue to date. EPDM is used in the automotive industry as a weatherseal coating due to its exceptional weathering and heat resistance properties (Jacob & Jourdain, 2011) as well as in artificial turfs such as sports fields (Ahrens, 2018). EPDM fragments (<100 µm in size) have recently been reported in all air samples from rubber industry occupational settings whereby the abundance was highest of airborne in the post-processing workshop at 559 ± 184 particles/m^3 (Sun et al., 2023). EVA-EVOH has previously been identified in human urine samples (Rotchell et al., personal communication). These are used as a tie-layer between other polymers in products where an oxygen barrier is required, such as food packaging, agricultural film and in the automotive industry (Gaucher-Miri et al., 2002). EVA on its own is commonly used in medical controlled drug delivery systems (such as contraceptives since it is thought to have favourable inflammation characteristics and is regarded as relatively inert) (Schneider et al., 2017). It is also used in everyday items such as clothing/mats where padding is required (such as running shoes and sports mats). EVOH alone is also used as a biocompatible polymer that forms a spongelike plug that can seal cavities. It has been successfully used in brain malformation clinical cases and in women with stress urinary incontinence (Elzayat & Corcos, 2008). The EVA/EVOH/EVA multi-layer film is composed of three different types of polymer, affording the generation of several types of radicals for each type of polymer, i.e., PE, which links back to most abundant MP polymer detected in this study (for review of the structure: Gaston et al., 2021).

The presence and potential impacts of MPs in the blood is critical to understand. The blood provides many key functions, to aide in the movement of substances and cells around the body. Disruption of the blood system can have significant effects on the overall well-being on the body. Previous work has already established that selected MPs (PET > PE > PS > PMMA) are present in human blood (Leslie et al., 2022) and within vein tissue (alkyd resin > polyvinylpropionate/acetate, (PVAc) > nylon-ethylene–vinyl acetate, (nylon-EVA), tie layer) in humans (Rotchell et al., 2023). Here we here show a much more varied mix of MP polymer types than previously identified and identify a mean particle length of 127.99 ± 293.26 µm (7 – 3000 µm), and a mean particle width of 57.88 ± 88.89 µm (5 – 800 µm). Importantly no spherical beads, were found within the samples. Given the range of size of the MPs, both in length and width, it must be noted that whilst parts of the vascular system, such as major arteries and veins can accommodate particles of this size, capillaries are typically 5–8 µm in diameter, presenting a theoretical barrier to any particle more than ~ 5–8 µm in more than one dimension. Therefore, although the MPs maybe able to squeeze through the capillary in part due to its flexibility and the pressure of the blood system, it is likely that it may have a slow transit through the capillary system, and therefore can then present a clear opportunity for interaction with both the vascular endothelium and blood cells.

Having established MP presence and characterised their physical dimensions within blood, the next important question relates to any potential implications. Once within the blood MPs can interact with proteins present within the plasma, for example immunoglobulins, fibrinogen, or coagulation proteins to form a corona (Lundqvist et al., 2008). Such corona may help the MP to not be recognised by the immune system (Mirshafiee et al., 2016), potentially elongating the time the MP spends within the body. Of the literature available, most biological impacts of exposure investigations use NP or smallest MP sized particles (<5 µm), typically of spherical shape and virgin origin. Smaller sized NPs (0.2 µm size, PS beads) can be ingested by murine macrophages and neutrophils leading to an inflammatory response (Florance et al., 2021). While murine macrophage cells exposed to PS beads (3 µm size), with an associated corona, enhances internalisation by macrophages (Ramsperger et al., 2020). Functionalised NPs (0.6—1 µm size PS and PS-amine beads) have also been shown to impact platelet aggregation and inhibit thrombus formation in rodent models (Vlacil et al., 2021; Nemmar et al., 2002), indicating that these smaller sized plastics could contribute to either a bleeding diathesis via an increased susceptibility to bleeding or bruising due to decreased clotting, or a prothrombotic environment involving abnormal coagulation. PE and PS NPs (1–5 µm size) taken up by immune cells in fish (*Salmo salar*) demonstrated differential tissue phagocytosis profiles (Abihssira-Garcia et al., 2020), highlighting another pathway of impact within blood.

The particles identified in these human blood samples were an order of magnitude larger in size and of different shape dimensions relative to those used in studies discussed so far. This raises the question in terms of how relatively large particles can enter the bloodstream. One direct route, of particles sized 4–148 µm in size, has been evidenced via infusion (Zhu et al., 2024). Indirect routes could include inhalation, diet and dermal contact involving barriers. While nanoplastic-sized spheres have been demonstrated to translocate through human intestinal barrier cells (Domenech et al., 2020), the micro-size range of plastics have yet to investigated in this way. In terms of impacts resulting from MP presence, of the literature available in this larger MP size range, those of < 10 µm size (PS beads) induced cytokine and histamine impacts in human peripheral blood mononuclear cells (PBMCs), while the larger sized 100 µm (PS beads) had no such impacts (Hwang et al., 2020). In contrast, 100 µm size PE beads and 25—200 µm fragments have been shown to reduce cell viability, trigger cytokine IL-6 and TNF alpha release and increase hemolysis in human PBMCs, mast cells, red blood cells albeit at high MP concentration (of 1000 µg/mL) exposures (Choi et al., 2021).

Cytokine IL-6, TNF alpha and histamine release have also been shown to occur following exposure to irregular-shaped PP exposure, whereby 20 μm size elicited a larger response relative to 200 μm size, using human dermal fibroblast and murine macrophage cells (Hwang et al., 2019). Using fish models, PS and polycarbonate (PC) MPs (sizes 0.04 – 1710 μm) induced degranulation of neutrophils in flathead minnows (*Pimephales promelas*), while PVC MPs (0.04 – 150 μm beads) exposure led to decreased phagocytic capacity of leucocytes in gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Espinosa et al., 2018). Therefore, MP size, type, shape and concentration affect *in vitro* test results using vertebrate species, and the data presented herein can now better inform the required levels, polymer types, shapes and dimensions to be able to more accurately understand the body's response to the presence of these MPs.

Although we can give examples of specific effects of MPs on certain cell types, it must be noted that the overall localisation of MPs and the effects of MPs within humans is not yet understood, and is the crucial next step for this field. In part this is why establishing the shape and size of the MPs identified within humans is so crucial, given that shape and size is known to affect cellular response (for review: Danopoulos et al., 2021). Furthermore, within the blood stream size and a malleable shape matter as whilst major arteries and veins can accommodate large unmalleable MPs, capillaries present a theoretical barrier to MPs given they are typically 5–8 μm in diameter. MPs maybe able to squeeze through the capillary, but it must be noted that capillaries are not just linear and can contain bends, and as such MPs may not have the flexibility to move round bends effectively as completed by red blood cells (Wang et al., 2022). Once stuck it is likely that red blood cell movement will be compromised and therefore local changes in oxygen concentrations might occur, leading to impacts on cell metabolism and cell function. The mean levels of MPs reported herein may also indicate bioaccumulation. While bioaccumulation was not within the scope of this study, there are studies that do show MP bioaccumulation across marine species (Miller et al., 2020), though this is not yet known for humans.

Alongside the type, shape and size of the MP being crucial to interaction with the body, it must also be noted that MPs contain thousands of additives, many known to be toxic (Hahladakis et al., 2018; Zimmermann et al., 2021) and representing a pool of potentially toxic leachate chemicals. Four types of phthalates (diundecyl phthalate, dicapryl phthalate, butyl benzyl phthalate and octyl benzylphthalate) were identified within particles in the blood samples. It should be noted that plastics are not the only source of these types of chemicals. Phthalates, added as plasticising agents, have been associated with toxic endpoints in humans including endocrine, nervous, cardiovascular, and respiratory systems as well as immune response pathways (Chang et al., 2021; Mariana et al., 2023; Wang and Qian, 2021). Trimellitate, another plasticising agent, used in PVC manufacturing in the automotive industry and insulation cables as well as food packaging materials, was identified in 25 % of samples though is not yet considered harmful to humans (EFSA, 2019). Leachate chemicals, such as these, may also affect the make-up of any corona and determine fate and impacts of the MPs present as discussed previously. The final category for discussion is bioplastics. Poly(3-hydroxybutyrate), a bacterially-derived thermoplastic (Barham et al., 1984), was identified in 20 % of blood samples investigated. Such biologically-derived plastics are marketed as an alternative to PES type plastics, and yet although biodegradable, their fragments can be detected within a human body.

To conclude, MPs have been detected in human blood samples, displaying different polymer composition, and greater size range, than has previously been appreciated. They have also been characterised, for the first time, with respect to their concentrations, size, and shape dimensions, with surprisingly no beads detected. In addition to the MP particles, several commonly used MP additives including four different types of phthalates, have been detected. This information now allows more representative *in vitro* and *in vivo* toxicity studies regarding the implications of MP presence in human blood and further highlights the urgency of conducting such analyses given the abundance evidenced in this investigation.

CRediT authorship contribution statement

Sophie V. L. Leonard: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Catriona R. Liddle:** Formal analysis. **Charlotte A. Atherall:** Formal analysis. **Emma Chapman:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Data curation. **Matthew Watkins:** Methodology, Formal analysis. **Simon D. J. Calaminus:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Jeanette M. Rotchell:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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