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This paper investigates one source of microplastics and nanoplastics: period products. The products have a global and widespread presence. The final fate of this persistent type of polymers is invariable the environment

Hypotheses:

- Products made with synthetic polymers in direct contact with skin will release fibres during conditions that simulating the vaginal cavity.
- if, under such conditions, polymer fibres will fragment into smaller nanoplastics

Many of the products released fibres during in-vitro tests and also fragmented to release up to 17 billion nanoplastics per tampon.

Health concern could manifest in three ways: by the nanoplastics itself, for release of contaminants adsorbed to the nanoplastics and finally, for leaching of additives associated to the production of the plastics

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ARTICLE

Release of microplastic fibres and fragmentation to billions of nanoplastics from period products: preliminary assessment of potential health implications

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Health effects related to the plastic content of disposable period products have not been recognized or scientifically addressed. To begin to understand their potential impact on the environment and human health, this study employed standardised *in-vitro* tests (Syngina), infrared spectroscopy (FTIR), confocal Raman microscopy, scanning electron microscopy (FEG-SEM) and nanoparticle tracking analysis (NTA) to characterize the bulk chemical composition of different components in period products, and quantified the amount of fibres released using *in-vitro* experiments, and measured their fragmentation into smaller particles (nanoplastics) under conditions that mimic vaginal fluids. It was found that 12 out of 24 of the tested products contain synthetic polymers (plastics) that would be in direct contact with the vaginal wall when in use. Many of the products released fibres during *in-vitro* tests and also fragmented to release up to 17 billion nanoplastics per tampon. These micro fibres and nanoplastics could be released into the environment upon disposal. The health implications within the body are unknown, but due to the large quantity of nano size plastics being released, public health concern could manifest in three ways: from the nanoplastics themeselves, from release of contaminants adsorbed to the nanoplastics and finally, from leaching of additives associated with the production of the plastics.

Introduction

Among period/menstrual disposable products, tampons are very popular (1). It has been estimated that the sales value of the tampons market will rise from US$ 4.25 billion worldwide in 2018 to 5.7 billion by 2024 (2). In Europe and the UK, the General Product Safety Directive (EEC Directive 2001/95/EC) provides guidelines and regulations for tampons. One of the Directive purposes is ensuring only the distribution of “safe products” in terms of several parameters including their labelling and their chemical composition. However, there are many products that do not include information on the product labels in relation to the chemical composition of all their components. Moreover, many other countries only follow guidelines set by the code of practice of the manufacturers themselves (Nonwoven industry association, Europe (EDANA), US (INDA) and Brazilian Association of the Nonwovens and Technical Industries (ABINT)). The EDANA code of practice uses a standardised protocol for testing tampon absorbency called Syngina (21CFR801.430 Code of Federal Regulations, FDA) (3). However, such codes of practice, as they currently stand do not address material composition labelling.

The plastic content in tampons has also been the focus of recent environmental concern. As bulk products, their incorrect disposal can cause sewer blockages and they can eventually find their way into water bodies, beaches and oceans (4,5,6). Tampons have also been associated with some negative health effects, for example toxic shock syndrome (TSS) and bacterial growth (7), mainly because they can release chemicals absorbed to them rather than direct risk from the material composition of the tampon itself (8).

Toxicity of microplastics (<5 mm in diameter) and nanoplastics (<100 nm in diameter) to humans is poorly understood. This is further complicated by the diverse types of plastics and their chemical compositions. Thomas et al. (2021) in their review indicated that the overwhelming majority of literature is based on aquatic biota and widely used biochemical tests targeting only one facet of the toxicological profile of microplastics and nanoplastics (9). In a murine model, polystyrene (PS) microplastics induced hepatic endoplasmic reticulum stress (10) and reproductive toxicity (11), while polystyrene nanoparticles caused defective neural tube morphogenesis (12) and are translocated to placental and foetal tissues (13).

Commercial nanoplastics are different from nanoplastics formed in the environment. Environmental nanoplastics have a more complex surface chemistry in which different functional groups are exposed according to the type of degradation experienced (14,15).

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† Electronic Supplementary Information (ESI) available: [Details of the spectral and imaging match analysis, details of the synthetic fibre measured diameter, details of the *in-vitro* Syngina set up and details of the statistical Two Sample Poisson rate comparisons]
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Current toxicity studies using nanoplastics are based mainly on PS, and although this plastic type is not necessarily representative of the common plastics found in the environment, the data provides a broad understanding of the indicative impact that generalised nanoplastics may have on mammals (14,16). Exposure to a mixture of PS in micro and nano size was found to result in the deterioration of intestinal barrier function and cause gut microbiota dysbiosis in mice (reduction of microbial diversity) (17). So far, evidence has suggested that PS nanoplastics caused oxidative stress in human peripheral blood mononuclear cells (18), toxicity in human lung epithelial cells (15) and DNA damage in monocytes (19). Human lymphocytes, human B lymphoblastoids (Raji-B), human lymphoblast cells heterozygous at the thymidine kinase (TK6), and human foreskin fibroblast cells (Hs27) showed increased genotoxicity after treatment with PS nanoplastics (20,21,22). Using human preimplantation embryos and induced pluripotent stem cells, Bojic et al. (2020) found that nano PS cause substantial genotoxic effects which are associated with cellular stress, abnormal development, and increased risk for certain diseases (23).

The aim of this research was to understand the potential environmental and health impacts of period products by characterising the bulk chemical composition of products obtained across the world (with focus on synthetic polymers). Products made with synthetic polymers that would be in direct contact with tissue were selected for further *in-vitro* experiments simulating the vaginal cavity and fluid. Such analysis aimed to measure the amount of fibres released as well as their chemical composition. Finally, we aimed to investigate if, under simulated in-vivo conditions, polymer fibres break down or fragment into smaller nanoplastics particles.

Materials and Methods

Filtered (0.2µm) deionised water (Purite HP+Boost, reverse osmosis, deionisation and microfiltration) was used to rinse and prepare all solutions. Solutions were prepared in borosilicate glass bottles (Fisher Scientific), rinsed 3 times and blown with nitrogen gas to dry and remove any fibre/particle contamination. All tests were performed inside a laminar flow fume chamber.

Table 1 shows a summary of the products used in the present study. Products acquired (2019-2021) in the UK, Europe (Germany and Spain), USA and Australia were used.

Table 1 Summary of the products used in the present study along with bulk material analysis and single fibre matches

Product code	Retail country	Manufacturing country	Composition (as labelled)	Format	Absorbency	Batch number	Skin contact material (FTIR bulk)	Applicator (FTIR bulk)	Wrapper (FTIR bulk)	Gravimetric	Single fibre Raman polymer
P1	UK	Hungary	Rayon, polyester, cotton	Applicator	Super	800190705662	Cover -polyester Inner string - cotton/polyester / polyethylene Outer string- polyester Protective skirt - Same as cover polyester	Polyethylene	Cellulose nitrate / polypropylene	YES	Matched polyester
P2	UK	Taiwan	Viscose, polyester, polyethylene, cotton, cellophane, 0.15g plastic per tampon	No applicator	Super	5000304116006	Cover -polyethylene / polyester String - cotton/ polyester	No applicator	Viscose / cellulose xanthogenate	YES	NM
P3	UK	Germany	NA	Applicator	Super	5057753467579	Cover -polyethylene / polypropylene Inner string - cotton/polyester Outer string- cotton/polyester	Polyethylene coated cellulose	Cellulose	YES	Matched polyester
P4	UK	Slovenia	NA	Applicator	Super	5025971102749	Cover -polyethylene / polyester	Polyethylene	Polyethylene /polyester	YES	NM
P5	UK	Hungary	Rayon, polyester, cotton, polypropylene, polyethylene	Applicator	Super	4015400758389	Cover -polyethylene / polypropylene String - polyester	Polyethylene	Polyethylene	YES	NM
P6	UK	UK	NA	Applicator	Super	4088600014029	Cover -polyethylene Inner string - cotton/polyester Outer string-	Polyethylene coated cellulose	Cellulose / cellulose nitrate	YES	Matched polyester

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							cotton/polyester				
P7	USA	USA	Rayon, polyester, cotton, polypropylene, polyethylene, fibre finishes	Applicator	Super	4015400758389	Cover -polyethylene / polypropylene String - polyester Leakguard - polypropylene	Polyethylene	Polyethylene / unknown polymer	YES	Matched polypropylene
P8	USA	Mexico	Rayon, polyethylene, polyester cover, polyester string	Applicator	Regular	036000515909	Cover - polyethylene / polyester String - cotton / polyester	1 Polyethylene 2 Polypropylene	Polyethylene	YES	NM
P9	Germany	EU	NA	No applicator	Mini	3574661322629	Cover - polyethylene String -polyester	No applicator	Polypropylene	YES	NM
P10	Australia	Australia	Rayon, Cover - polypropylene / polyester String - polyester / cotton	No applicator	Super	9325344002680	Core – cotton / cellulose xanthogenate Cover - polyester / polypropylene String - cotton / polyester	No applicator	Polypropylene	YES	Matched polyester
P11	Germany	Germany	NA	NA	NA	4260600580012	Core - polyether urethane	No applicator	No wrapper	YES	Matched nylon Matched polyester
P12	USA	USA with global materials	Rayon, cotton, polyester, polysorbate 20	Applicator	Regular	78300098492	Core - cotton / cellulose xanthogenate String - cotton / polyethylene	Polyethylene	Polypropylene	YES	Matched polyethylene
P13	Germany	USA	100% organic plant-based applicator	Applicator	Regular	8001841385730	Cover - cotton Inner string - polyester Outer string - cotton / elastane Leakguard - polypropylene	Polyethylene	Polyethylene	YES	NM
P14	UK	Spain	Organic cotton biodegradable	Applicator	Super	X0016FY53J	Cover – cotton / elastane, Inner string - cotton/polyester Outer string - cotton/polyester	Polyethylene coated cellulose	Cellulose	YES	NM
P15	UK	UK	100% organic cotton	Applicator	Regular	793052119225	Core - cotton String - cotton / Ethylene bis(stearamide)	Polyethylene	Cellulose nitrate / polypropylene	NO	NA
P16	UK	UK	100% natural cotton, plastic applicator	Applicator	Super	X000UJVVST	Core - cotton String – cotton / elastane	Polyethylene	Cellulose nitrate	NO	NA
P17	UK	Made in EU Packed in UK	100% compostable 100% organic cotton	No applicator	NA	NA	Core - cotton String - cotton	No applicator	Viscose / cellulose xanhogenate	NO	NA
P18	UK	Europe	100% GOTS organic cotton	No applicator	Super	314202	Cover - cotton String - cotton	No applicator	Cellulose	NO	NA
P19	UK	Germany	100% cotton	Applicator	Super	78212600900	Cover - cotton String - cotton	Cellulose	Cellulose / cellulose nitrate	NO	NA

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P20	USA	USA	Organic cotton	Applicator	Super	LPNRRBC8153447	Cover - cotton Inner string - cotton / polyester Outer string - cotton	Polyethylene coated cellulose	Cellulose	NO	NA
P21	Germany	Spain	Organic cotton, viscose free	No applicator	Super plus	8432984000530	Cover - cotton String - cotton	No applicator	Cellulose	NO	NA
P22	Australia	Germany	100% cotton	No applicator	Super	782126001009	Core - cotton String - cotton	No applicator	Polypropylene	NO	NA
P23	UK	Copenhagen, Denmark	Vegan, No BPA, latex or dyes, 100% soft medical silicon	No applicator	A	5711782000014	Silicon rubber	No applicator	NA	NO	NA
P24	USA	Germany	100% cotton	Applicator	Super	078300029939	Cover - cotton Inner string - cotton Outer string - cotton	Polyethylene	Polyethylene	NO	NA

NA= Not applicable, NM= Not matched

Bulk chemical analysis was carried out as follows. All products were carefully dismantled and analysed using a Bruker alpha Fourier transformed infrared spectrometer (FTIR) with a diamond attenuated total reflectance probe (ATR). Background signal was collected before each sample analysis. Infrared spectra (22 times) were acquired from 450-4000 cm^{-1} and analysed using Ominc 7.0 (Thermo Scientific) software and Aldrich polymer database.

In-vitro tests for fibre release were performed as follows. A Syngina test (3) was performed using 500mL bottles connected using silicon tubing, kept at constant temperature of 37°C using a water bath and a USB powered fish tank pump (Figure 1). Non-lubricated condoms (*On clinic*, Amazon.com, Inc. [UK]) were used as membranes after rinsing 3 times with deionised water. Period products were inserted into the membrane (such products were only in contact with the membrane and never with the bottles) and 12mL of a filtered solution pH4 (phthalate buffer diluted 10 times with deionised water, Fisher Scientific) was injected. The test was left to run for 2h and shaken at 120rpm. At the end of the test, the membrane was rinsed 3 times with pH4 solution, and the tampon was gently removed and rinsed. Both solutions were collected and filtered using pre-weighed 10µm mesh stainless steel filters. Filters were placed inside glass petri dishes and a desiccator for at least 48h. Finally, filters were weighed to calculate the amount of fibres released (gravimetric test) using an analytical balance (PAS214C, Fisher Scientific, reproducibility 0.1mg).

One replicate from the gravimetric analysis was used for single fibre identification. Part of the filter was transferred to glass slides and glass coverslips and analyses were performed using an ARAMIS confocal Raman microscope (Horiba UK Ltd) using a 633nm laser, 10 and 100X objectives, 600 l/mm grating, 100µm pinhole and 80-2060 cm^{-1} Raman shift range. The sample was illuminated in transmission mode using a LED light.

Part of another replicate was transferred on top of the double-sided carbon tape applied (Ted Pella). This set was analysed using a FEG-SEM (Phenom Pharos, scanning electron microscope) after gold coating (20nm), 15KV and backscattered detector. The presence of manmade-polymers was deemed positive if the Raman spectra matched the database and the FEG-SEM examination showed characteristic smooth surface and uniform diameter.

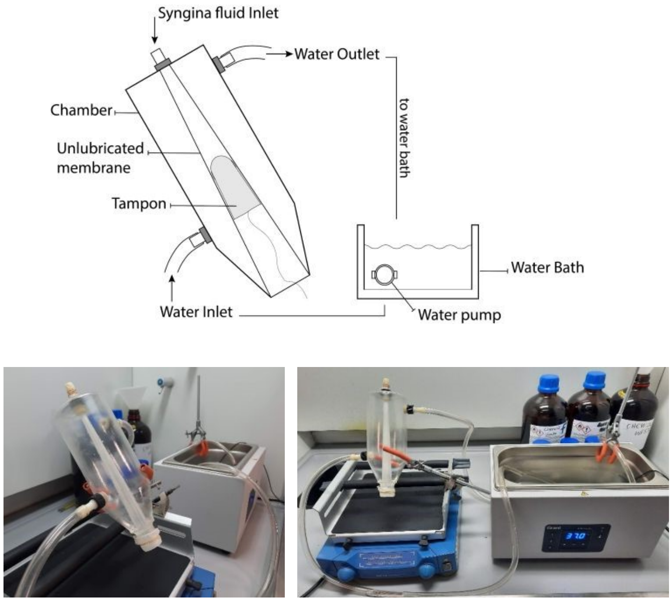

Figure 1 Diagram of *in-vitro* test (Syngina), temperature of 37°C and 120rpm shaking

For the fragmentation experiments, the products were dismantled and only the component containing synthetic polymers was isolated and used. A small subsample of this isolated component was taken and was placed in 10mL of buffered solution (pH4 phthalate buffer diluted 10 times and pH7 phosphate buffer saline, PBS) in glass containers. The average pH range of the vagina is 3.8-4.2 (24,3) . We decided to use a buffer that mimics such pH and another one that does not promote harsh acidic or basic conditions (PBS, pH7).

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The solution was shaken for 4h (100rpm), kept at 37°C and covered with aluminium foil, to avoid any fragmentation known to be possible due to photo degradation (UV light) (25).
At the end of the experiment, samples were filtered using 0.8µm filters (Advantec, cellulose acetate, C080A090C). The fraction retained not further analysed since our focus was the fragmented products. The filtrate was collected and analysed. High resolution nanoparticle size and concentration analyses were performed using a Nanosight NS300 with 488nm laser and sCMOS camera. The instrument uses light scattering and every single particle in the field of view is tracked while moving under Brownian motion (nanoparticle tracking analysis NTA). Particle size and concentration were calculated by the software NTA 3.4. Five sample replicates were tracked for 60s. Several types of control samples were used for contamination and particle size accuracy, namely, deionised water used in the study, buffer solutions after filtration (0.2µm filter), negative controls (negative for fragmentation experiment) that were filtered and processed in identical conditions as the samples and finally polystyrene beads as positive controls for size (nominal size 100nm, Sigma Aldrich, 43302). On the fragmentation test, the samples and controls were not in contact with any synthetic polymer (such as the membrane in the Syngina test).
Chemical composition of nanoparticles obtained were analysed in two ways. Firstly, a small aliquot was placed on carbon tape and allowed to air-dry for FEG-SEM visualisation. Particles were then filtered using 30kDa spin filters (Amicon Ultra, regenerated cellulose UFC503096), 2mL of sample was filtered (4X 0.5mL) and washed 3 times with deionised water, then washed with methanol (UPLC grade Fisher Scientific). Samples were reconstituted in methanol and were ready to analyse. Because we found residual glycerol contamination on the methanol wash, the samples were further washed with acetone (HPLC grade, fisher Scientific) for further analysis. A 5µL aliquot was placed in the ATR crystal and was allowed to dry. This was repeated 3 times then FTIR spectra were taken (50 scans).
Minitab 18 was used for the statistical tests. All tests were done at 95% confidence. Because the results for the particle size analysis follow a discrete Poisson distribution, statistical analysis of difference for this part was done using a 2-sample Poisson rate. This test compares rates (total occurrences divided by the number of observations) rather than concentrations alone (particles/mL).

Results

The results for the bulk FTIR analysis are presented in Table 1 (Skin contact material, FTIR bulk column). Two general types of products were found. In the first type of product, the absorbent material is wrapped with a thin "cover" sheet (Figure 2A); in the second type, the absorbent material is not wrapped in a "cover" (Figure 2B). Most of the absorbent material matched Cotton, as well as some of the covers. However, we found that the following parts of some products were made of synthetic polymers: the "cover", the "outer string" which is the string used to hold the tampon together and helps pull the tampon to be discarded and the "inner string" which is the string used to

Figure 2 Main types of products found A) comprising a separate cover, B) products not comprising a cover. 1=cover, 2=core, 3=outer string, 4=inner string, 5=applicator and 6=core with no cover

hold together the outer string and the tampon core (absorbent). The synthetic polymers found in such parts were polyester, polyethylene, polypropylene, polyether as well as a synthetic wax (ethylene bis(stearamide)).
On the basis of the polymers found in the bulk results, it was decided to test fourteen products for fibre release using the Syngina test. A graphical summary of the results for this test is shown in Figure 3.
Some products released an amount of fibres that was not enough to be measured due to the sensitivity of the balance (0.1mg). However, when the filter was examined under microscope, fibres were visibly detected (see supplementary information).
In summary, out of 12 products tested (12 known to contain synthetic polymers plus two negative controls [negative for fibre release experiment)] known to contain only cellulose, P13 and P14 from the bulk FTIR analysis), we found evidence for the release of synthetic polymers in 7 of them. Figure 4 presents the

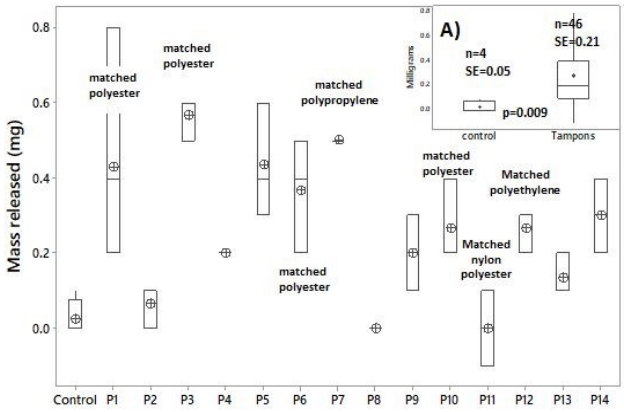

Figure 3 Mass of fibres released for 12 products (product 13 and 14 only contained cotton). Single fibre polymer match highlighted. A) all tampons results were merged and a significant difference with controls (no product) was found (p=0.009, difference=0.2, 95% CI (0.1;0.4), Mann-Whitney test), n=3-7 for each product, n=4 for control. Mean mass is indicated by circle.

matching results for product 1 (P1). This was corroborated by

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the morphology of the fibres under scanning electron microscope.

Some products (P12, P13 and P14) were found to only contain synthetic polymers in the strings holding the tampons in the bulk analysis. To our surprise, we found that one of these (P12, Figure S12) released the same polymer fibres (as found in the string material). This was confirmed by confocal Raman microscopy and FEG-SEM.

Interestingly, there was a unique product made of polyether urethane and therefore it was expected to only observe such polymer. However, four particles were found, one particle matched nylon and the other polyester. The remaining two particles displayed a strong Raman spectrum that was not matched to any spectra in our library (Figure S11). We believe the particles are perhaps contamination products in the manufacture of polyurethane rather than pieces released from the actual product.

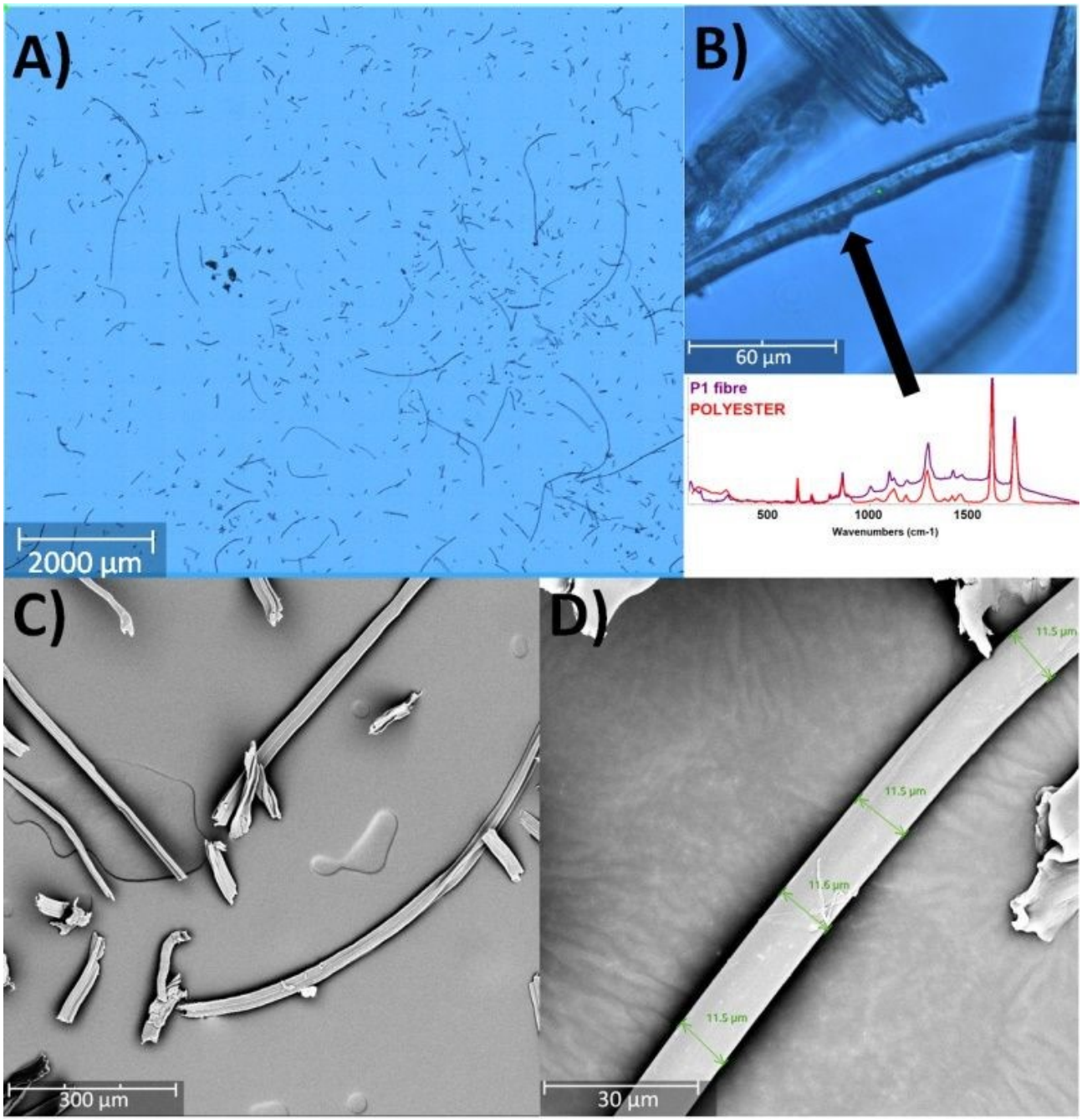

Figure 4 Single fibre analysis of product 1 (P1), A) mosaic view using 10X objective, B) single fibre confocal Raman analysis, 50X objective and 80-2060 cm-1 Raman shift range C) FEG-SEM analysis of several fibres 350X magnification showing two types of fibres cellulose and polymer based and D) FEG-SEM analysis 2500X magnification of single polymer fibre showing characteristic smooth surface and uniform diameter

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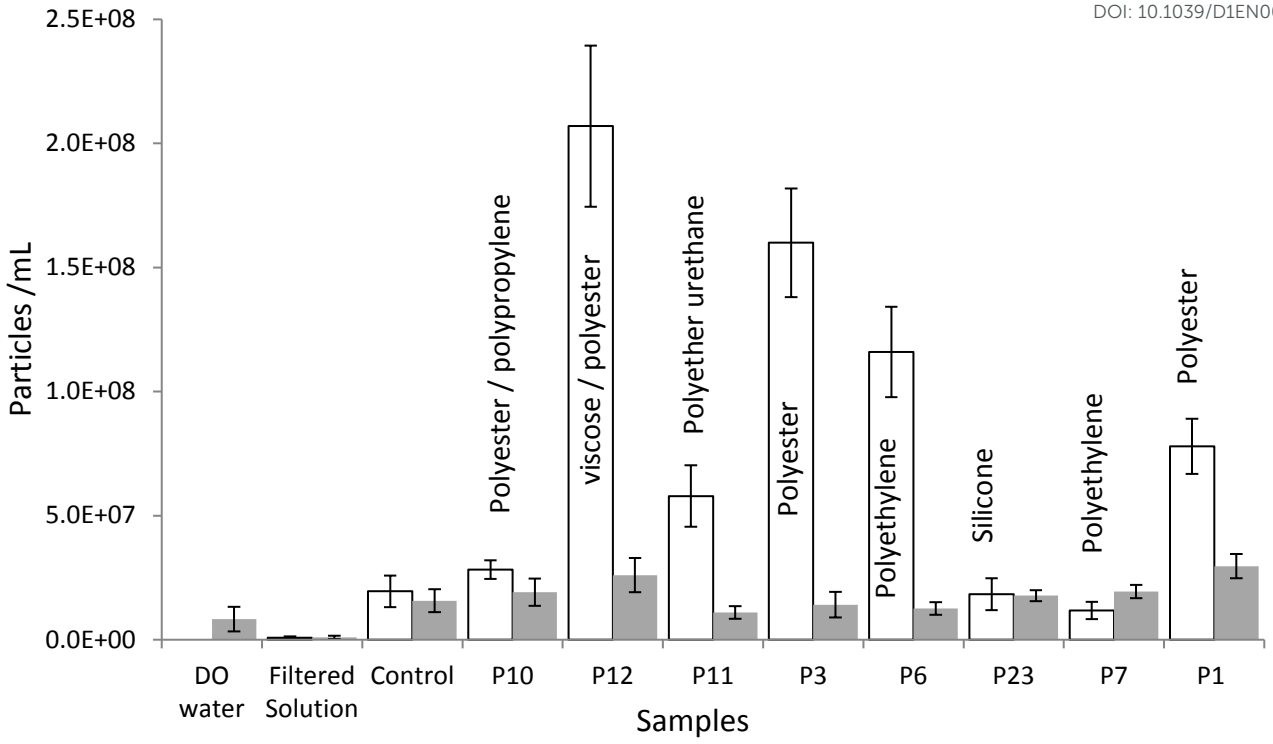

Figure 5 Fragmentation experiment results, white bars represent pH4, grey bars pH7, vertical bars represent standard errors. Bars contain the name of the polymer found in the bulk analysis. Figure displays raw particle counts not corrected for contamination in negative controls

Results for the fragmentation experiments are shown in Figure 5. Deionised water used in the experiment shows some but minimal contamination, after filtration the amount of contaminating particles decreased in the solutions of pH4 and pH7. However, some contamination was evident after the filtration step, possibly due to the filter itself (cellulose acetate). Nevertheless, the amount of particles found for some products were significantly higher than those from such negative controls (P10, P12, P11, P3, P6 and P1, p-value <1E-17, Table S1). The fragmentation seems to be pH dependent as all the products tested show lower particle concentrations at neutral pH. The fragmentation does not seem to be dependent on the temperature used (37°C) because some products (P7) did not show any fragmentation products at the same temperature.

A small aliquot of the solutions containing released nanoparticles were examined using FEG-SEM (Figure 6). It was noted that most of the particles agglomerated after being dried. This is common as many of the polymers are hygroscopic (26).

Nanoparticles released were characterized using FTIR after centrifugation and reconstitution sequentially in two solvents, methanol then acetone (to allow rapid drying of the solvent) (27). The results of the analysis are shown in Figure 7. For acetone wash and reconstitution, Figure 7A, particles show characteristic polypropylene bands 2950 and 2920 cm^{-1} CH_3 stretching and 1456 cm^{-1} symmetrical CH_3 bending. Such particles also have characteristic polyethylene 2850 cm^{-1} CH_3 stretching. The following bands were not present in controls (Figure 7A), peak at 698cm^{-1} as well as 1450-1500 cm^{-1} region. Methanol wash and reconstitution showed characteristic glycerol spectra (used in the manufacture of the 30KDa filters). However, we subtracted the spectra of glycerol (as found in the database) and the resulting spectra are shown in Figure 7B. Polyethylene bands at 2915 and 2846 cm^{-1} can be observed as well as polypropylene bands at 2949, 1373, 1164 and 995 cm^{-1}. However, although present, characteristic mentioned signals for product P6 were too weak and therefore not conclusive. We were able to match the nanoparticles released from products P1, P3, P7, P10, P11 and P12 to either polyethylene or polypropylene. In order to fully characterise every single particle fragmented from such hygiene products, the use of AFM-Raman or Nano-FTIR would be required.

It is noted that such released nano sized particles do not have an environmental origin. Moreover, we knew with certainty what the initial material was present at the beginning of this test. The material was characterized previously in bulk and we only selected the materials which contained synthetic polymers and therefore all particles found, other than contamination shown in controls, can only come from such synthetic polymers.

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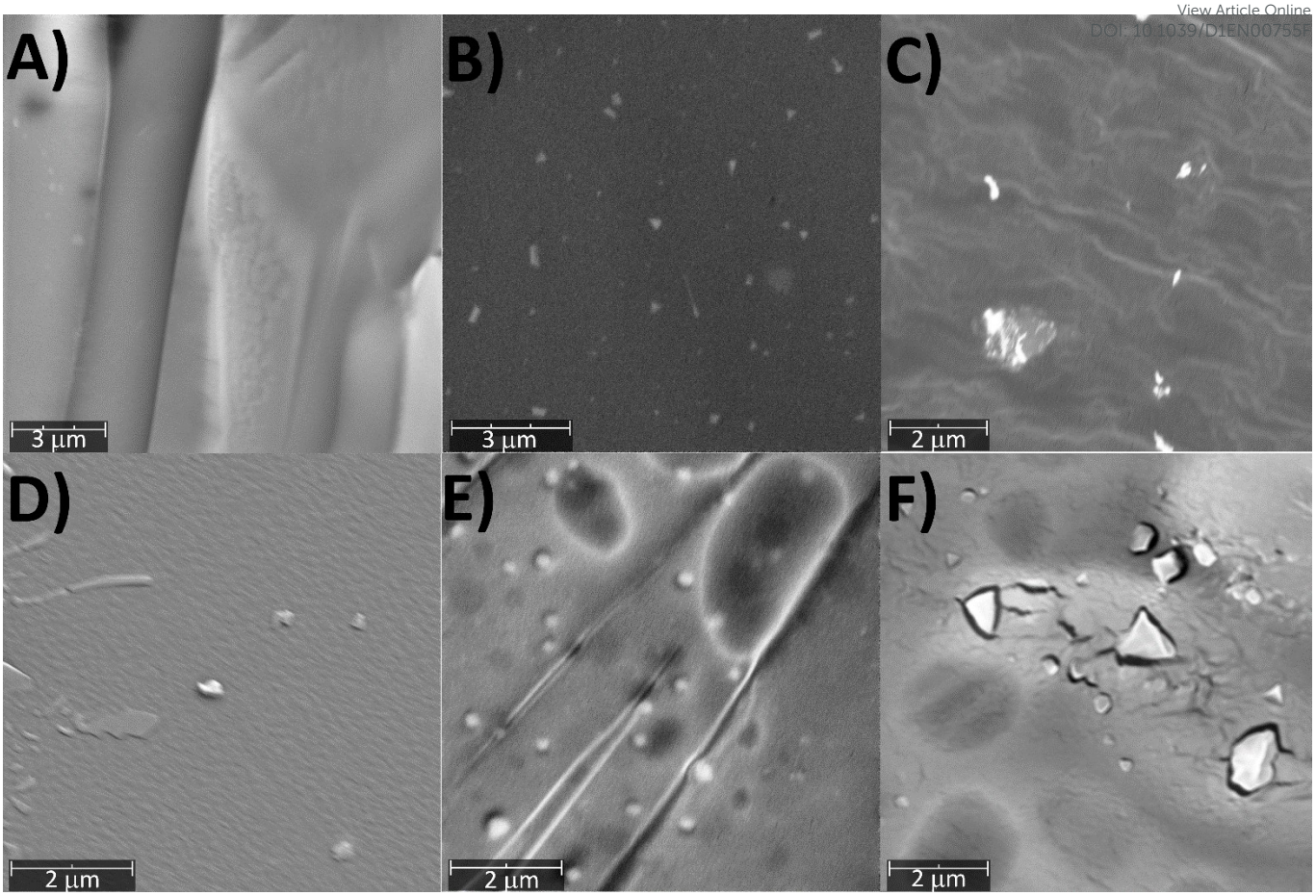

Figure 6 FEG-SEM micrographs of fragmented particles for different products at pH4 A) negative control, B) P1, C)P3, D)P6, E)P11 and F) P12

For the products that showed significantly higher fragmentation than controls, we calculated the amount of particles released as follows. Firstly, we subtracted the amount of particles in the negative controls and then the difference was used. We then calculated the total amount of particles in the volume used (10mL). Because we used a small subsample of the isolated tampon part, we then calculated the typical amount (of the isolated part) and then reported the total amount of potential particles released per tampon. A summary of the total particle calculated is shown in Table 2.

The particle sizes ranged from 36 to 848nm. The mean particle size and the mode for all samples are shown in Table 2. As the release of particles seemed to be pH dependent, when comparing the size distribution of particles, it can be seen that smaller particles are released in more acidic conditions (84nm). It should be noted that the particle size in the negative control (from unknown sources of contamination) could influence the size distribution of the particles truly coming from the synthetic polymers although this influence is minimal as the number of particles in controls is considerably lower.

Discussion

We characterised the bulk chemical composition of different common period products found all over the world. We dismantled the products and found that products are made of as many as 6 different components: wrapper, applicator, cover, core, inner string and outer string (Figure 2(3)).

Surprisingly, we discovered that many of the examined products contain synthetic polymers (mainly fibres) that would be in direct contact with the inner wall of the vagina when the products are used. Therefore, we decided to do *in-vitro* tests that simulate such conditions (pH4, 37°C and friction) (24,3).

When we examined the chemical composition of single fibres released, we noticed that the majority were cellulose based. This is consistent with the fact that all tampon cores are made of some form of cellulose. We also found evidence for the release of synthetic polymers in 7 out of 12 products tested (polyester, polypropylene, polyethylene and nylon).

The amount of fibres released was unexpectedly high. We found that on average 0.28mg of fibres were released per tampon. We therefore estimated that, on average, 9.4 billion fibres are released per period (considering 15 tampons used, d=1.5g/cm^3, fibres as cylinders, average diameter 27.5µm and average length 500µm).

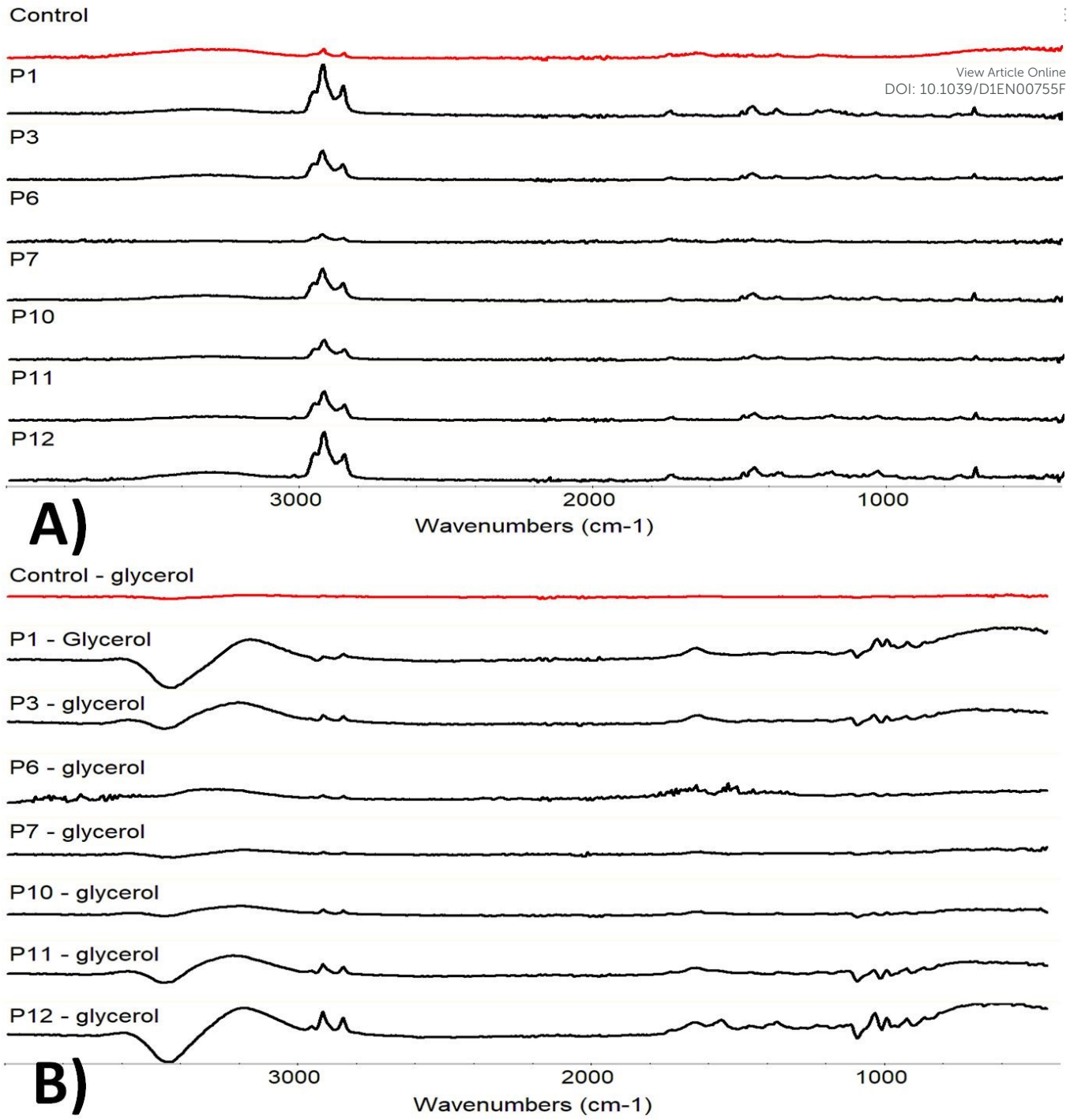

Figure 7 FTIR analysis of the released nanoparticles, A) Acetone wash, particles show polypropylene characteristic 2950, and 2920 cm^{-1} CH_3 stretching and 1456 cm^{-1}, symmetrical CH_3 bending as well as characteristic polyethylene 2850 cm^{-1} CH_3 stretching , peak a at 698 cm^{-1} not present in blank as well as 1450-1500 cm^{-1} region. B) Methanol wash, spectra shown after glycerol subtraction. Polyethylene bands at 2915 and 2846 cm^{-1} can be observed as well as polypropylene bands at 2949, 1373, 1164 and 995 cm^{-1}. All spectra shows the same y-axis scale as the controls

Even for "environmentally friendly" cotton (cellulose) fibres, the amount found is likely to cause adverse health effects. This is because cellulose is a very stable natural polymer with half-life of 5-8 million years and even in environments where microbes have developed highly specialised sets of enzymes, its degradation is in the order of weeks or months (28). Although larger fibres are more likely to be flushed out and are therefore less likely to be retained in the body, some released fibres can remain inside the body at least for as long the product is used (up to 8h) and possibly harbour bacterial infections, rupture cells or cause irritation and inflammation (29). Little attention has been given to the fate of such fibres inside the vagina. Whether these fibres are completely (and rapidly) flushed out or have any potential health effects while inside the body, still need to be properly addressed by the scientific community.

In terms of the release of synthetic polymer fibres inside the vagina, there is little research on their health effects. This is because the main entry routes studied in microplastic research are the lungs, gastrointestinal track and skin (30). Moreover, most of the research involving period products focuses on

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addressing the release of organic contaminants after extraction with strong organic solvents. Such contaminants include traces of phthalates, parabens, carbon disulphide, hexane, xylene, ethyl acetate and methylene chloride (31,1). However, extraction conditions used in such tests do not mimic conditions inside the vagina and therefore such chemicals may not leach. Recently, microplastics were reported in human placenta (32). In terms of shape, only microplastic particles were reported to be present. Microplastics fibres were found but were excluded from the above study. The authors decided to omit the presence of fibres because of the possible contamination and analytical challenges to determine their composition and origin. A suggestion was made about the origin of the microplastics found in placenta. It was pointed that most probable access was via the blood stream or the maternal respiratory system. However, our results highlight a different possibility for the occurrence of microplastics during pregnancy.

With respect to mimicking real world conditions of usage, there are some limitations to the Syngina test as well as to the preparation of the tampon itself for our fragmentation studies. First, the Syngina test conditions are not fully representative of the vaginal canal, in particular the membrane used does not mimic real conditions of friction, shape and elasticity as well as the presence of menstrual fluids and mucosa and the test is therefore less likely to promote fragmentation than normal use. Secondly, in our fragmentation experiments we deconstructed the tampon to select the component in contact with the vaginal wall, but did so with extreme care to avoid unnecessary physical damage and any associated fragmentation. Overall, we therefore believe our results are likely to represent a conservative estimation of fragmentation in comparison to real-world usage.

The formation of nanoplastics in a handful of consumer products due to mechanical, UV light and temperature factors has been recently reported (27,26,25). The validity of such results has been questioned, as it was pointed out that particles found using FEG-SEM could be attributed to the formation of soluble monomer crystals as a result of sample drying. However, the complementary use of NTA in the current reported study, provides unequivocal evidence to refute such concerns, since the particle counts are performed in suspension and thus cannot report presence of precipitated crystals (33,34).

Table 2 Calculated potential total amounts of particles released per tampon (pH4), amount of particles found in negative controls was subtracted and considered for calculations. Summary of the particle size distribution for the fragmentation experiments at different pH conditions as well as quality control samples

	Particle concentration						Particle size (nm)					
							pH4			pH7		
Sample	particles /mL difference	Total in 10mL	Weight of subsample (g)	Total weight of part (g)	Total particles per tampon	Part used / Bulk composition	Mean	Mode	SD	Mean	Mode	SD
Polystyrene	NA	NA	NA	NA	NA	NA	NA	NA	NA	99.3	96.7	9.5
DO water	(8.26E+06)	NA	NA	NA	NA	NA	NA	NA	NA	152	84.7	48.1
Filtered buffer	(7.80E+05)	NA	NA	NA	NA	NA	78.7	71.3	11.9	75.4	76.1	3.5
Negative control	(1.95E+07)*	NA	NA	NA	NA	NA	137.1	149.3	43.8	166	130.9	65.9
P1	**5.84E+07**	5.84E+08	0.0187	0.3363	1.05E+10	Cover / Polyester	105.1	92.9	26.5	251	162.6	211.1
P3	**1.41E+08**	1.41E+09	0.0191	0.0965	7.10E+09	Cover /polyester / polypropylene	48.2	35.3	27.7	133	91	44.6
P6	**9.65E+07**	9.65E+08	0.0162	0.1145	6.82E+09	Cover / polyethylene	74.3	49.4	35.3	201	166.7	77.9
P10	**8.70E+06**	8.70E+07	0.0122	0.1875	1.34E+09	Cover /polyester / polypropylene	67.2	56.6	43.1	128	152.4	36.8
P11	**3.84E+07**	3.84E+08	0.0223	1.0157	1.75E+10	Whole /polyetherurethane	61	41.1	22.9	125	150.4	25.8
P12	**1.88E+08**	1.88E+09	0.0159	0.0825	9.73E+09	String /cotton / polyethylene	75.2	46	31.6	111	100.4	24.7

DO= deionised water, NA= Not applicable, SD=standard deviation, nm=nanometer. Numbers in brackets indicate raw results, the number of particles

reported in products **P1-P12** in bold were subtracted from negative control (*) for fragmentation experiment

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In the present study, it was found that the fragmentation and release of nanoplastics is pH dependent (Figure 5). It also seems to be dependent on the initial type of synthetic polymer. For example, silicone rubber (Figure 5, P23, as found in period cups) show to have excellent resistance to fragmentation and did not show release of particles at any of the tested pH. We found differences in the amount of particles released for the same polymers in different products. We also noted that different products have different polymer fibre diameter (Figure S16 and S17). It is well known that contact area increases exponentially when the particle size is decreased and therefore the fibre diameter could also affect the amount of particles released in different products with the same polymer. This confirms the hypothesis that the release of particles is polymer-dependent as well as dependent on the initial material size. Other research on fragmentation of plastics into nanoplastics discovered much less concentration of nanoplastics when the initial material was bulk plastic (25). In contrast, a similar number of nanoplastics (in the billions) were released when the initial material used were fibres of approximately 50µm in diameter (26).

Polyesters (such as polyethylene terephthalate, PET) are known to be degraded by hydrolysis (30). We hypothesise that the cleavage of the internal polymer bonds when reacted with water can be accelerated at lower pH as the loss of molecular weight causes a reduction of the mechanical properties and increased stress gradients in the surface of the polymer, eventually leading to fragmentation.

In the present study, we found products containing synthetic polymers released 1.3 to 17 billion nanosize particles per tampon at pH4 and 37 °C in 4h. Using the median number of particles found in our tested products (8.6 billion, from Table 2) a woman using tampons (40 years, 500 menstrual cycles, 20 tampons per cycle as an indicative example) (35) could be exposed to 86 trillion (range 13 - 170 trillion) fragmented synthetic polymers over a lifetime of product use. While these are generalised figures only and considerable uncertainty surrounds the estimates, this number is of concern because such amounts have the potential to be chiefly released inside the vagina and therefore to potentially trigger chronic effects of the kind reported by Lin et al., (2020) (1).

The vaginal tract has a bigger surface area compared to normal skin. In contrast to the self-cleaning properties of the vagina, the mucosa is known to show mucoadhesion properties. Several polymers have been used to exploit that property (polyacrylates, polycarbophil, chitosan, cellulose derivatives, pectin and alginate among others) (36). It is therefore a possibility, for the nano size plastic particles observed, to find their way and reach the epithelial surface and interact with cells. In order to understand with certainty, the colloidal stability and fate of the particles, additional *in-vitro* tests mimicking more closely the fluids of interest are needed.

Although not the primary focus of the current research, the potential for environmental fragment release post-use should also be considered. Given the non-recyclable nature of the products tested and likely continued ex-vivo degradation (with the accepted caveat that environmental e.g., landfill conditions will vary substantially) some estimates can be made. Based on a current estimate of global sales of 18 billion/annum of tampons (5.8 billion sold in US in 2018 as 1/3 of global market (35)) and an equivalent environmental release per tampon of particles as reported in our lab study (8.6×10^9) a broad approximation of a total of 1.55×10^{20} fragmented released particles is attained. While the contribution of sanitary products to bulk litter issues has been investigated (6) the potential environmental impact of their fragmentation products warrants further investigation.

The effect of the nanoplastic itself in human health is poorly understood for several reasons. Firstly, the characterisation and investigation of their fate is challenging even using modern analytical techniques. Secondly, most of the studies have used "environmentally" relevant concentrations of nanoplastics (1pg/L to 15ug/L or 1.4 particles/mL to $2X10^7$ particles/mL, considering 100nm particles and 1.38g/cm^{-3}) (37,38). In the present study we provide an indication that certain toxicology tests should consider cells exposed to billions of nanoplastics per mL instead.

The surface epithelial squamous mucosa forms a barrier that can be compromised by nanoplastics. Particles smaller than 100nm are known to cross cell membranes (39). If this barrier is compromised, then potentially harmful chemicals can enter and initiate pathological and chronic effects (40). Moreover, Wick et al, showed that nanoparticles smaller than 240nm (polystyrene beads) can cross the placental barrier using ex-vivo human placental perfusion model (41,42). Such research focused on possible implications and applications of engineered nanoparticles highlighting the fact that no exposure to nanoparticles with environmental origin was of concern at the time of publication (2015). We believe that this assumption has to be challenged.

Extrapolation from knowledge into engineered nanoparticles (gold and titanium dioxide) that bear essential similarities to nanoplastics (inertness and size) indicates that nanoplastic toxicity to human cells can manifest in three main ways: the plastic particles themselves, the release of persistent organic pollutant adsorbed to the plastics, and to the leaching of additives of the plastics (43). Nanoplastics themselves can activate the innate immune system by inducing inflammatory responses, or mediating oxidative stress.

Even in a scenario where nanoplastics themselves are found not to be cytotoxic, the fact that volatile organic contaminants have been found in tampons (1), and because the concentration of such contaminants on the surface of nanoplastics can be increased several orders of magnitude than the bulk material (43), makes these particular nanoplastics coming from tampons potentially very toxic.

A novel recently introduced product (menstrual cup) was found to release a lower amount of nanoparticles. However, we suggest research into a comprehensive chemical characterisation of such products because of the known ability of medical silicones to diffuse chemicals. This property is so

effective that medical silicone has even been used as drug delivery substrate (44).

A plethora of research has highlighted the risk of microplastics and nanoplastics finding their way from the environment and eventually into animals and humans. Few studies have however investigated the generation of nanoplastics that have a readily available entry route for exposure to humans. To our knowledge, this is the first investigation regarding the potential generation of nanoplastics inside the human body and most worryingly may have implications for women's health.

Conclusions

We characterised the bulk chemical composition of several commonly used tampons and found that many products contain synthetic polymers (plastics) that would be in direct contact with the vaginal wall when in use. We tested our hypothesis and confirmed that some products released fibres during *in-vitro* tests and also found evidence for fragmentation and a possible release of billions of nanoplastics per tampon under conditions that mimic normal use. The health implications are unknown. However, knowledge extrapolated from other areas indicates an increased probability of health problems from decreased particle sizes and because of the huge amount of potential particles released inside the human body. Negative health effects could manifest in three ways: by the nanoplastics themselves, from release of contaminants adsorbed to the nanoplastics and leaching of additives associated to the production of the plastics.

Author Contributions

Leonardo Pantoja Munoz: Conceptualization; Investigation; Resources; Methodology; Writing - original draft; Writing - review & editing, Alejandra Gonzalez Baez: Investigation; Methodology; Data curation; Writing - review & editing, Diane Purchase: Writing - original draft; Writing - review & editing, Huw Jones: Data curation; Writing - review & editing and Hemda Garelick: Writing - review & editing

Conflicts of interest

There are no conflicts to declare.

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References

1. Lin N, Ding N, Meza-Wilson E, Devasurendra AM, Godwin C, Park SK, et al. Volatile organic compounds in feminine hygiene products sold in the US market: A survey of products and health risks. Environment International. 2020; 144.

2. Statista. Forecast market of tampons worldwide from 2018 to 2024.; 2020 [cited 2021 June 15. Available from: https://www.statista.com/statistics/920646/global-market-value-of-tampons/.

3. EDANA. EDANA Guidelines for Testing Feminine Hygiene Products. ; 2018.

4. Tudor DT, Williams AT. Public Perception and Opinion of Visible Beach Aesthetic Pollution: The Utilisation of Photography. Journal of Coastal Research. 2003.

5. de Carvalho RH, Dutra Lacerda P, da Silva Mendes S, Correa Barbosa B, Paschoalini M, Prezoto F, et al. Marine debris ingestion by sea turtles (Testudines) on the Brazilian coast: an underestimated threat? Marine Pollution Bulletin. 2015.

6. Treilles R, Gasperi J, Saad M, Tramoy R, Breton J, Rabier A, et al. Abundance, composition and fluxes of plastic debris and other macrolitter in urban runoff in a suburban catchment of Greater Paris. Water Research. 2021.

7. Chiaruzzi M, Barbri A, Muggeo A, Tristan A, Jacquemond I, Badiou C, et al. Vaginal Tampon Colonization by Staphylococcus aureus in Healthy Women. Applied and Environmental Microbiology. 2020.

8. Gao CJ, Kannan K. Phthalates, bisphenols, parabens, and triclocarban in feminine hygiene products from the United States and their implications for human exposure. Environmental International. 2020; 136.

9. Thomas P, Perono G, Tomassi F, Pagano G, Oral R, Buric P, et al. Resolving the effects of environmental micro- and nanoplastics exposure in biota: A knowledge gap analysis. Science of The Total Environment. 2021.

10. Pan L, Yu D, Zhang Y, Zhu C, Yin Q, Hu Y, et al. Polystyrene microplastics-triggered mitophagy and oxidative burst via activation of PERK pathway. Science of The Total Environment. 2021.

11. Xie X, Deng T, Duan J, Xie J, Yuan J, Chen M. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. Ecotoxicology and Environmental Safety. 2020.

12. Nie Jh, Shen Y, Roshdy M, Cheng X, Wang G, Yang X. Polystyrene nanoplastics exposure caused defective neural tube morphogenesis through caveolae-mediated endocytosis and faulty apoptosis. Nanotoxicology. 2021.

13. Fournier S, D'Errico J, Adler D, Kollontzi S, Goedken M, Fabris L, et al. Nanopolystyrene translocation and fetal deposition after acute lung exposure during late-stage pregnancy. Particle and Fibre Toxicology. 2020.

14. Magri D, Sanchez-Moreno P, Caputo G, Gatto F, Veronisi M, Bardi G, et al. Laser Ablation as a Versatile Tool To Mimic Polyethylene Terephthalate Nanoplastic Pollutants: Characterization and Toxicology Assessment. ACS Nano. 2018;: p. 7690-7700.

15. Shi Q, Tang J, Wang L, Liu R, Giesy J. Combined cytotoxicity of polystyrene nanoplastics and phthalate esters on human lung epithelial A549 cells and its mechanism. Ecotoxicology and Environmental Safety. 2021.

16. Shi Q, Tang J, Liu R, Wang L. Toxicity in vitro reveals potential impacts of microplastics and nanoplastics on human health: A

review. Critical reviews in environmental science and technology. 2021.

17. Qiao J, Chen R, Wang M, Bai R, Cui X, Liu Y, et al. Perturbation of gut microbiota plays an important role in micro/nanoplastics-induced gut barrier dysfunction. Nanoscale. 2021.

18. Kik K, Bukowska B, Krokosz A, Sicinska P. Oxidative Properties of Polystyrene Nanoparticles with Different Diameters in Human Peripheral Blood Mononuclear Cells (In Vitro Study). International Journal of Molecular Sciences. 2021.

19. Ballesteros S, Domenech J, Barguilla I, Cortes C, Marcos R, Hernandez A. Genotoxic and immunomodulatory effects in human white blood cells after ex vivo exposure to polystyrene nanoplastics. Environmental Science: Nano. 2020.

20. Mishra P, Vinayagam S, Duraisamy K, Patil SR, Godbole J, Mohan A, et al. Distinctive impact of polystyrene nano-spherules as an emergent pollutant toward the environment. Environmental Science and Pollution Research. 2018.

21. Poma A, Vecchiotti G, Colafarina S, Zarivi O, Aloisi M, Arrizza L, et al. In Vitro Genotoxicity of Polystyrene Nanoparticles on the Human Fibroblast Hs27 Cell Line. Nanomaterials. 2019.

22. Rubio L, Barguilla I, Domenech J, Marcos R, Hernandez A. Biological effects, including oxidative stress and genotoxic damage, of polystyrene nanoparticles in different human hematopoietic cell lines. Journal of Hazardous Materials. 2020.

23. Bojic S, Falco M, Stojkovic P, Ljujic B, Jankovic MG, Armstrong L, et al. Platform to study intracellular polystyrene nanoplastic pollution and clinical outcomes. Stem Cells. 2021.

24. Rakoff AE, Feo LG, Goldstein L. The Biologic Characteristics of the Normal Vagina. American Journal of Obstetrics and Gynecology. 1944.

25. Lambert S, Wagner M. Characterisation of nanoplastics during the degradation of polystyrene. Chemosphere. 2016.

26. Hernandez LM, Xu EG, Larsson HCE, Tahara R, Maisuria VB, Tufenkiji N. Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea. Environmental Science and Technology. 2019.

27. Ekvall MT, Lundqvist M, Kelpsiene E, Sileikis E, Gunnarsson SB, Cedervall T. Nanoplastics formed during the mechanical breakdown of daily-use polystyrene products. Nanoscale advances. 2019.

28. Chmolowska D, Hamda N, Laskowski R. Cellulose decomposed faster in fallow soil than in meadow soil due to a shorter lag time. Journal of Soils and Sediments. 2017.

29. Schilevert PM. Comparison of cotton and cotton/rayon tampons for effect on production of toxic shock syndrome toxin. Journal of infectious Disease. 1995.

30. Lehner R, Weder C, Petri-Fink A, Rothen-Rutishauser B. Emergence of Nanoplastic in the Environment and Possible Impact on Human Health. Environmental Science and Technology. 2019.

31. Park CJ, Barakat R, Ulanov A, Li Z, Lin PC, Chiu K, et al. Sanitary pads and diapers contain higher phthalate contents than those in common commercial plastic products. Reproductive toicology. 2019.

32. Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, et al. Plasticenta: First evidence of microplastics in human placenta. Environment International. 2021.

33. Busse K, Ebner I, Humpf HU, Ivleva N, Kaepler A, Ozmann BE, et al. Comment on "Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea". Environmental Science and Technology. 2020.

34. Hernandez LM, Xu EG, Larsson HCE, Tahara R, Maisuria VB, Tufenkji N. Response to Comment on "Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea". Environmental Science and Technology. 2020.

35. Borunda A. National Geographic. [Online].; 2019 [cited 2021 July 12. Available from: www.nationalgeographic.com/environment/2019/09/how-tampons-pads-became-unsustainablestory-of-plastic/..

36. Acarturk. Mucoadhesive vaginal drug delivery systems. Recent Patent Drug Delivery Formulations. 2009; 3(3): p. 193-20.

37. Mendoza LMR, Karapanagioto H, Ramirez Alvarez N. Micro(nanoplastics) in the marine environment: Current knowledge and gaps. Current Opinion in Environmental Science & Health. 2018.

38. Shen M, Zhang Y, Zhu Y, Song B, Zeng G, Hu D, et al. Recent advances in toxicological research of nanoplastics in the environment: A review. Environmental Pollution. 2019.

39. Shilo M, Sharon A, Baranes K, Motiei M, Lellouche JPM, Popovtzer R. The effect of nanoparticle size on the probability to cross the blood-brain barrier: an in-vitro endothelial cell model. Journal of Nanobiotechnology. 2015.

40. Squier CA, Mantz MJ, Schlievert PM, Davis CC. Porcine vagina Ex Vivo as a model for studying permeability and pathogenesis in mucosa. Journal of Pharmaceutical Sciences. 2007.

41. Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier Capacity of Human Placenta for Nanosized Materials. Environmental Health Perspectives. 2010.

42. Grafmueller S, Manser P, Diener L, Diener PA, Maeder-Althaus X, Maurizi L, et al. Bidirectional Transfer Study of Polystyrene Nanoparticles across the Placental Barrier in an ex Vivo Human Placental Perfusion Model. Environmental Health Perspectives. 2015.

43. Bouwmeester H, Hollman PCH, Peters RJB. Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. Environmental Science and Technology. 2015.

44. Rahimi A, Mashak A. Review on rubbers in medicine: natural, silicone and polyurethane rubbers. Plastics, Rubber and Composites. 2013.

View Article Online DOI: 10.1039/D1EN00755F