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Research Paper

Identifying laboratory sources of microplastic and nanoplastic contamination from the air, water, and consumables

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Low

HIGHLIGHTS

G R A P H I C A L A B S T R A C T

Labware

Plastic

Glass

Location

Biosafety cabinet

Lab bench

Fume hood

Water

Milli-O

Reverse osmosis

an water

Other

Experiment duration

Dust

Foil

- Any experimental work adds microplastic and nanoplastic contamination.
- Mitigation strategies to minimise contamination depends on detection techniques.
- Plasticware consumables outperform glassware consumables.
- Biological safety cabinets did not significantly reduce contamination.
- Aluminium foil introduces contamination to samples.

ABSTRACT

Microplastic particle contamination

Microplastic and nanoplastic research has proliferated in recent years in response to the escalating plastic pollution crisis. However, a lack of optimised methods for sampling and sample processing has potential implications for contaminating samples resulting in an overestimation of the quantity of microplastics and nanoplastics present in environmental samples. In response, a series of recommendations have been made, but most have not been quantified or validated sources of contamination. In the present study, we investigated sources of plastic contamination in common laboratory procedures including water sources (e.g., Milli-Q), consumables (e.g., unburnt glassware), airflow (e.g., fume hood) and dust. Using flow cytometry, we identified water, air flow and dust as sources of significant contamination. Milli-Q and reverse osmosis were the least contaminated

Abbreviations: QA/QC, quality assurance and quality control; μ-FTIR, micro-Fourier-transform infrared spectroscopy; BSC, biological safety cabinet; LAF bench, laminar flow cabinet; UTAS, University of Tasmania; IMAS, Institute of Marine and Antarctic Studies; TSoM, Tasmanian School of Medicine; FSC, forward scatter; SSC, side scatter; RO, reverse osmosis.

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sources when compared with tap water. Interestingly, current recommendations are to use glass consumables in replacement of plastic consumables, however, we have identified glassware and glass consumables as a significant source of contamination. Current best practice is to cover the glass tube with aluminium foil to reduce airborne contamination, but we found fresh aluminium foil to be a significant source of contamination, bringing light to the limitations foil has as a contamination control measure. Lastly, we identified significant quantities of microplastics and nanoplastics present in dust collected within the laboratory, suggesting this is a widespread and underestimated source of contamination. We have provided validated sources of contamination for both consumables and common laboratory procedures and provided mitigation strategies based on these. Additional recommendations include the appropriate design of experimental controls to quantify levels of introduced contamination based on methods and the detection techniques utilised. The application of these mitigation strategies and appropriate experimental design will allow for more accurate estimations on the level of microplastic and nanoplastic contamination within environmental samples.

1. Introduction

Microplastic and nanoplastic particle research has increased exponentially in the last two decades [46]. Plastic pollution is ubiquitous and has been driving this surge in scientific interest: each year new research documents pollution in new and often unexpected locations, such as in the fresh snow of Antarctica [2], remote uninhabited islands [22] and throughout the human body [29,36]. Oceanic currents and weather systems play a key role in the transport and degradation of plastics [10] from multiple sources including accidental or intentional littering (e.g., on beaches), poor waste management in cities [26,55], and dumping or unintentional loss during transport (e.g., shipping waste; [9,14]). An increasing number of studies now suggest the plastic crisis is encompassing all aspects of the natural world, from human health to the stability of ecosystems [21,39]. However, many inconsistencies (i.e., non-standardised methodologies) and limitations (i.e., laboratory contamination) are increasingly apparent within the plastics literature [28,34,49]. Given the unveiling suite of consequences of this diverse pollutant, calls from established microplastic research groups have been made to harmonise methods to increase comparability [35]. Without this, our understanding of the scope or severity of plastic impacts, or pace of change, is being limited.

Most experiments rely on single-use plastics, with 2014 estimates suggesting researchers generated approximately 5.5 million tonnes of laboratory-based plastic waste [1,47]. This is largely due to the convenience of single-use plastics (e.g., pipette tips and centrifuge tubes) in managing biological contamination (i.e., reduces the necessity for sterilising instruments between experiments; [17,24]). However, the surge of single-use plastics, combined with synthetic textiles found in most commercially available laboratory coats [43] and airborne plastic particles from filters [50] and dust [45,58] increases the risk of contamination of environmental samples with very small particles. Wearing even a single item of protective equipment (e.g., single-use gloves) in research laboratories has been shown to interfere with quantification and polymer identification of microplastics using techniques such as Fourier-transform infrared spectroscopy (FT-IR; [52]). In light of this information, it is unsurprising that sample contamination has been highlighted as an issue of increasing concern for studies that utilise plastic materials while also aiming to quantify the presence of small particles (i.e., nano-particles; [16,18,51]). Consequently, the validity of the data produced is often questioned as single-use plastics as a source of particle contamination has not been validated.

To try and address the contamination issue, a recent review of contemporary plastic research by Prata et al. [33] identified common quality control and quality assurance (QA/QC) protocols employed by past researchers and provided recommendations on how to reduce or limit procedural contamination. These included wearing cotton laboratory coats [12], extensive cleaning and sterilisation of equipment and utensils [30] often with the use of particle-free water [6,53], switching from plastic to glass utensils where possible [15,32], using laminar flow cabinets and fume hoods to limit airborne contamination [33,52] and comparisons between environmental samples and procedural blanks

[48]. While these extensive recommendations may reduce some contamination in experiments, sources of contamination often remain speculative rather than experimentally validated. Consequently, implementing QA/QC protocols, as suggested above, may not consistently ensure the same level of contamination reduction across each experiment, potentially impacting the replicability of studies.

Despite these recommendations, many studies do not implement sufficient preventative measures, such as those recommended by Prata et al. [33], Hermsen et al. [16], and Koelmans et al. [18]. This is likely due to a lack of standardised methods available to guide sampling of very small particles (e.g., nano-plastics), limited but increasing awareness of this issue in the broader research community, and inherent challenges in avoiding contamination [15]. Information and guidance regarding the effectiveness of different measures currently employed to limit contamination are also lacking, but urgently needed. An exception is a single study by Gwinnett and Miller [15] that demonstrated employing strict anti-contamination protocols, such as replacing plastic utensils for glass and reducing airflow in experimental rooms, may reduce sample contamination by \sim 37%. However, not all suggested QA/QC protocols have been validated in this way (i.e., sources of contamination are identified and quantified). In the absence of robust data on a variety of effective preventative measures, environmental samples will continue to be accidentally contaminated by researchers leading to biased datasets, less significant findings, and together, this may impede the development and reliability of plastic research [15,52].

Developing best practice approaches that ensure researchers can limit contamination and thus compare results across space and time and build on existing datasets is a priority [27,5,7]. Therefore, the current research aims to (1) identify contamination in common plastic research procedures; water sources (i.e., Milli-Q), experimental consumables (i. e., plastic centrifuge tubes or glass centrifuge vials), and air ventilation (i.e., fume hoods) as sources of airborne contamination; (2) estimate the abundance and approximate size range of microplastics and nanoplastics in samples using flow cytometry; (3) provide recommendations to reduce microplastic and nanoplastic contamination and standardise the experimental approach to plastic research.

For clarity, this study defines microplastics and nanoplastics as 1 μm – 5 mm and 1 - < 1000 nm, respectively [51]. In addition, our study refers to contamination as anthropogenic or airborne microparticles or nanoparticles that become entrapped in samples throughout sampling and processing and are therefore not part of the original environmental sample (e.g., water; [15,33]), or are detected as part of a sample that has different origins.

2. Materials and methods

2.1. Quality assurance and quality control (QA/QC) procedures

As this research aims to identify and quantify sources of contamination, extensive QA/QC procedures were used to minimise external contamination in all experiments, thereby ensuring results best reflect contamination from sources tested. A workplace health and safety agreement was generated in collaboration with laboratory management to ensure all experiments were conducted safely. No nitrile gloves were worn when conducting experiments with the exception of plastic milling and when conducting the location experiment due to operating the Biological Safety Cabinets (hereafter referred to as BSC) and fume hoods. Those conducting experiments wore non-synthetic clothing where possible with the exception of laboratory coats, which were made of a cotton blend (65% polyester and 35% cotton) as pure cotton laboratory coats could not be sourced.

2.1.1. Preparation of consumables

All unburnt glassware (e.g., pipette tips, tubes) was triple rinsed with the least contaminated water identified immediately prior to experiment (Milli-Q®; as described below). Single-use plastic (i.e., plastic pipette tips and tubes) were not rinsed as to best replicate standard laboratory practices. Flow cytometry tubes were triple rinsed with Milli-Q® directly before use.

2.2. Plastic milling for positive control

To include a number of positive samples of a known plastic type, we employed a combination of plastic milling methods with a mortar and pestle and a blender to obtain microplastics and nanoplastics suitable for analysis on flow cytometry.

Cell culture plates (polystyrene), Eppendorf® tubes and FALCON® tubes (polypropylene) were first blended with a Nutribullet® to create micrometre particles. Particles were manually milled with liquid nitrogen to obtain nanometre particles. Manual grinding was performed for twenty minutes with a mortar and pestle until a finer powder was achieved. The cyro-milled plastics were filter through a 70 μ m cell strainer (Corning®) to ensure that the size of the particles is detectable by flow cytometry.

2.3. Comparison of common laboratory water sources

Water samples were obtained from three different laboratories at three University of Tasmania campus locations separated by at least 950 m; Chemistry and Central Science Laboratory (UTAS -42.903108, 147.325736), the Institute of Marine and Antarctic Studies (IMAS; -42.886214, 147.335753), and the Tasmanian School of Medicine (TSoM; -42.878792, 147.329797). From each campus, water samples were collected from three different sources: Milli-Q®, tap and reverse osmosis (RO) in centrifuge tubes and were processed as quickly as possible using flow cytometry.

Technical replicates were collected from each site from different taps (i.e., 3 Milli-Q samples from 3 Milli-Q taps), with the exception of IMAS and TSoM laboratories where collection number was based on the number of taps available.

From this experiment, Milli-Q was determined to be the water source with the least contamination and formed the basis of experiments moving forward. In experiments 2.4 - 2.7, Milli-Q was utilised as a control and processed immediately following collection.

2.4. Comparison of laboratory consumables (i.e., plastic or glass)

Pipetting is a common technique used in laboratory experiments to introduce an accurate quantity of a reagent either to or off a sample. This practice is commonly used in experiments to quantify microplastics, for example creating and applying a chemical digestion to remove biological material and reveal embedded plastic particles. This experiment is designed to examine the level of contamination from common laboratory consumables (i.e., glass vs plastic tips and tubes) used in an experiment with pipetting. To test plastic consumables, 2 centrifuge tubes were filled with 1 ml or 0.5 ml of Milli-Q, with 0.5 ml pipetted with a plastic pipette (Gilson) and plastic pipette tip between each tube twenty times in twenty minutes with the lid remaining open for the

duration of the experiment. For glass, 2 glass tubes (Borax® dispense-apax disposable culture tubes) were filled with 2.5 ml or 1.5 ml of Milli-Q, with 1.5 ml pipetted with a plastic stripette with a glass stripette tip between each tube twenty times in twenty minutes. Samples were processed immediately following the experiment using flow cytometry. Experimental replicates were considered independent if sampled on different days. For each day, three technical replicates were performed, and the average was taken.

2.5. Experiment location

The following experiment was designed to investigate the sources of airflow contamination within the laboratory by examining the level of contamination in common laboratory workspaces. We examined airflow contamination in the fume hood, BSC (LabCulture® ESCO Class II Biological Safety Cabinet), and bench top (open air) settings. In each setting, two centrifuge tubes were filled with 1 ml or 0.5 ml of Milli-Q, with 0.5 ml pipetted between each tube twenty times in twenty minutes with the lid open, as to best replicate a typical laboratory experiment. Once the experiment was complete, the centrifuge tube was closed, and samples were obtained by performing experiments on different days. Three replicates were obtained in each setting (i.e., bench top, fume hood, BSC).

2.6. Laboratory dust

To investigate if airborne dust contamination is present within the laboratory, three locations within the laboratory at the Tasmanian School of Medicine were sampled for dust. Experiment location was determined on bench locations adjacent to a fume hood in order to identify airborne contaminants that may be present in areas where experiments are commonly conducted.

An aluminium foil dish (10×10 cm) was constructed and placed to collected dust over the course of 30 days. At the end of this time, the aluminium foil trays were removed and thoroughly rinsed with 25 ml of Milli-Q into a glass beaker. This was then heated at 60 °C, with a foil cover, until the water was fully evaporated. The remaining particles were then resuspended with 5 ml of Milli-Q water and transferred to triple Milli-Q rinsed glass tubes. These tubes were then analysed with flow cytometry and a sub-sample with μ -FTIR (see Supplementary Methods 1.1). This experiment had an additional control to account for the use of foil. Fresh aluminium foil was taken and 2 ml of Milli-Q was rinsed over the unexposed side, collected, and processed immediately as above.

2.7. Washout experiment

This experiment was designed to test if consumables can be reused or cleaned following a nanoplastic or microplastic experimental sample. Centrifuge tubes were filled with 1 ml of Milli-Q and processed on flow cytometry to determine baseline. The same centrifuge tubes were dosed with a known concentration of known nanoplastic and microplastic polymers which was confirmed by flow cytometry. The centrifuge tube was then tripled rinsed with Milli-Q, 1 ml of Milli-Q added, vortexed, and re-processed through flow cytometry. An additional detergent cleaning step was performed, centrifuge tubes were placed in detergent bath with Milli-Q and rinsed again with Milli-Q a further three times. Finally, 1 ml of Milli-Q was added, vortexed and re-processed through flow cytometry. Experimental replicates (n = 5) were achieved by repeating the experiment with different plastic types including environmental microplastics, nylon, polypropylene, polystyrene, and polyvinyl chloride.



⁽caption on next column)

Fig. 1. Laboratory water including Milli-Q systems, reverse osmosis (RO) and tap water as sources of introduced nanoplastic and microplastic contamination. Water samples (Milli-Q, n = 8; RO, n = 10; tap, n = 11) were analysed using flow cytometry and data was analysed using one-way ANOVA followed by Holm- Šídák post-hoc comparisons. Plastic particle count (a) and all particle count (b) was measured for each water source. Displayed as half violin plots and box plots with median and interquartile ranges on an exponential y axis. Significance is displayed as * when compared to Milli-Q or # when compared to RO. (c) Density plots of log10 Side scatter (LOG SSC) of the plastic particles of each water source compared to polystyrene beads of known sizes (200 nm, 500 nm, 800 nm and 3.2 μ m, and A549 cultured human cells (~10 μ m). Each density plot is set to the same axis scale expressed as a percentage of the maximum mode of all the groups within each plot.

2.8. Flow cytometry (Cytek Aurora) analysis

A Cytek Aurora® flow cytometer equipped with multiple lasers of the violet fluorescence spectra was used to analyse samples. Prior to processing samples, the instrumental fluidics were cleaned to reduce the possibility of contamination by residual particles from previous analyses (see Supplementary Methods 1.2).

Samples were placed into the sample injection port and passed through the fluidic system on medium flow rate. Forward scatter (FSC) and side scatter (SSC) parameters were detected to characterise the size and complexity of each particle that passed the internal laser light source. The lasers were of the violet fluorescence spectra (V2-A; violet excitation 405 nm, and V15-A; excitation 405 nm, bandpass 765–795 nm).

2.8.1. Gating strategy

We designed specialised gates to identify if microplastics and nanoplastics were present in our samples. This was achieved by processing both positive and negative controls. Positive controls include environmental plastics (mixed plastics found in beach sediment, Lord Howe Island, AUS, $31.53^{\circ}S$, 159.07°), polypropylene and polystyrene that were milled (as described above), and primary nylon microplastics (Goodfellow (AM30-PD-000110)). Silica (<15 µm, MIN-U-SIL 15; 300 µg/ml) was included as a negative control (Fig. S1). The plastic positive controls were identified as being distinctly fluorescent under the V15-A and V4-A spectra channels, therefore all particles were characterised with a V15-A and V4-A gate. Polyester beads of various known sizes (200 nm, 500 nm, 800 nm, and 3.2μ m) were purchased and used to set size limits to determine the particle size of plastics.

It should be noted that previous research has established that skin cells (a common component of dust) are not fluorescent without labelling when analysed using flow cytometry [57]. However, we acknowledge that the fluorescent profile of all potential non-plastic particles has not been quantified and therefore some particles detected within our gate may be of non-plastic origin. However, with thorough QA/QC protocols, procedural blanks, and specialised gates, we expect that a small portion of particles would be of non-plastic fluorescing particles.

2.9. Statistical analyses

Experiments were analysed with one-way ANOVA. The assumptions of normality and heteroscedasticity of the residuals were evaluated graphically with Q-Q plots and residual vs. predicted plots, respectively. Appropriate transformations were applied when necessary. Pairwise comparisons were made with Holm-Šídák post-hoc test. Distribution differences were evaluated with Kolmogorov-Smirnoff tests. Statistical evaluations were deemed significant if p < 0.05. All analyses were conducted in R v4.2.2 and RStudio 2023.03.0 + 386 [41].



(caption on next column)

Fig. 2. Common plastic and glass laboratory consumables as sources of introduced nanoplastic and microplastic contamination. Milli-Q was pipetted between glass and plastic tubes with a glass or plastic pipette for 20 min as to best replicate standard experimental procedures. Milli-Q collected into a flow cytometer tube was used as a control. (a) Plastic particle count per μ L and (b) all particle count per µL was determined for each experiment. Data was analysed with a one-way ANOVA followed by Holm- Šídák post-hoc pairwise compari-*** p < 0.0001, ** p < 0.001, * p < 0.01 compared to control; son. ### p < 0.001, # p < 0.05 compared to plastic consumables. Displayed as half violin plots and box plots with median and interquartile ranges on an exponential y axis. (c) Density plots of log_{10} Side scatter (LOG SSC) of the plastic particles compared to polystyrene beads of known sizes (200 nm, 500 nm, 800 nm and 3.2 μ m, and A549 cultured human cells (~10 μ m). Each density plot is set to the same scale expressed as the percentage of the maximum mode of all the groups within each plot. To visualise the plastic and Milli-Q distribution, the glass distribution scale was reduced 10-fold. Plasticware introduced significantly less plastic and non-plastic particles compared to glassware.

3. Results and discussion

3.1. Water source as a source of microplastic contamination

All water sources had some level of plastic and non-plastic particle contamination (Fig. 1a & b). Milli-Q was identified as the least contaminated water source with an estimated plastic particle count of 21.7 (95%CI: 4.7 - 64.4) particles/ml, followed by reserve osmosis with 29.9 (95%CI: 9.6 - 77.3) particles/ml, and tap water with 151.7 (95%CI: 62.2 - 387.0) particles/ml. Tap water had significantly higher plastic and non-plastic particles from both Milli-Q and reverse osmosis (p < 0.05).

While Milli-Q is the least contaminated water source, overall, water contamination is important to consider when designing procedural and experimental blanks. For example, Leslie et al. [23] quantified microplastics present within human blood samples in the detection range of > 700 nm. While Milli-Q procedural blanks were processed alongside plasma samples to quantify the level of contamination introduced during sample processing, there have been some methodological concerns [19]. The number of plastic particles detected in the plasma samples were adjusted based on the measurements found in the control group. This resulted in 23% of samples having lower levels of plastics than the Milli-Q blank control and were classified as having no microplastics present. However, our results indicate that Milli-Q has plastic contamination and while it is challenging to design an appropriate procedural blank, one needs to be cautious when drawing conclusions based on this. The plasma samples were not processed with Milli-Q and therefore it is difficult to ensure that the microplastics quantified in the Milli-Q procedural blanks were introduced during sample handling or processing. A more appropriate conclusion would be that 23% of plasma samples contained less microplastics when compared to Milli-Q. In contrast, Ragusa et al. [37] analysed microplastics in placenta tissue and used a digestion technique that was Milli-Q based. In this case, using Milli-Q as a control or procedural blank is more appropriate.

It is important to be aware of the size distribution of particles within the control as we found that particles within all water sources were on the order of 10 μ m, which is detectable in most common methods of microplastic detection including μ -FTIR, flow cytometry, and pyrolysis – gas chromatography/mass spectrometry (pyr-GCMS). Filtration steps are not necessary if procedural blanks and size detection strategies are well planned and utilised. The additional procedural steps associated with filtration likely introduce greater risk of contamination.

3.2. Comparison of laboratory consumables

Until now, it has been widely proposed that plastic instruments are a source of microplastic contamination through sampling and processing, thus glass is often recommended as an alternative [15,33]. However, our results indicate that contamination levels significantly reduce when

Journal of Hazardous Materials 465 (2024) 133276



Fig. 3. Significant airborne nanoplastic and microplastic contamination occurs within 20 min of pipetting, and this is not mitigated by the use of biosafety cabinet (BSC) or fume hood. Milli-Q was pipetted between plastic tubes for 20 min within each setting as to best replicate standard experimental procedures. Milli-Q collected into a flow cytometer tube was used as a control. (a) Plastic particle count per μ L and (b) all particle count per μ L was determined for each experiment location. Data was analysed with a one-way ANOVA followed Tukey pair-wise comparison. *** p < 0.0001, ** p < 0.001, * p < 0.01 compared to control. Laboratory dust was examined for (c) plastic particles count per μ L and (d) all particle count per μ L. Dust was collected over 30 days and found to be a significant source of airborne plastic. Data was analysed with a one-way ANOVA followed Tukey pair-wise comparison. (e, f) Density plots of log₁₀ Side scatter (LOG SSC) of the plastic particles compared to polystyrene beads of known sizes (200 nm, 500 nm, 800 nm and 3.2 µm, and A549 cultured human cells (~10 µm). Each density plot is set to the same scale expressed as the percentage of the maximum mode of all the groups within each plot.

using plasticware compared to glassware (p < 0.0001; Fig. 2a). Experiments conducted in glassware had the highest level of contamination with an estimated plastic particle count of 1356.9 (95%CI: 975.3 – 1861.1) particles/ml. Whereas experiments conducted in plasticware had an estimated plastic particle count of 6.9 (95%CI: -0.7 - 19.2) particles/ml which is similar to the estimated plastic count of the Milli-Q control that was collected and analysed immediately. This had an estimated plastic particle count of 6.7 (95%CI: -0.8 - 19 particles/ml.

Plastic laboratory consumables are manufactured, transported, and stored at a medical and analytical standard. For example, tubes and tips are all made in accordance with ISO 13485 and ISO 9001 manufacturing standards, ensuring medical level of cleanliness and sterility of the product. All plastics used in our experiments were sterile, RNAase free, endotoxic free and sealed in air-tight plastic sachets. In contrast, glass instruments were provided in cardboard boxes, sometimes with a single film of plastic covering and not individually wrapped. These differences in consumable production, distribution and use may account for the difference in contamination observed.

The few particles detected when using plasticware were smaller than the 3.2 μ m standard, thus not likely to be detected during μ -FTIR. This highlights the importance of considering the relationship between potential contamination sources and the minimum level of detection of the microplastic detection technique utilised.

The consideration of the use of plastic or glassware may be different when analysing leachates or additives in the context of plastic research as plastic laboratory consumables have been shown to contribute to the chemical contamination of samples. For example, Schauer et al. [42] found that microcentrifuge tubes were a source of Tinuvin 770, a UV stabiliser commonly used in the production of plastics and this confounded the mass spectroscopy results. These considerations may be field specific depending on the analytes being investigated. Controls and procedural blanks can be designed to account for the level of introduced contamination when using glassware. It should be noted that we utilised washed glass, however, a minority of researchers use burnt glassware [33] which has been heated in an oven typically over 400 °C for several hours. We cannot comment on the efficiency of such methods, and this should be researched in future studies.

3.3. Experimental location and additional sources of contamination

Various methods are used in plastic particle quantification to control airborne dust as a potential source of contamination. These include: reducing air disturbances by not using air conditioning or fans [15]; using fume hoods both powered on [25,38] and powered off [8,40], and using biosafety/laminar flow cabinets [11,20]. There is an incomplete understanding of how these methods potentially effect air quality, for example in Reichert et al. [38] stated "All further processing was performed under a fume hood to minimise potential contamination", however, fume hoods do not improve air quality above the sample as they function by pulling unfiltered air from the external laboratory environment through the hood, in a continuous flow, thereby potentially contaminating samples. In contrast, BSCs draw air through a High Efficiency Particle Arrestor (HEPA) glass fibre filter of which entraps 99.99% of particles larger or smaller than 0.3 μ m [3]. Filtered air is then blown over the sample and expelled through a grate at the entrance where the operator is situated [13,33]. Laminar flow cabinets have similar technology to a BSC with the exception of the grate, where air is expelled from the front of the cabinet towards the operator [50]. Thus, BSCs and laminar flow cabinets are often preferred over fume hoods and recommended to limit airborne contamination [12,50].

We examined airborne contamination in the fume food, biosafety cabinets (SterilGARD® Class II Type A2 Biosafety Cabinet) and bench top (open air) settings, by simulating an experiment through pipetting 0.5 ml of Milli-Q between two tubes for twenty minutes. From this experiment we found no significant differences between settings. The experiment performed in the bench setting had the highest level of contamination with an estimated plastic particle count of 55.6 (95%CI: 26 - 128.6) particles/ml, followed by the fume hood settings with 31.7 (95%CI: 14.7 - 68.5) particles/ml and the biosafety cabinets setting with 32.0 (95%CI: 14.9 - 69.3) particles/ml. All of these experimental settings had significantly higher counts from the Milli-Q control that was collected and analysed immediately (p < 0.001). This had an estimated plastic particle count of 3.3 (95%CI: 2.8 – 9.3) particles/ml. No substantial differences in distributions of the particle size could be observed (Fig. 3e&f). The relationship of experimental setting to all contaminant particles was the same with no significant effect between experimental setting, but considerable contamination compared to the Milli-Q control.

From these results two key conclusions can be made: 1) Fume hoods and biosafety cabinets do not provide significant protection from microplastic and other particulate contamination. 2) Performing simple laboratory tasks, such as pipetting between tubes, will introduce significant levels of microplastic and other particle contamination. A recent study by Wesch et al. [50] examined microfiber contamination in different working environments, including basic laboratory bench, fume hood, and biosafety cabinet. Similar to our findings, Wesch et al. [50] found fume hoods and laboratory bench work do not differ in the levels of microplastic induced contamination. Interestingly, unlike our results (Fig. 3a), Wesch et al. [50] found the microbiological safety workbench outperformed other approaches. There are several potential reasons for this disparity. Wesch et al. [50] investigated passive collection of plastics utilising wet paper discs (90 mm, 45 min to 4.75 h), while our study investigated plastic contamination during the relevant laboratory method of pipetting between tubes (20 min). Furthermore, the size of the plastic particles investigated covered different ranges as Wesch et al. [50] included microfibres that were a "few µm up to cm in length" whereas our study differentiated between nanoplastic and microplastic categories (70 µm - 200 nm). Additional differences in reporting metrics and analytic approaches makes direct comparison of any study results difficult.

The glass filtration system and air quality control of the biosafety cabinet has been designed to remove particles larger than 300 nm specifically to reduce the risk of microbe contamination. This raises the question - if biosafety cabinets effectively eliminate microbes why were they ineffective at reducing microplastics of similar sizes in our study? One potential explanation is that the air is not a substantial source of microplastic contamination in a brief (20 min) pipetting experiment using 1.5 ml centrifuge tubes. The surface area of liquid in the tube and the tube opening is relatively small and this may limit the interaction between particles in the air and the surface of the liquid. To evaluate the significance of air as a source of plastic particles, we conducted an experiment involving 50 repetitions of pipetting 0.5 ml of Milli-Q water between two Eppendorf tubes across a varied timeframe (from 5 min to 24 h; Fig. S2). The centrifuge tubes were left open to the air during the whole experiment. We found no relationship between time exposed to the air and plastic particle or all particle contamination. This result is also corroborated by Wesch et al. [50] who performed a similar experiment ranging from 45 min to 4.5 h. Collectively, this suggests that airborne particles are not a substantial source of particle contamination during experiments and explains why areas with more air particles, such as the laboratory open bench and flow hood, did not have significantly more particulate contamination. Therefore, the particulate contamination observed during these experiments must come predominately from other sources.

The levels of particulates in the Milli-Q control demonstrate that the laboratory water explains approximately a third to one half of the particulates observed. The lack of difference observed between the control and laboratory location, and the significant increase in particle contamination in the biosafety cabinets (which is specifically designed to remove airborne particulates such as bacterial and fungal contamination), suggests that the air is not a substantial source of plastic particle contamination during these types of experiments. Therefore, we argue



(caption on next column)

Fig. 4. Laboratory consumables may retain particles following a microplastic experiment. Plastic tubes were dosed with known concentrations of plastic and tripled Milli-Q rinsed with followed by triple rinse in a detergent bath. (a) Plastic particle count per μ L and (b) all particle count per μ L was determined at each experimental stage. Data was analysed with a one-way ANOVA followed by Holm- Šídák post-hoc pairwise comparison. n = 5. Experimental replicates were considered independent as tubes were dosed with different plastic types. * **p < 0.0001, **p < 0.01, **p < 0.01 compared to baseline. Displayed as half violin plots and box plots with median and interquartile ranges on an exponential y axis. (c) Density plots of log₁₀ Side scatter (LOG SSC) of the plastic particles compared to polystyrene beads of known sizes (200 nm, 500 nm, 800 nm and 3.2 μ m, and A549 cultured human cells (~10 μ m). Each density plot is set to the same scale expressed as the percentage of the maximum mode of all the groups within each plot, with the exception of doped as the axis is scaled 10x.

that the pipette tip and centrifuge tube are the primary source of contamination during the pipetting process, explaining the remaining half to two thirds of the plastic contamination observed.

Another factor influencing the results of the airflow experiments is the presence of dust in the vicinity of sample processing locations. Indoor dust has been identified as a significant source of microplastics, exemplified by Soltani et al. [44] identified an average of 3095 plastic fibres/m²/day in Australian homes. The smallest fibres detected in this study were 50 – 200 μ m [44], whereas other studies have detected micro-fibres in dust as small as 2 – 3 μ m [56]. In our study, we have identified an estimated plastic particle count of 1175 (96%CI: 884.5 – 1522.8) particles/ml present within laboratory derived dust, with the observed particle size range of ~200 nm to > 10 μ m. μ -FTIR identified polypropylene (Fig. S3) to be present within laboratory dust samples, which is a plastic polymer often utilised in producing plastic consumables (e.g., FALCON tubes).

We performed the dust experiment utilising aluminium foil as a tray for dust collection. Aluminium foil is commonly recommended as a covering on glassware consumables to prevent air contamination and is considered standard QA/QC protocol when working on microplastic or nanoplastic experiments (e.g., [31,54]). Prata et al. [33] provided a series of contamination control measures including "all materials, solutions and samples shall be covered with glass lids or aluminium foil, only opened when necessary, in order to prevent airborne contamination". However, the foil control in this experiment highlights the contamination level on aluminium foil, estimating the presence of plastic particles at 168.9 (95%CI: 93.3 - 274.7) particles/ml. The foil control best represents the use of foil when coming into contact with reagents, as fresh aluminium foil was taken and had Milli-Q flushed on the unexposed side and was processed immediately. In this very short exposure to foil, we are still observing high counts of plastic particle contamination bringing light to the potential contamination introduced from the use of foil as a contamination control measure. With the exception of glassware, the use of foil and laboratory dust had the highest estimated plastic particle count of contamination identified in this study.

3.4. Reusing microplastic doped consumables

Microplastic and nanoplastic studies are proliferating in response to the plastics crisis [46], however when designing an experiment, it is important to consider our contribution to these issues. As laboratories rely heavily on single-use plastic, we evaluated if consumables could be cleaned and re-used following a microplastic experiment.

Our results suggest that plastic consumables can be re-used following a microplastic experiment and return close to the starting baseline level of contamination (Fig. 4a). However, water washing alone retained a significant quantity of the doped microplastics, indicating that three-time water washing step is not sufficient as the centrifuge tubes had an estimated particle count of 225.4 (95%CI: 102.5 - 415.4) particles/

Table 1

Summary of the risk of introducing detectable contamination of plastic particles when performing an experiment to quantify nanoplastic or microplastic contamination. The two particle size ranges are reflecting the two methods employed by this study including Flow cytometry (200 nm - 700 μ m) and micro-Fourier-transform infrared spectroscopy (μ -FTIR; 5 μ m +).

| | 200 nm – 700 µm | 5 µm + |
|---------------------------|-----------------|----------|
| Water | | |
| Milli-Q | Low | Low |
| Reverse osmosis | Moderate | Low |
| Tap water | High | Moderate |
| Consumables | | |
| Plastic labware | Low | Low |
| Glass labware | High | Low |
| Laboratory dust | High | Moderate |
| Airflow | | |
| Bench (open air) | Moderate | Low |
| Biological safety cabinet | Low | High |
| Fume hood | High | Low |
| Experiment duration | Low | Low |

ml. The additional detergent and water washing step proved sufficient at removing the doped microplastics reduced the estimated particle count to 122.3 (95%CI: 42.6 – 257.4) particles/ml. Adding detergent to water reduces the surface tension likely reducing the adhesion between the microplastics and the consumable.

There was an important but not significant difference observed between the baseline and detergent washed consumables. Therefore, we can with low confidence say that washing out of consumables is an effective strategy. There are multiple variables that introduce potential contamination back to the consumable including the detergent and selected water source. We recommend research groups to investigate if this is a feasible strategy to implement prior to employing these methods.

4. Conclusion

At present, avoiding contamination in laboratory settings is challenging. Careful experimental planning is essential as the detection techniques utilised and experimental protocols will greatly influence the amount of contamination detected in samples. Procedural blanks remain a fundamental tool to quantifying the amount of contamination introduced during the experiment [4]. However, the design must be well-structured to capture the level of contamination introduced through experimental design and consideration should be taken when statistically comparing to experimental samples. In Table 1, we have summarised the risk of common laboratory procedures for introduced contamination depending on the method of detection employed.

We have provided quantified and validated sources of microplastic and nanoplastic sources of contamination within a laboratory. From this, we have a series of suggestions: (1) Any experimental work adds contamination, it is critical to plan and streamline experiments to keep exposure time to a minimum. (2) Perform experiments and store samples in and using plasticware consumables. (3) Avoid the use of aluminium foil to cover samples. (4) Use Milli-Q when water is required. (5) Experiments should be performed in a biological safety cabinet (BSC) or similar laminar flow cabinet (LAF bench). (6) Frequent cleaning to reduce the build-up and distribution of laboratory dust using 70% ethanol and paper towel.

An additional key finding is that the level of concern over potential contamination depends on the methods of detection and the corresponding size ranges of microplastic being investigated. For example, we detected almost no contamination in our samples with μ -FTIR despite

performing experimental paradigms designed to induce typical contamination such as pipetting continuously for 20 min on an open bench using plastic labware. This same procedure introduced a significant amount of contamination when measured using flow cytometry. This is due to the different minimum size of detection of these methods (μ -FTIR 2.7 μ m, Flowcytometry 200 nm). Consequently, when designing contamination reduction protocols considerations should be made based on the methods of detection utilised.

Finally, this study underscores the importance of maintaining strict QA/QC protocols and transparently documenting these measures. This practice is vital for enabling future research to compare and standardise methodologies and results in the field of microplastic and nanoplastic research.

Environmental Impact Statement

Microplastic and nanoplastic particle research has increased exponentially with increasing awareness of the plastic pollution crisis. These particles are widespread and have been identified to be present and accumulating not only within the environment, but also within humans and wildlife. This research provides quantified and validated sources of microplastic and nanoplastic contamination in laboratory settings. We have provided guidance on effective quality assurance and quality control measures of which ensures the robustness of microplastic research thus facilitating accurate quantifications when comparing dose size to effect interactions. Ultimately, this enhances our understanding of microplastics as a hazardous material.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.133276.

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