Microplastic Methods Workshop Report

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Introduction

Microplastics (plastic particles < 5 mm in size) are an emerging contaminant of growing concern given their abundance and widespread distribution globally across marine, freshwater and terrestrial systems. The majority of microplastic particles within the environment derive from the degradation of plastic products during use, including car tyre abrasion, fragments of road markings, city dust, effluent from manufacturing industries, and microfibres from synthetic clothing. These are usually manufactured and used on land, entering surface waters via runoff or drainage systems.

Microplastics comprise a wide variety of polymer types of differing chemical complexity (reflecting their many different uses), and capable of acting as carriers of other pollutants which could then enter food chains. Due to their ubiquity and persistence, concern is rising over human and organism exposure to microplastics via ingestion, inhalation and physical contact, and the health impacts this may cause. However, there is currently a shortage of evidence on the effects of microplastics under realistic environmental exposure conditions. While it is essential that we develop our understanding of the health and wider environmental effects of microplastics, exposure can only be understood by studying the presence, abundance and behaviour of microplastic particles across various environmental media. This is the focus of many recent and current studies in this field.

One of the key issues highlighted by the Marine Strategy Framework Directive Technical Subgroup on Marine Litter, in the document "Guidance on Monitoring of Marine Litter in European Seas", is the lack of standardised methodologies and appropriate reference materials to determine the levels and state of anthropogenic plastic litter in aquatic environments (Marine Strategy Framework Directive, 2013). The problem of plastic pollution is one that needs a multidisciplinary approach to solve it.

Given the novelty of microplastic research, and the variety of available approaches, there is no one recognised method for analysing microplastic samples, with different studies using a variety of different techniques and reporting units. This extends even as far as the definition of microplastics, with most studies defining these as plastic particles < 5 mm, while others use < 1 mm as a working definition (Horton et al., 2017; Claessens et al., 2013). To date, the quantification of microplastic particles in the environment has revealed mixed results ranging from stable to increasing concentrations (Cole et al., 2011). Despite a large number of studies in this area over the last few years, it needs to be noted that robust and consistent methodology is only now starting to emerge. It is therefore recognised that there is a need for standardisation, or at least harmonisation, of methods used for microplastic analysis across studies, to allow for accurate comparison of data (Rochman et al., 2017). This is especially important given not only the growing number of studies in this area, but the

growing importance of reliable and reproducible data for industries and governments who may ultimately use these data to inform policies, regulations and business strategies.

The focus of this workshop was specifically on the methods for microplastic sampling, extraction and identification from environmental samples, to identify the range of current techniques being applied across microplastics research, the advantages of these methods, how these may be optimised in the future and any challenges or disadvantages faced when applying these to real-world samples. The aim was to initiate discussions on the state of the currently available methods for microplastic sampling and analysis and their suitability for ongoing microplastic research, and to ascertain whether there are any methods that should be specifically pursued or discounted when progressing the research in this field. It was also intended to determine whether it is currently possible, or advisable, to work towards standardisation of methods for microplastic analysis in different environmental matrices, i.e. water, air, sediment, soil, wastewater sludge, biota. Alternatively, whether we are still at a stage where further research, development and improved analytical techniques are needed before recommendations for standardisation can be made.

The workshop brought together a number of experts who are actively working and developing techniques within this field to present and discuss their work and exchange ideas and expertise. This also provided a networking opportunity where new connections could be made between research groups, and also between researchers, regulators and industry, helping to progress shared understanding and potentially leading to future collaborations.

The workshop was split into two sections: presentations and discussions. The morning and early afternoon sessions consisted of presentations from a range of researchers giving different perspectives on working with different types of environmental samples, in addition to the research and industry need for better-defined methods. These talks were intended to provide a basis for the afternoon discussions. For the discussions, delegates were divided into three targeted and individually-moderated discussion groups to broadly cover different matrices: 1) Air and water, 2) Biota and sludge (the challenges of high organic content samples), 3) Soil and sediment (the challenges of high inorganic content samples).

Overview of presentations

(Copies of the presentations are available on the RSC Water Science Forum and UK Microplastics Network websites.)

Sampling, sample preparation and detection of microplastics, current activities in the ISO/TC 61/SC14/WG 4

Dr Ulrike Braun (presenter), Bundesanstalt für Materialforschung und prüfung (BAM), Berlin & Dr Claus G. Bannick, Umweltbundesamt, Berlin UBA, Germany.

The presentation by Dr Ulrike Braun was divided into three parts and covered studies undertaken at BAM into methods based on thermal degradation and chemical analysis. The first part addressed the collaborative work of Dr Braun and Dr Claus Bannick on microplastic analysis since 2014; Dr Bannick specialises in sampling of environmental samples, whereas Dr Braun is working on a new method for mass fraction determination of microplastics. An initial overview was given of the results so far obtained.

The second part dealt with current activities associated with ISO/TC 61 "Plastics". A "Microplastics" working group has been set up within the newly created SC 14 "Environmental Aspects" subcommittee and is chaired by Dr Bannick. A technical report entitled "Plastics in the environment – current state of knowledge and methodologies" is currently being compiled by the WG, based on a survey of international and related technical groups in ISO and CEN. This report includes chapters about terms and definitions, applications of plastic materials, occurrence of plastics in environment (water, sediments, sludge, soil, air, biota), testing methods with focus microplastics (sampling, sampling preparation, analysis) as well as entry pathways, environmental assessment. A finalized version is expected in the end of the 2018. The third part of the talk summarised wider microplastic research activities being conducted in Germany. Harmonisation of individual projects has been initiated by a large funding call from the Federal Ministry of Education and Research. This addresses the terms and definitions as well as analysis of microplastics. The former topic is currently accompanied by a living document with recommendation for sampling, sample preparation and detection of microplastics. This report also embraces more general aspects of analysis, practical rules in field and lab work, definition of microplastic size classes, a brief summary of capabilities and limitations of various detection methods and the information obtained from each, as well as recommendations about representativeness of environmental samples volumes or masses. A first official document is expected in late summer 2018.

Maximising Information from microfibres: Methods for characterisation and contamination prevention from the forensic science industry.

Dr Claire Gwinnett, Staffordshire University.

Dr Gwinnett's presentation brought a new perspective to microplastic analysis, focussing upon the benefits of employing other scientific disciplines in improving and standardising microplastic analysis, specifically the processes employed in forensic fibres examination which have been a fundamental part of criminal investigations for over 50 years. Fibres retrieved from crime scenes are typically less than 5 mm in size and are a mix of both synthetic and natural fibres which have to be effectively screened and characterised to gain intelligence information. The forensic fibre approach in analysing microfibres allows more reliable screening for microplastics and characterisation beyond polymer type (Woodall et al., 2015). Features such as cross-sectional shape, width and the presence and absence of inclusions can allow fibres that fall within the same polymer type to be sub-categorised in order to fully understand the extent of possible sources of these fibres. Utilising a series of sequential analytical approaches to microplastic analysis allows greater differentiation between microplastic types and a more informed approach to identifying the source of the microplastic. This 'source level' information in forensic fibres examination is well established and has been scrutinised by the courts of law. This session outlined the analytical approach used commonly in forensic fibres work and evaluated for use in microplastic analysis.

This presentation also introduced other areas of forensic fibres research that can be informative to microplastic analysis and interpretation, these included; sheddability tests of fabrics, contamination prevention procedures during sampling and analysis, fibre persistence in water environments, statistical approaches to evaluate fibre samples and polymer degradation studies.

Finally, the presentation outlined work being conducted at Staffordshire University in combining expertise in machine learning, computer vision, plastics analysis and evidence evaluation in producing an automated analysis system. Spectral 360, a patented machine learning system developed at Staffordshire University (http://www.spectral360.com/) is currently being adapted for use with automated analysis, quantification and identification of microplastics, eliminating missed particles and false positives. This will allow a standardised approach for microplastic analysis and the generation of large amounts of data in a fast and more cost-effective manner.

The thought processes to design a pipeline for accurate extraction, processing and ultimately reporting of microplastics

Dr Lucy Woodall (presenter) and M Rivers, University of Oxford.

How we collect and report data depends on the scientific questions we aim answer, however there are some aspects that should be considered to make data more comparable. Sampling and processing methodologies are continuously evolving and have been reviewed by a number of previous publications, as have the techniques of identifying the polymer. Other aspects are less frequently considered, however it is important that researchers also consider accurately measuring sediment or water volume sampled and the heterogeneity of surface microplastics. Furthermore all should include greater detail in reporting of the methods used, as these can differ greatly. To allow for comparability between studies, a standard method of reporting microplastic concentrations would be useful, and data beyond simply counting particles is essential when dealing with heterogeneous fragments.

The complexities of isolating microplastics from environmental samples.

Dr Matt Cole, Plymouth Marine Laboratory.

Microplastics have been identified in water, sediment and biotic samples from across the globe. However, accurately determining microplastic concentrations within these different environmental compartments is confounded by the presence of organic (e.g. plankton, macroalgae, detritus) and inorganic materials (e.g. sediment, silt) that obscure the plastic. A key challenge for the research community has been to develop a toolbox of methods effective in isolating microplastics from environmental samples, without damaging the plastics themselves. Here we have highlighted how traditional digestion techniques using concentrated acids and bases are destructive, causing loss of plastic. We alternatively suggest enzymatic digestion as a safe, robust technique for removing organic material, with the use of potassium hydroxide as a valid alternative. An investigation into the efficacy and cost effectiveness of using different salt solutions in the density separation of microplastics from sediments has also been undertaken. While there is no "catch all" method, we have identified the construction of a sediment-microplastic isolation unit, in combination with 1.5 g/cm^3 zinc chloride to be the most effective for the majority of our samples. We conclude by highlighting the importance of considering sample contamination.

Detecting microplastics in ambient particulate matter using Raman spectroscopy

Dr Stephanie Wright, Kings College London.

Microplastics are a global environmental issue contaminating aquatic and terrestrial environments. Recently, they have been reported in atmospheric deposition, and indoor and

outdoor air, indicating they are airborne. This raises concern for public health due to the potential for exposure via inhalation. However, very little is known about airborne microplastics, including spatial and temporal concentrations; chemical composition; and, importantly, whether they occur in the inhalable size range. This is partly due to the complexity of airborne particulate matter (PM), which consists of a diverse range of particles, presenting an analytical challenge. Here, we explore the challenges of sample substrate composition for inhalable microplastic detection using Raman Spectral Imaging (RSI).

Water industry action on microplastics

Dr Matt Hill, Yorkshire Water.

The UK water industry is responsible for taking the country's wastewater and recycling it to the environment in a way that protects public health and the ecological health of the receiving water and land. The water industry are regulated to ensure we meet this commitment, but no regulations specific to microplastics currently exist. Despite this, the water industry has acted upon the recommendation from the select committee of 2016 on the environmental impact of microplastics that, 'the Government and Environment Agency work with Water Companies to understand what feasible options there are to monitor and ultimately reduce microplastic pollution.' Water UK, the trade association for the major water and wastewater service providers, has a microplastics mission statement, 'To quantify the water industry contribution of microplastics to the environment in the context of ecotoxicology studies being carried out elsewhere, and so assist in the development of a strategic response.'

In order to deliver this mission statement the water industry is co-funding an international analytical standardisation project run by the Global Water Research Coalition (GWRC). The water industry is also funding relevant PhDs and a UK Water Industry Research (UKWIR) project quantifying the presence of microplastics in raw water, potable water, raw sewage, sewage final effluent and sludges from clean and wastewater treatment, and the effect of current treatment processes, which will complete by 31st March 2019. This will inform a larger project that will run from April 2020 to autumn 2021 at a wider number of sampling locations to further improve our understanding of our impact. The development of an accepted standard for microplastics quantification in different media would significantly boost the ability of the water industry to deliver our mission to quantify the water industry contribution of microplastics to the environment, and so assist in the development of a strategic response.

Lost without Nile red: finding small microplastics in environmental samples

Gabriel Erni-Cassola, University of Warwick.

Marine plastic debris surveys have shown that plastic particles <5 mm in size, known as microplastics, are significantly more abundant in surface seawater and on shorelines than larger plastic particles. Nevertheless, quantification of microplastics in the environment is

hampered by a lack of adequate high throughput methods to distinguish and quantify smaller size fractions (<1 mm), and this has probably resulted in an underestimation of actual microplastic concentrations. The method presented here allows high throughput detection and automated quantification of small microplastic particles (20–1000 µm) using the dye Nile red, fluorescence microscopy and image analysis software. The protocol is highly effective in the quantification of small polyethylene, polypropylene, polystyrene and nylon 6 particles, which due to their strong hydrophobicity, stain well and strongly fluoresce in the green spectrum. To avoid false positives however, it is crucial to thoroughly digest environmental samples using hydrogen peroxide or enzymatic digestion protocols. In addition, the use of Raman to confirm the identity of a subset of identified particles is strongly recommended. Our preliminary results from sea surface tows showed a power-law increase of small microplastics (*i.e.* <1 mm) with decreasing particle size. We consider that this method presents a step change in the ability to detect small microplastics by substituting the subjectivity of human visual sorting with a sensitive and semi-automated procedure (Erni-Cassola et al., 2017).

Advantages and limitations of spectroscopic and microscopic approaches for the characterisation of microplastics.

Dr Jesús Ojeda, Swansea University.

The majority of the studies for isolating microplastics from marine water and sediments cover a wide range of protocols including density separation, elutriation, or the use of acids, alkalis, hydrogen peroxide and enzymatic digestion (Cole et al., 2014). Once the microplastics have been properly isolated, they can be analysed for quantification and/or identification using a range of spectroscopic or microscopic techniques, such as visual sorting, fluorescent dyes, Scanning Electron Microscopy (SEM), Pyrolysis-GC-MS, Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy (Shim et al., 2017; Silva et al., 2018; Fries et al., 2013).

In order to quantify and characterise microplastics in aquatic and sediment samples, the majority of published methods have relied on visual sorting to select putative microplastic particles for further analysis, once they are separated from the environmental matrix. However, this approach is time-consuming and also prone to bias, as visual differentiation can be challenging when microplastics, similar size organic and inorganic matter are present in the same sample. When the number of particles is needed (but not the identity of the polymer), the use of fluorescent dyes (such as Nile Red) (Erni-Cassola et al., 2017; Maes et al., 2017) and optical microscopy could be more suitable. However, this method is limited to small microplastic sizes (1 mm to 20 µm).

A combination of multiple methods is recommended when both quantification and identification are required. If identification of the polymer is required, the use of spectroscopic tools such as Pyrolysis-GC-MS, FTIR or Raman is recommended, although

availability of materials and instruments may be an issue. It is important to consider however, that the presence of other chemical additives, exposure of the microplastics to elements over a long period of time, or colonisation of the surfaces by microorganisms, could complicate spectroscopic analyses. Therefore, additional pre-treatment protocols may be needed, but the effect of these protocols on the microplastics should also be evaluated. Validation studies and blanks should also be included for the reliability assessment of the method, alongside inter-laboratory studies and proficiency tests.

Optimising the Workflow for Microplastic Analysis by FTIR Microscopy.
Ian Robertson, Perkin Elmer Ltd.

Analysis of environmental samples containing microplastics is essential to determine their prevalence and their impact. A range of analytical techniques have been applied to the analysis of microplastics. Of the techniques adopted, infrared (IR) spectroscopy, and more specifically IR microscopy, has established itself as a primary analytical technique for the detection and identification of microplastics. The microplastics analysis workflow for IR microscopy consists of several steps involved in getting from the raw sample to answers, including the initial sampling through to data analysis. The steps involved may be different depending on the type of initial sample and the amount of sample cleanup/purification required to prepare the sample for infrared (IR) analysis. This presentation described the different types of environmental samples, the sample collection methods, the range of different sample purification methods, and then more specifically the best ways to optimise sample filtration for measurement by IR microscopy. The principles of IR microscopy and the different sample measurement modes were described, comparing and contrasting each type. IR microscopy and imaging experiments can generate significant quantities of data that need to be analysed to get the required information. The different methods for extracting data and information were explained and suggestions made for best practice.

Quantitative trace analysis of microplastics in environmental samples using thermal techniques with a special focus on pyrolysis GC-MS.
Barbara M. Scholz-Böttcher (presenter) and Marten Fischer, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University of Oldenburg, Germany

Identification and quantification of microplastics in the water column, sediment and biota is time-consuming and lacks standardization. Exclusively microscopic recognition and counting forfeit reliability below 100 µm particle size. Combined microscopic and spectroscopic FTIR and Raman techniques are the most established approaches in microplastic analysis. Pyrolysis gas chromatography mass spectrometry (Py-GCMS) is frequently used for identification but rarely for quantification of single plastics in natural samples. Comparably fast, quantitative

chemical and weight-related data complementary to number- and size-related records are generated.

The study presented applies Py-GCMS combined with thermochemolysis for simultaneous analysis of nine majority plastics (PE, PP, PET, PS, PVC, PC, PA-6, PMMA, MDI-PUR). Selected fragment ions of specific pyrolysis products enable a sensitive polymer-specific identification and quantification on the µg trace level and even below. Prior to Py-GCMS environmental samples need a multistep enzymatic, oxidative treatment and occasionally density separation in order to reduce accompanying organic as well as inorganic matrix components and achieve a sufficient microplastic enrichment.

The potential of this method has been demonstrated for different environmental sample types concerning applicability, calibration range, recovery, LOD/LOQ, reliability and possible interferences with common occasionally remaining natural organic polymers. In this context Curie-point and an improved oven pyrolysis was compared regarding their sensitivity and linearity for trace quantification purposes. These aspects are of general relevance for the analysis of organic polymers in environmental samples. The microplastic concentrations so far detected in selected samples from the North Sea range from ppb (sediments and water column) to ppm (mussels and fish) level.

Achieving new standards

Professor Richard Thompson, University of Plymouth

Plastic debris is a very heterogeneous mixture of polymer types, sizes, shapes colours, densities and origins. This presents immense challenges when seeking to adopt standardised methods. Given there are also practical limitations in sampling, it may therefore be beneficial to link monitoring either to categories of litter where there is clear evidence of harm, or to assessing the efficacy of specific interventions. This could include monitoring the abundance of plastic items that have been to focus of specific policies reductions for example the quantity of plastic bags found in the environment as a consequence of the single-use bag tax or reductions in the abundance of plastic microbeads in sewage as a consequence of legislative measure to reduce the quantity of microbeads used in cosmetics. Whereas widespread quantification of all microplastics, while important to our understanding of encounter rate and possible harm is likely to provide a relatively blunt tool for monitoring change. Whatever approach is used it is essential to be explicit about the limitations of the given sampling strategy and the associated limitations of any extrapolations made in subsequent modelling studies.

Overview of discussion sessions

Discussion Group Questions

The following questions were put to each of the 3 groups at the start of the session:

1. What are the most commonly-used methods for these particular media – do you consider current methods to be effective?
2. What are the main drawbacks of these current methodologies?
3. What advances are being made to eliminate/reduce these issues?
4. Where do you see the developments for these media heading in 1, 5, 10 years time?
5. Is it possible to standardise methodologies based on current protocols? – if not, how long do you think it might be until this is possible? What is needed?

Due to the scale of the issue and time limitations during the workshop it was not envisaged that each question would be addressed in detail, but these questions aimed to prompt considered discussions.

Soil and sediment

Soils and sediments were grouped due to their complex and heterogenous nature, with a high proportion of dense inorganic matter. This group highlighted that before deciding on the methods to be used within a study, it is imperative to ascertain what the question is that is being asked, as this will inform the level of detail needed in the resulting data, and therefore the scale and specificity of the sampling and processing required.

It was suggested that a 'recommendation toolbox' could be developed as a guidance for future studies. An example is density separation - it could be recommended that if carrying out a density separation step, the solution used should be at least 1.3 g cm^{-3} to capture the denser polymers such as PET (1.3 g cm^{-3}) and PVC (1.3-1.58 g cm^{-3}). This is something that warrants future discussion when considering the future of microplastic research.

It was suggested that sediment samples may not be entirely representative of contamination within a specific area as this is highly temporally variable and the results will depend on when the sample is taken. For example a recent study showed that the sediment of a heavily contaminated river contained a significantly lower concentration microplastics following a flooding event (Hurley et al 2018). However, studies such as this can give an indication of the extent to which river sediment can act as a sink for particles (e.g. those remaining following the flood). Additionally, the particles found can give an ideas as to sources, for example whether they are derived from road runoff or wastewater inputs (e.g. Horton et al 2017).

Soil is generally likely to be less dynamic than sediment and therefore microplastic concentration will be more stable and less dependent on time and weather events. However, land-based activities still need to be taken into account, for example sludge spreading or

seasonal agricultural plastic usage. For soil studies it would be worth having a quality control standard soil, which each lab would analyse alongside their samples to allow for comparison of extraction and analytical efficiency between studies and methods. An example of such a soil is Lufa 2.2, which is recognised as a standard soil for ecotoxicity and environmental testing (Lokke and van Gestel, 1998).

Water and air

Water and air were grouped due to their relatively 'clean' nature, which generally allows large volumes of sample to be collected and processed. However, it is difficult to quantify and identify microplastics from air and water samples with a single analytical method. The correct approach is driven by the question being asked. A combination of multiple methods is recommended when both quantification and identification are required. If identification of the polymer is required, the use of spectroscopic tools such as FTIR or Raman is necessary.

This group highlighted that an extremely important aspect of microplastics reporting is consistency of units. It was agreed that researchers should always report number or mass of particles per volume, for example L or m^3, rather than by area. For sediment or soil studies it could be argued that concentration of particles by sample weight (e.g. kg) is a more suitable measure than volume.

In addition to methods, this group touched on policy needs; given that water and air quality directly impact on human health it is especially important that these data are reliable and accessible for regulators. Before policies and regulations are put into place it will be necessary to provide evidence of harm to fauna, ecosystems and/or humans, justifying the need for regulation. This extends to understanding which characteristics of particles (e.g. shape, size, polymer) are most likely to lead particles to cause harm, and the likelihood of contact based on environmental presence. It is also important that there is a recognised definition of a microplastic (Wagner et al., 2018) and that for clarity it may be worth redefining microplastics as 'small microplastics' (1 µm – 1 mm) and 'large microplastics' (1 mm – 5 mm). Nanoparticles are defined as particles less than 100 nm in size, therefore there is still no common consensus on how the particles between 100 nm and 1 µm should be defined. However, for the purposes of environmental sampling this is not relevant as it is not possible to extract and identify particles < 1 µm.

Biota and sludge

Biota and sludge were grouped due to their complex nature, containing a high proportion of organic matter. As with all other matrices, it is important to determine what question is being asked, and why, before undertaking surveys to analyse microplastics within biota or sludge. For example – is it important to know where within organisms microplastics may have

accumulated, in which case dissection before analysis is necessary, or is it simply enough to know that microplastics are present? Is it important to determine ingestion by individuals, or is a pooled population sufficient? These questions will determine the way samples are taken, preserved and analysed. It was discussed that in order to understand the effects of microplastics within organisms, studies on harm should be run in parallel to environmental sample analysis: although the knowledge of presence develops our understanding of the distribution and ecological interactions of microplastics, simply looking at presence within organisms gives no indication of the ecological relevance and possible effects of this contamination.

Sludge is especially relevant for human health given that it is commonly used on agricultural land as a fertiliser and soil conditioner. It is therefore important to know what is within sewage sludge and at what concentrations so that longer term we may determine likely human exposure. However as with biota, given that we do not yet understand the ecological or human health relevance of this, additional biological and physiological studies are necessary. Due to technological and sample-handling limitations, studies to date have generally measured microplastics down to a common minimum size of 20 µm. Although there will undoubtedly be plastics smaller than this present within many environmental samples, given that we don't yet know the health impacts of these particles, there is no pressing need yet to determine the abundance of smaller particles. However, standardised methods for analysis of microplastics in sludge are likely to be necessary going forward given that this is a product that will be sold; with the growing awareness of microplastics the customers for this product are likely to want to know whether microplastics are present within the sludge and the implications of this. Methods should be developed that can be applied by the range of sludge producers given that this is a product that can be controlled and regulated. As with water, it was agreed that proving evidence of harm to human health or ecosystems is necessary before regulations are enforced; simply determining presence does not justify the cost or effort required to legislate.

Some key considerations:

1) Cost of method (equipment).
2) Ease of use in variety of conditions.
3) Extent of existing data using a given approach (number of papers, research groups, countries using).
4) Information on efficiency of methods for collecting microplastics (JPI comparison is used in the EU).
5) Any issues with contamination.
6) Information on inter-comparability of data with other methods.
7) Determine ratio - experimental / operator error: natural variability (error).

8) Does the method capture data of relevance to microplastics and harm / risk (does particle size captured overlap with those shown to cause harm)?
9) Does the method capture data of relevance to effectiveness of a specific policy measure? If so, state the measure (e.g. for a ban on microbeads, is the mesh size able to capture microbeads).
10) For monitoring, calculate sampling effort required to show a 10%, 25%, 50% change in microplastic abundance.

Summary and outcomes

The most important consideration when carrying out a microplastic survey is to ensure that the methods used allow for the collection of data that will suitably answer the question being asked. Additionally, knowing who will use the data will have an impact on the way the data needs to be collected and reported. For example, if coming from a forensic angle in order to identify particle sources then it will be important to ascertain the number and characteristics of every microplastic particle, including shape, size, colour and polymer type. This type of analysis can be carried out on few, small samples. However, it is not possible to carry out multiple large-scale analyses in this way. In the case of a survey, many large samples may be analysed less thoroughly to produce simple abundance data. Rather than very specific particle characteristics, this would give a more general idea of the number of microplastics in a sample, which may be the concern of, for example, the water industry or a river authority. In this instance, a simple staining and fluorescence technique may be sufficient for particle quantification.

An overarching agreement amongst attendees was that this is too complex a field to simply apply a method standardisation to 'microplastic analysis'. This is primarily because environmental samples are extremely complex and very heterogenous and different matrices will require very different processing techniques in order to be able to extract and analyse particles. For example, sediment requires different treatment to water. However, this can also be true for 'the same' matrix; for example, if considering sediment again, coarse-grained sediment will require different treatment to a fine-grained sediment, and the organic content will also influence processing techniques.

Some areas, particularly those relevant to the water industry such as wastewater and drinking water (in addition to wider industry concerns surrounding bottled water and beverages), were recognised as having priority for standardisation due to growing public concern; with investigatory research studies in this area underway. Although even water samples may be heterogeneous depending on time and treatment (especially effluent), it may be possible to recommend a treatment that could be applied to these for basic analysis and monitoring within water companies themselves, ideally building on filtration and water quality analysis procedures already in place, and thereby also limit the need for new infrastructure costs. Research is currently being commissioned by the UK water industry research (UKWIR) to further investigate these possibilities.

Issues remain over sampling strategies for microplastics and constraints imposed by time, labour and cost of equipment and analysis. New thermal decomposition and analysis techniques and automated micro FTIR technologies are under investigation to improve accuracy and reproducibility, and produce faster data output. However methods still require use of specialist analytical equipment and skilled laboratory staff. Due to resource limitations it is not possible for all researchers and organisations worldwide to adhere to highly technical and specific techniques relying on expensive equipment. Therefore, it is essential that even

with varying techniques, data are presented and reported in such a way that allows them to be interpreted correctly and compared to other relevant studies.

Given the difficulties in adopting blanket standardisation for microplastic sampling and analysis, it may therefore be better to consider 'harmonisation', whereby studies are carried out in such a way that the results are easily comparable to similar studies, for example in related environments or sample matrices. This especially relates to the way in which data are published, ensuring that specific information on sample collection processing and analysis are reported. It should include information such as (but not limited to): mesh size of nets, sediment depth sampled, sample storage, preparation of density separation solutions, temperature and pH for digestion protocols. An easy and effective way of facilitating comparability between studies is in adopting uniform units: for example, a simple recommendation would be for researchers to always report number or mass of particles per volume, e.g. in L or m^3, or mass, rather than by area.

Harmonisation is especially important in monitoring spatial and temporal changes in composition and abundance of microplastics, necessary for instance when monitoring the efficacy of policies or changes in practice in controlling plastic pollution at macro or micro level. By contrast, many of the current studies have been largely investigative and limited to answering discrete questions. There is now a need to move forward with agreement on the most appropriate choice of method to meet particular monitoring requirements, including possible standardisation. Method selection should define parameters such as the choice of sampling location(s), numbers of samples and replicates, degree of precision, sampling frequency, when to sample, and what to monitor (categories of microplastic and types of specimen to be taken). The importance of adopting standards of Good Laboratory Practice was also stressed in order to minimise cross contamination, and ensuring systematic use of blanks, numbers of replicates, and choice of appropriate standards etc.

Some areas of investigation still remain problematic: identifying and quantifying nanoplastics due to difficulties in sampling; understanding health and environmental risks; which sizes and categories of microplastic are most harmful, and tracing the ultimate fate of microplastics considering their wide chemical diversity. Techniques for measuring the specific impact on biota and ecosystems under the wide variety of environmental conditions are also in their infancy. Where possible laboratory studies should also seek to represent real world conditions in seeking evidence of likely harm, and risk assessments should be linked to monitoring needs.

In summary, this workshop was worthwhile in bringing UK and European experts together from diverse backgrounds to discuss standardisation and harmonisation of microplastic analysis methods. The presentations provided a comprehensive overview of methods currently in use and their effectiveness across a wide range of sample types not previously considered together. The complexities in handling real world samples were discussed along with what might, or might not, be achievable using the latest techniques. Also highlighted

were opportunities to apply methods and procedures in use elsewhere, notably in polymer analysis and forensic science. This has been an important first step in assessing how effective currently-used methods are in answering the key questions that need to be addressed across the range of sample types with regard to the environmental impact of microplastic pollution. Further international collaboration will be necessary in order to agree on harmonisation and standardisation of specific methodologies. A follow-up workshop on this topic is therefore recommended to allow more detailed discussions to take place and the involvement of other researchers working in this field. There will also be opportunities to continue information exchange via the UK Microplastics Network and Royal Society of Chemistry.

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