Microplastic Reference Materials

Invited Expert Workshop

May 25-26, 2022

American Chemistry Council

EXECUTIVE SUMMARY

Micro- and nanoplastic particle (MNP) research is at a nascent stage, with numerous studies indicating a need to adopt robust quality assurance / quality control (QA/QC) practices regarding sample collection, analysis and effects testing. Good QA/QC is needed to support the reliability and relevance of data generated, which further supports comparability across studies and strengthens the ability to perform relevant risk assessments. It is generally understood that an important element of QA/QC protocol relates to a demonstrated understanding of the characteristics of the stressor under investigation. The development and application of sampling and analytical methods, for instance, relies on the use of analytical standards, which are used to quantify the efficacy of the sampling and analytical method, such as in the reporting of recovery efficiencies or in the use of quantifying calibration curves. At present, there are limited standardized MNP reference materials or universally agreed on methods for separating and analyzing MNP from environmentally relevant matrices. Consequently, it is difficult to ascertain the environmental and human health risks from existing studies because the exposure data may be of varying quality and reproducibility. The lack of availability to reference materials represents an important barrier towards strengthening the quality of MNP research and are thus urgently needed. In an effort to address this urgent research need, a multi-stakeholder workshop was held during May 2022 in Atlanta, aimed at exploring opportunities to support the generation of a suite of environmentally relevant reference MNP materials for use to support the validation of sampling, preparation, and analytical protocols. MNP reference materials would encompass different resins, morphologies, and sizes to represent in some degree the particle variability present in the environment. Reference materials would serve a variety of needs but would be particularly valuable in supporting the adoption of good QA/QC practices for both environmental monitoring and effects testing, thus helping to strengthen the quality and reliability of data to support risk-based decisions. The objective of this Experts Workshop regarding “Microplastic reference materials” was thus aimed at summarizing and evaluating the challenges of generating a suite of environmentally relevant MNP particles and to initiate a discussion regarding best practices for their use in supporting analytical method development and effects testing. The workshop follows recommendations made following an International Council of Chemical Associations workshop, regarding the development of an environmental risk assessment framework in 2018 for MNP, as well as in response to various activities currently ongoing aimed at generating MNP reference materials, such as efforts directed at supporting numerous high-profile research projects being undertaken nationally and internationally.

Workshop participants concluded that advancing the development and generation of MNP reference materials represents a non-trivial but significantly important activity that is urgently needed. A number of opportunities and challenges were discussed and the strengths, weaknesses and recommendations regarding best practices for use of MNP reference materials towards supporting both analytical method development and effects testing were considered. The opportunities identified include:

- Building on the relative success of the Polymer Kit 1.0, distributed by the Center for Marine Debris Research at Hawaii Pacific University.
    - There was general consensus to generate MNP targeting the most commonly detected plastic polymers currently identified in environmental samples, i.e., start simple.

  - Particles should be prepared as both homogeneous sets of particles, with respect to size and shape, but also should include at least one heterogeneous mixture.
  - A subset of particles should be produced in great enough quantity to support effects studies.
  - Weathered/aged materials using relatively simple approaches suggested as needed as part of a suite of reference materials, however, best practices should be developed and communicated to the research community – emphasis here is on best practices.
- When considering the various approaches used to generate MNP, the application of cryo-milling was generally well received as representing a promising tool for potentially generating large amounts of MNP for certain types of plastic polymers, such as polystyrene.

There is an overall perception and expectation that developing reference materials will require several years (e.g., 10 years), depending on the relative complexity of the types of MNP needed (e.g., size, shape, polymeric composition, etc.) and the extent of particle characterization required. OECD TG Reference materials, for instance, suggests the need to characterize a number of parameters (e.g., 20), representative of a resource-intensive activity, with respect to both cost and time. Alternatively, less intensive activities aimed at characterizing a limited number of critical parameters (e.g., 8), may greatly facilitate the generation and access to a suite of MNP reference materials of limited but relevant characteristics. Additional considerations, such as clarifying whether or not MNP reference materials require certification, are also important to address. Given the complex heterogeneity associated with environmental exposure to MNP, generating a consistent suite of reference materials that have been characterized for a limited number of critical parameters may represent the optimal path forward – i.e., start simple.

Numerous challenges associated with the generation of a suite of environmentally relevant reference materials for MNP, however, were also identified. These include:

- Although the application of cryo-milling to generate MNP was positively received, there was also a recognition that not all plastic polymers are equal. It is thus anticipated that the generation of MNP will require the application of different methods, representative of the optimal method for generating environmentally relevant MNP for that specific plastic polymer.
- The generation of microplastic fibers represents an important research need. Current methods used to generate fibers lack standardization, with a limited number of examples identified for generating fibers used in toxicity tests being identified. The availability of relatively large volumes of microplastic fiber reference materials would prove beneficial towards supporting analytical method development, whereby current approaches generally rely on spherical shaped particles to evaluate analytical method performance. Important to note – across all types of particles (shapes, size, and polymeric composition), relatively high quantities (several kg) will be needed.
- There is currently a lack of clarity regarding which physicochemical properties should be characterized for any reference material – size, shape, surface chemistry, surface area, density, presence of chemical additives and residual monomers, etc. Only preliminary insight was possible within the context of this workshop, additional discussions are warranted to identify a critical set of properties, which may vary depending on the intended purpose of the reference materials. For instance, use as an analytical reference standard may require characterization of properties that may differ from those when MNP reference materials might be used in effects testing.
  - Analytical method development support
    - Reference materials identified as an important factor supporting QA/QC activities, such as related to analytical method development or to support the development of spectral libraries.
    - The use of matrix-specific reference materials, such as NIST SRM Organic Contaminants in House Dust, are also considered as potentially beneficial towards strengthening method development and QA/QC components. Matrix-specific materials, such as standard dust

available from NIST, however, must be evaluated with respect to homogeneity of MNP in the standards prior to advocating its use in analytical method development.
    - Importantly, the availability of a suite of reference materials would enable intra and inter-lab comparisons to be performed, supporting the development of a community of practice and/or establishing a database regarding the use and performance of methods where reference materials are used.
- Effects testing
    - Workshop discussions emphasized the importance of best practices for handling and preparing standards as well as dosimetry in effects testing. There continues to be a need to evaluate and clarify how OECD technical guidance for nanomaterials may be relevant and where guidance may not be appropriate.
    - The appropriateness of using MNP standard reference materials in effects testing was discussed, with concerns being raised regarding the interpretation of results using MNP reference materials as surrogates for evaluating environmental and human health risks. There continues to be a need to better characterize and quantify environmental exposure in order to ensure that the effects testing of MNP, with respect to their shape, size and polymeric composition, are consistent with typical exposures. Given the current state-of-the-science aimed at enabling a robust exposure assessment, it may be premature to use MNP reference materials as part of an effects testing program aimed at informing risk assessment.
    - Alternatively, the use of MNP reference materials in effects testing for the purposes of elucidating toxicological mechanisms of action and/or to develop an improved understanding of biological uptake and systemic distribution and elimination, may be entirely appropriate.

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WORKSHOP SUMMARY

INTRODUCTION

This document summarizes the background and aims of an invited multi-stakeholder expert workshop, held in Atlanta, GA on May 25-26 on the generation of Microplastic Reference Materials. Microplastic research is at a nascent stage, with numerous studies observing a need for the adoption of robust quality assurance / quality control (QA/QC) practices that are aligned with sample collection, analysis and effects testing. At present, there are no environmentally relevant standardized microplastic particle (MP) materials or recognized standard methods for their generation. To address these challenges, ACC and its member companies are exploring opportunities to support the generation of a suite of environmentally relevant standard reference MP materials that could be used to support the development of standard analytical methods and for use in supporting analytical method development and effects testing. The multi-stakeholder group of experts met to discuss the development of MP reference materials, including how to generate reference materials in a consistent and reproducible manner, which properties are needed to characterize and quantify and considerations towards best practices related to their use, such as in the context of effects testing.

DAY ONE

I. Welcome and Introductions

Dr. Todd Gouin welcomed the group and introduced the topics for the workshop. He described the various external factors identified as motivations for supporting the need for this workshop, including scientific and regulatory drivers, increasing pressure on assessing the environmental risks of MPs, and the lack of standard reference materials, standard methods for their generation and the associated challenges complicating the development of harmonized standard analytical methods. Dr. Gouin stated there is a need to strengthen the quality and robustness of data, both exposure and effects. He then discussed problem formulation. From a regulatory perspective, several different instruments can be identified, which appear to address different issues related to concerns associated with exposure to MPs. For instance, whether the concern is aimed at reducing the unintentional release of plastic into the environment or if the aim is to reduce risk may require different management actions and data needs. Regardless, it is clear that there exists consensus among all stakeholders that plastic does not belong in the environment, thus the overarching goal is to reduce the unintentional emission of plastic debris and MPs. Based on our current understanding of environmental and human health risks, Dr. Gouin described output from various research activities that suggest current exposures to MPs are unlikely to be consistent with concentrations that may cause harm. In order to characterize and quantify risks, however, the application of a risk assessment framework, supported by robust data aligned with the degradation, aggregation, sedimentation, agglomeration, long-range transport, bioaccumulation, absorption of chemical containments, and source characterization and apportionment of MPs is needed. To address the data needs, several CEFIC LRI projects have been progressed, including ECO 48, ECO 56, ECO 57, ECO 58, and ECO 59. ECO 56 aims to develop a generic multimedia modelling framework for MP. ECO 57 aims at improved mechanistic understanding of the long-range transport of MPs. ECO 58 aims at establishing a comprehensive additive release and bioaccessibility model for risk assessment of micro- and nano-plastics. Lastly, the aim of ECO 59 is to develop a mechanistic model of micro- and nano-plastic fragmentation in the environment. The hazard side of the risk assessment framework was also summarized, which focused on the development and standardization of effect studies. He noted the need to characterize the physiochemical properties of MPs and to strategically evaluate property-activity relationships, aimed at comparing similar, instead of dissimilar properties. He then provided an overview of various activities that have taken place, with an emphasis on highlighting the support that has been initiated by industry stakeholders. Furthermore, Dr. Gouin explained CEFIC's goal to focus on clarifying the characteristics of a MP reference material and what they might be used for, including weathering, ageing, particle generation, effects

testing, and analytic method development. Additional considerations include the need to refine the definition of MP, whether it might be beneficial to consider binning the characteristics of the MP reference materials in the context of their intended use, such as with respect to the particle size, with particles <10µm potentially representing greater toxicological concern due to higher probability of cellular translocation as opposed to particles >10µm, for which different toxicological mechanisms of action may occur. He concluded with an overview of the Day 1 presentations and commented he will create a summary presentation of the May 25th discussions for various stakeholders.

II. Reference Materials for Micro and Nanoplastic Research: Efforts by NIST and HPU Center for Marine Debris Research

Jennifer Lynch *(NIST)*

Dr. Jennifer Lynch introduced herself and provided a disclaimer that the identification of equipment, instruments, or materials mentioned in the presentation does not imply recommendation or endorsement by the National Institute of Standards and Technology (NIST). Dr. Lynch drew attention to the current concerns related to the presence of plastic debris in the global oceans and the associated implications with the generation of MPs. She then described reference materials, which are physical materials with well characterized composition or properties, and the challenges of generating a standard certified reference material from plastic. For instance, there are thousands of chemical additives associated with plastic products, and which vary depending on their intended use and efficacy, and which can be added to any of the hundreds of polymers or combination of polymers in commercial use. When considering the generation of MPs, the added challenge of attempting to capture the large variety of plastic products is then further complicated by needing to generate MPs representing multiple particle size classes and shapes. Consequently, determining which plastic to use is difficult and the polymers that have been used thus far, in the context of MP research, are limited and can be costly to obtain. Dr. Lynch presented solutions to these challenges, including the Polymer Kit 1.0, Polymer Kit 2.0 being developed in partnership with Hawaii Pacific University (HPU), Standard reference materials (SRMs), and nanoplastic collaborations. Polymer Kit 1.0 is an affordable and easily obtained kit that includes a variety of plastic materials commonly found in the environment. Dr. Lynch highlighted that an advantage of the kit is that it has enabled the establishment of a network for researchers to communicate who are all using the exact same materials. She recommended that more attention be brought to the topic of the Polymer Kit network, and the added value that the information obtained from within a community of researchers can provide to advancing our overall understanding. The researchers were polled in November 2021 and Dr. Lynch displayed output from the survey, illustrating the applications of the Polymer Kit, which to date have largely been associated with use in helping to support analytical method development. In this context, she described the results of Case Study 1, which used Polymer Kit 1.0 to compare against an in-house mass spectral reference library. Dr. Lynch proceeded by describing Case Study 2, which used Polymer Kit 2.0, from which no major chemical changes in the spectrum were noted in this study. Dr. Lynch summarized that the overall feedback from those who have used the kits has been positive and encouraging. She identified the number one request in the Polymer Kit 2.0 survey, was to provide access to smaller sized particles. Beyond that, Dr. Lynch noted there were also request for more fibers, more variety of polymers with the inclusion of known chemical additives, the inclusion of weathered particles, among others. She noted that both NIST and HPU Center for Marine Debris Research were currently working on the number one request for smaller particles. Dr. Lynch then provided graphs with FTIR data and pointed to the large list of existing polymer standard reference materials (SRMs). She illustrated that the graph listed 20 different reference materials, which however, represented only six resin types. Four of the reference materials had chemical additives that were limited to inorganic elements and phthalates. Most were in pellet form, except for a few present as powders and three were present as nano-sized particles. Dr. Lynch noted that the cost associated with obtaining sufficient quantities of these SRMs was perceived as being inaccessible for the majority of academic researchers. She described other specific SRMs: dust, sludge, sediment and soil, and bivalves. With specific discussion related to the sediment SRM, Dr. Lynch noted that the SRM 1941b organics was observed to

contain MPs. Dr. Lynch listed new projects NIST was currently working on, including the need to evaluate the homogeneity of MPs that might already be found in the various NIST SRMs and the generation of large quantities of five different polymers from common consumer products, such as plastic polystyrene cutlery. The activities associated with generating large quantities of MPs was presented at the SETAC Europe meeting, where they reported results of generating particles from plastic water bottles (weathered and brand new) through a Retsch cryomill. Lastly, Dr. Lynch raised awareness of a new collaboration between NIST and JRC on the topic of MP reference materials.

Discussion:

The group discussed the cost of the Polymer 1.0 and 2.0 kits. The Polymer 1.0 kit costs $375, and the price of the Polymer 2.0 kit is unknown as of now. A comment was made that there were no toxicology studies mentioned and that the generalization is that fibers are inducing more of adverse health effects than particles' will be important to consider as part of future activities. The group discussed the characterization of the MPs, noting there is a certificate of analysis for each one and it lists what it is certified for. Another member mentioned that the chemistry could be different, and Dr. Lynch responded that Polymer kit 1.0 was a collaboration between ACC member companies and ACC. Member companies donated materials towards the development of the polymer kits and that ACC had stripped all information about which companies provided which particles. Both Dr. Lynch and the research community are thus "blind" with respect to the origin of the particles, which does create a lot of questions that arise from the kit. A question was asked about the grinding of materials, a process which is understood to have the potential to introduce metal contamination from the machine, to which Dr. Lynch responded that when the high-density particles came out, they indeed let the process run a little longer in an effort to obtain the last bit of particles from the process, and indeed did observe contamination from titanium. Consequently, caution is needed during the generation of particles, aimed at minimizing this contamination potential. The level of contamination should be evaluated with each batch generated. Finally, the group discussed that the Polymer kit 2.0 is coming from HPU, although the name of the kit is as yet undecided.

III. Nanoplastic Standards – Known Unknowns and New Order

Samuel Stavis *(NIST)*

Dr. Samuel Stavis began by outlining the two parts of his presentation: known unknowns of current standards and new order of future standards. He thanked his co-authors and summarized interactions with NIST stakeholders. Dr. Stavis stated that NIST stakeholders need access to well-characterized nanoplastic standards, often for use in calibrating optical microspectroscopy instruments. Dr. Stavis stated that, by definition, such standards should be homogenous and stable within property specifications, and thus fit for purpose. Reporting on the development of a lateral nanoflow assay, which is a measurement system combining a nanofluidic device and optical microscope, Dr. Stavis summarized results obtained when the method was applied to polystyrene nanoparticles, which are commonly used as a nanoplastic standard. Observations from this research implied that surprising trends and heterogeneity of fluorescence are hiding in plain sight, which could result in questionable inferences about the diameters of single nanoparticles derived from their fluorescence intensities. The lateral nanoflow assay provides an opportunity for new statistical tests of the relationship between fluorescence intensity and particle diameter. Specifically, a hierarchical model was developed to test the goodness of fit and fitted exponent of a power-law model. This analysis showed that the fluorescence intensity of these common standards scales with steric diameter to nearly the fourth power, confounding basic concepts of surface adsorption or volume absorption of molecular adsorbates to polymeric nanoparticles. Moreover, the analysis showed significant heterogeneity in intrinsic fluorescivity of single nanoparticles, which can confound any inference of dimensional and chemical properties from fluorescence intensity. To better understand these results, Dr. Stavis discussed the lithographic fabrication of ordered arrays of nanoplastic standards, using nanoscale pillars and films with diameters and thicknesses in the range from 100 nm to 1 µm. Using a phenolic resin to prove the concept, he demonstrated how

nanofabricated structures could be used in combination with correlative microspectroscopy, such as by fluorescence emission and electron, Rayleigh and Raman scattering, to improve the calibration of instrument response and detection limits. The lithographic fabrication of the nanoplastic arrays yields uniform films, which enable flatfield corrections and provide reference spectra, followed by uniform pillars, which enable aberration corrections and correlative microspectroscopy, and variable pillars, which enable characterization and quantification of instrument responses and detection limits. Dr. Stavis also noted that the nanoplastic arrays enable sorption assays to probe the interaction of molecular adsorbates with plastic nanoparticles. In conclusion, Dr. Stavis emphasized the known unknowns, stating that structure-property relationships of nanoplastic standards are poorly understood at the state of the art. Considering the results of recent activities at NIST to develop and apply analytic tools to accurately measure the structures and properties of nanoparticles, care is necessary to avoid erroneous interpretations of data based on poorly understood structure-property relationships, such as with respect to correlating particle diameter and fluorescence intensity.

Discussion:

A question was asked regarding the observations pertaining to the power law, and to clarify if it was for size. Dr. Stavis responded that researchers have put forward hypotheses or have expressed naïve expectations regarding the relationship between fluorescence intensity and particle diameter, and that measurements based on simple correlations might be inaccurate. Researchers have also used these expectations as reference trends, searching for deviations of results from expectations. What if the expectations are wrong? An incorrect expectation should not serve as a reference trend. A comment was made about finding correlations versus causation. The individual asked if there might be a third axis that could have been applied to the results presented. Dr. Stavis noted the nanoplastic array would be an ideal standard to enable such measurements, which would require correlative microspectroscopy for each axis of the measurement. One could measure the same structures by the different methods, obtaining accurate information on the particle diameter and various optical properties on different axes, and registering the data accurately and efficiently for the different aberrated instruments.

IV. To Properly Address the Multidimensionality of Microplastic in Risk Assessment, Your Reference Material Must also be Multidimensional

Bart Koelmans *(Wageningen University)*

Dr. Bart Koelmans began by stating that the risk assessment of MPs has been performed based on well-known principles, with application of evaluating risk following a basic paradigm scheme and from data obtained from a literature review. A key challenge, however, is that while data reporting on adverse effects tend to be represented by tests using monodisperse particles (e.g., polystyrene spheres of a specific size), environmental exposure data are characterized by a heterogeneous mixture of particles, thus resulting in an ‘apples-to-oranges’ comparison. There may, however, be a pragmatic path forward, if we assume that MPs in the environment behave in a predicable way. For instance, Dr. Koelmans suggested that environmental MPs appear to adhere to a set of ‘habits’, which he described as predictable distributions of mass, volume, density, area, specific surface area, elongation, width, and length. The observation presented thus enables an approach towards addressing the issue of uncertainty and diversity in the occurrence of MPs in the environment, such as via the development and application of probability density functions (PDFs). He then presented illustrative plots that summarized various examples of these habits. Dr. Koelmans stated that by summarizing empirical data reporting the presence of MPs in the environment, that the data can be captured in the form of statistical distributions, which can be quantified as probability distribution functions and used prospectively to estimate environmental exposure. Using data reporting MPs in air, he illustrated how the data can be used to inform a human exposure assessment. He then described more examples and stated this could be used to look at mixtures of around six thousand MPs that have been reported at the surface of the ocean. Furthermore, it was noted that the PDFs can be used to scale between MPs reported in the environment and MPs used in effects testing in the lab. For example, by comparing against a

toxicologically relevant effect metric, such as particle volume or particle surface area. In this instance, scaling against a relevant effect metric would help reduce the need to address inconsistencies in particle size and/or shape, where the effect is due to a property not intrinsic to the polymer composition. ..Dr. Koelmans provided an illustrative example of how the approach has been applied in the context of characterizing risk. A strength of the approach is that it would include the ability to align all existing data towards assessing risk, although a limitation is that there exists a potential for error propagation in the alignment calculations due to variability of slopes and PDF parameters. One solution is to perform effect testing using an environmentally relevant mixture of particle shapes, sizes, and polymers in an effort to better approximate the 'real' PDFs that occur in the environment. He then provided an overview of a study that was just submitted, which was standardized and completed using the same material. Dr. Koelmans then discussed the pitfalls of a 'monodisperse' approach when the stressor in the environment is actually polydisperse. For instance, if the material is varied in size but the other qualities are the same, it still means there will be a change in mass, volume, surface area, and chemical bioavailability. Since there are so many combinations that can occur when using 'single type' particles , we would need to test >12,000 exposure test system combinations. Due to the challenge of extrapolating between monodisperse particles used in test systems and actual environmental exposure the observed adverse effects would still not be directly translatable to effects of environmentally relevant MPs. Dr. Koelmans then went over some points for discussion, which included the feasibility of using PDF-based heterogeneous MP reference materials for quantifying the recovery efficiency to support analytical QA/QC, as well as the need to avoid associated chemicals, an issue that can be difficult to control, and a recognition that there are likely limitations to using a heterogeneous mixture of particles that need to be acknowledged. The use of reference materials should thus be 'fit-for-purpose'. The examples provided included matrices with relevance for human health, cases with a small 'bioavailability window', and cases where a monodisperse reference material would better than using a polydisperse group of particles (e.g., instrument calibration, process research, and use of particles with a tracer).

V. Industrial Microplastic Powders; Chemistry, Manufacturing, and Characterization

Richard Czarnecki (Micro Powders Inc.)

Mr. Richard Czarnecki began by introducing Micro Powders Inc., who offer the most comprehensive range of powder additives in the industry. As some background, he mentioned that the use of polyethylene beads as exfoliants in body and facial scrubs had become the face of the microplastic problem, but that the industry is now transitioning away from their use in personal care products, with the majority of major companies voluntarily phasing-out their use several years ago. He also noted that the agrochemical industry was similarly moving away from the use of MPs in their products and that they are of concern because of pending ECHA Annex XV regulations. Additionally, the surface coatings industry may be of emerging concern. Providing a definition for MPs, Mr. Czarnecki described them as particles that contain solid polymer and at least 1% of particles have dimensions of 0.1 um to 5 mm or fibers with a length of 0.3 um to 15 mm and a length to diameter ratio of >3. Based on this definition of MPs, it should be noted that not all fine industrial powders are MPs. For example, powders based on substances and defined as natural or biodegradable per European Chemicals Agency (ECHA) guidelines are not considered microplastics. Mr. Czarnecki also discussed the sizes and uses of industrial microplastics by going over the top and mean sizes of fine powders which are used in cosmetic powders and seed coatings, medium powders which are for gloss reduction in paints, and coarse powders which are used for texturing effects and in personal care products. Mr. Czarnecki then gave an overview of the chemical manufacturing and processing of MPs, which can vary between different types of materials commonly used to make plastic particles. For example, by definition, not all fine industrial powders are MPs, thus a micronized synthetic wax powder is not defined as a microplastic according to ECHA guidelines, neither are powders defined as natural or powders that are biodegradable. Industrial processing of MPs can be achieved through a process referred to as air micronization, where raw powder wax (top particle size of 100 µm or finer) is accelerated with high velocity by air jets in a toroidal chamber. Alternatively, they can be produced via mechanical milling, where

wax or plastic (top particle size of 100 µm or larger) is crushed and reduced as it feeds through opposing and rotating serrated steel plates. Using a process referred to as spray micronization, the starting material is introduced into air where raw wax is melted and discharged with pressure through a micron-sized orifice into an air-cooling tower, or into a solvent. Finally, Mr. Czarnecki summarized a process known as spray drying of emulsion particles, where spherical plastic particles are formed using an emulsion polymerization process. Dr. Czarnecki then discussed the different particle morphology types that are typically generated using the different approaches, such as spheres or irregular shapes with jagged edges. He then noted the different methods used for particle characterization, which characterize particles with respect to their shape and size, material type, melting or softening point, and density. Mr. Czarnecki also mentioned that not all powders used as starting materials are represented by a single homogenous polymer composition but can be composed of two or more materials. Additionally, he commented on the generation of secondary MPs, which can be characterized as the degradation products of high molecular weight plastics, not waxes, so it can be difficult to create controlled conditions capable of generating secondary MPs, and that the use of primary MPs to mimic them represents cause for concern. An observation that is entirely consistent with the material presented by Dr. Koelmans.

Discussion:

A comment was made that ocean or atmospheric weathering creates a particle and a microplastic type that is not being intentionally produced, making it difficult to track and characterize.

VI. Applying Lessons in Nano Standard Materials Generation and Microplastics Quantitation Towards Future Standard Reference Materials for Hazard Assessment

Jeanne Hankett *(BASF)*

Dr. Jeanne Hankett began by discussing the current research landscape with respect to the development and application of analytical methods. She noted that sample preparation and analysis methods for MPs are still in the developmental stage and not all major matrices/plastic types can be reliably evaluated. Currently, regulators and policy makers are pursuing quantitative methods in their monitoring portfolios, and standard organizations are similarly developing methods. She discussed the importance of relating our reference materials of interest to the types of analytics that they would most likely undergo. Furthermore, she discussed the quantification of MPs which included count-based and mass-based methods. She also mentioned that if we want to better characterize MPs, we need to obtain a better understanding of their physical characteristics. Dr. Hankett discussed the output from two interlaboratory comparison (ILC) studies. The first interlaboratory study was conducted by the European Joint Research Centre (JRC) in collaboration with the German Federal Institute for Materials Research and Testing (BAM) to quantify PET MPs in water. She discussed the participants and sample details. She summarized that the aim of the ILC was to investigate and evaluate the performance of current state-of-the-art methods used for the analysis of MPs and to support the development of MP reference materials. For this ILC, there were participants across the globe and various procedures were applied. The results of the ILC demonstrated a wide variety of results and she stated it was not surprising, as it is an emerging field and quality controls are key factors likely influencing the high variation between labs. Dr. Hankett then provided an overview of the conclusions from the study, stating µFTIR and µRaman represented the most promising tools and that pyrolysis GC/MS also appears to be a suitable technique gaining wider use. She next discussed the Southern California Coastal Water Research Project (SCCWRP) interlaboratory study (ILS). SCCWRP hosted several ILS to support SB 1422 and 1263 (drinking water and ocean protection). She noted the research papers for these will soon be published. Dr. Hankett drew attention to an important observation, whereby the full picture might not be represented in the results reported since there were substantive issues concerning MPs between 1-20 µm. She discussed the conclusions of this study and how they were similar to the JRC BAM study, with both studies observing high deviations in particle counts. She also noted that in subsequent workshops BASF and SCCWRP identified best practices, potential areas for improvement, needs for laboratory accreditation, and further analyzed datasets. It was also highlighted that

the number one challenge pertains to the need for better analytical methods capable of characterizing and quantifying the smallest size fractions of MPs (e.g. <10 µm)

Dr. Hankett discussed the JRC nanomaterials repository, which started from a need of proficiency demonstration for laboratories and high-quality method development for testing, analogous to the need for a repository of MP reference materials. She discussed the importance of the sponsorship program, which was launched in 2007 and drew attention to the wide use of the nanomaterials repository in research, with over 258 publications having utilized the repository thus far. Furthermore, it was stated that OECD test guidelines for nanomaterials have been recently updated, based on the learnings of projects and OECD round robins of adapted methods that have used the nanomaterial repository. Dr. Hankett then discussed how to translate JRC NR to MP reference materials. Considerations included requirements of high-quality materials, different sizes and shapes, the need to target relatively large volumes, (e.g., kilograms scale) for source material, long-term stability, and the need for materials to be well-documented and characterized. Dr. Hankett listed the key take-home messages. She described nuances were important for successful development and deployment of specific analytical methods. Additionally, there is a need to consider anonymity, characterization, accessibility, type of material, single sources, repository control, traceability of deployment and potential impacts of reference materials.

Discussion:

A question was asked about how MP were introduced and evaluated in drinking water. Dr. Hankett responded by clarifying that the larger particles are picked and counted and deposited in jars of water. The smaller size fractions were introduced into drinking water through the use of pills that contained hydrophilic gels or polymers containing a known concentration of the hydrophobic particles. The pills dissolve in the water, thereby releasing the MPs. In actual deployment, once labs let that system sit out for a while, the hydrophilic particles associated with the gel would start to harden. This caused issues with filtering. Someone asked for Dr. Hankett's opinion on control materials versus reference materials. Dr. Hankett stated that for reference materials, we are looking at the right sizes, materials, etc., and when looking at controls, I think we need to look at natural and inorganic materials, which generated considerable discussion. The group discussed this point further and someone noted that they try to have NIST responsible for the repository of MP reference materials. Someone mentioned that the research community is using beads as control material not reference material, to which someone noted that calibration is a different situation and if using it in toxicological testing it would be very expensive. The group discussed stabilizing the particle in a meaningful matrix, issues with testing, parameters for testing, and the timelines for receiving more advanced materials.

VII. MNP Toxicology – What can we learn from that which has preceded it?

Martin Clift *(University of Swansea)*

Dr. Martin Clift began by describing the London Smog Episode of 1952 and Influenza hitting in the same area in 1953. Exposure to high concentrations of particulates in smog during 1952 thus resulted in an increase in deaths due to individuals being immunocompromised following their lungs experiencing a particle overload. He clarified that particle toxicology is not limited to air pollution but that it has also been an important area targeting occupational exposure to classical particles, such as coal mine particulates, such as silica and asbestos. He noted that depending on the physical and chemical attributes of the particles, the specific toxicology and reduced human health can be attributed to exposure to particles in the immediate vicinity of an individual, or may be related to occupational exposure, such as in mines or other specific industrial practices. Dr. Clift then presented research related to ultrafine particles, with reference to the ultrafine hypothesis. He added that when the size of particles is considered, there is a tendency towards engineered nanomaterials. He described the study of Ferin et al., who observed an inflammatory response to exposure to ultrafine particles in lung cells, which were attributed to a combination of factors, including the particle size, mass, number, and toxicodynamic of exposure. Dr. Clift suggested that the physico-chemical characteristics thus represent paramount factors in driving the effects of

particles on human health. He drew attention to a study comparing unknown particles to carbon black and classical particles to predict their effects. However, he drew attention to a number of major challenges in the field of effects testing of ultrafine particles, which can result in a high potential for obtaining both false positive and negative results. Researchers should thus be able to understand what they are viewing and focus on human relevant systems. Dr. Cliff then reviewed what is known in nanotoxicology such as the array of different model systems and an understanding of where the field needs to be. He proposed nanotoxicology would benefit from improvements related to the exposure strategy, identifying biological characteristics of the model, and the model having a predictive nature. He gave examples of the road less traveled" and the "longer road needed to be traveled".

Discussion:

A comment was made about one of the things to draw attention to is around the effects testing. For discussion in the breakout groups, the point raised about the interaction of the properties is important to consider. If we are going to take milk bottles that were never used and crush them into small particles, is that truly representative of an environmentally relevant exposure? How might we consider using MP reference materials in the context of effects testing?

VIII. Degradation and Fragmentation of plastics

Anthony Andrady *(North Carolina State University)*

Dr. Anthony Andrady discussed the basic mechanisms of weathered degradation, including photodegradation, thermo-oxidation, biodegradation, and hydrolysis. He briefly touched on the basic concepts of weathering in the environment. He described degradation further and stated that after degradation, the plastic becomes very weak and gets fragmented, but that it is not the only way by which fragmentation might occur. He provided an overview of the degradation and fragmentation cycle and noted that as long as there is a sufficient supply of the polymer and oxygen, the cycle will continue. He also discussed the rate of degradation which can change the expression if there is a low concentration of oxygen verses a high one. The interior of thick samples from plastic in freshwater or seawater do not have enough oxygen, for instance. Therefore, oxygen is used faster since it cannot come in at the same rate as it is used. It was also noted that most of the oxidation is limited to a thin surface. Dr. Andrady then explained that surface chemistry of the thin oxidized layer is different from that of the bulk material. If the same experiment is done in seawater, a minimal of particle size of 50 micrometers as opposed to 600 micrometers in air can be observed. Furthermore, when discussing graphs on the slides, Dr. Andrady pointed that for seawater there is almost no change, and the surface layer is too thin in the case of seawater exposures for its effect to be reflected in tensile extensibility measurements on an ASTM Type IV test piece. He also discussed Weathering fragmentation mechanisms and stated cracks propagate in the z-direction via the amorphous content and mechanical forces in the environment encourage fragmentation. Afterwards, Dr. Andrady described the two reasons crystallinity changes. First, preferential degradation of amorphous fraction increases the fractional crystallinity and second, surface chemi-crystallization. Some impacts of increased crystallinity include reduction of the rate of further photodegradation of the polymer, reduction of the pick-up and concentration of persistent organic pollutants (POPs), decrease in the rate of subsequent biodegradation of the polymer, and the size and shape of the secondary fragment depends on the crystalline pattern at the surface. He noted if crystallization continues, cracks will go into the materials and create macro-fragmentation and surface ablation. Furthermore, the types of crystallites also change with weathering and can be artificially created in the lab. Implications discussed were the choice of resin and the control of reference MP characteristics.

Discussion:

A comment was made about the crystalline phase and the amorphous phase, and smaller particles causing surface disruption. The individual asked if there would be a lower affinity due to larger surface area. Dr. Andrady

confirmed the lower affinity and stated the smallest materials can bio-mineralize in some reports, but that the crystallized fraction only slowly undergoes degradation. Dr. Andrady discussed the thickness of the oxidized layer being very large compared to the film. The film can fragment under the same conditions. Someone asked if environmental fate affects the density of the polymer. Dr. Andrady stated oxidation is important for ocean environments. It can only occur while they are floating, and density does contribute to it sinking. Dr. Andrady noted accelerated weathering is a generic term. The thickness of the sample is not discussed. If you are going to use accelerated weathering, one must be careful. Accelerated weathering does two things, including increase UV and increase temperature. The group discussed the associated routes of degradation, with an important observation that not all plastic polymeric materials degrade following the same degradation mechanism. Consequently, depending on the type of plastic different processes may require characterization and quantification, it is thus important to obtain data on environmentally relevant plastic materials.

Executive Summary of Particle Generation Methods:

Both groups identified the need for a central repository to house information. It was also noted that the work should start simple by targeting specific polymers and then growing from there. Furthermore, it was discussed how there might need to be different types of reference materials for different purposes (such as toxicological studies versus fate and transport studies). It is also important to have particles generated as both homogenous groups but also heterogenous mixtures. The implication of microplastic fibers were also discussed and the associated challenges to generate and characterize them. The need for irregular shapes as opposed to spheres was also emphasized.

Executive Summary of Weathering and Ageing Methods:

Both groups concluded that reference materials are important and necessary. They discussed both purchasing pre-weathered materials and having best practices and standard operating procedures for individual groups to weather materials themselves. Looking to organizations that already have similar procedures for this guidance, like OECD, EPA, ISO, and ASTM would be helpful. Additionally, both groups discussed the methods for weathering which include lab weathering, natural weathering, and controlled outdoor weathering. They also discussed UV, water/humidity, chemical weathering, and mechanical weathering. Furthermore, the idea of creating "kits" was brought up, similar to the Polymer 1.0 kit, but for weathered materials, although the creation of a MP reference material repository was also emphasized.

IX. Breakout Group 1 – Particle Generation, Methods/Best Practices

Chair: Denise Mitrano *(ETH Zurich)* Rapporteur: Erik Rushton *(LyondellBasell)*

The group began their discussions by acknowledging that different questions and objectives will require different types of MP reference materials. They discussed the need for access to both well-characterized control materials and MP reference materials and referenced JRC and the need to identify sources of the particles. It was mentioned that the goal for ACC's consortium is to partner with both JRC and/or NIST, who could provide a one-stop central location for a MP reference material repository. The group discussed trying to identify the most appropriate sample vials, and various other different elements aligned with storage and handling. Additionally, it was recognized that there is a need for a common and harmonized approach used for characterizing the different materials, using a consistent set of criteria. The advantages of generating MP reference materials from one large batch as a method for demonstrating consistency in the properties of reference materials and reducing inter-batch variability was emphasized. It was also mentioned that volatiles evaporate from the surface, and they have a finite shelf-life associated with them. Consequently, consideration of the types of solvents in which MPs are to be stored will require some thought. Furthermore, it was noted that MP reference materials would not include water soluble polymers, thus the definition of plastic is important to clarify in the context of the reference

materials, which will differ from that of a polymer. It was suggested that the definition should initially be broad and then following input from various stakeholders could then become narrower. The group also discussed that there continues to exist a difference of perception between different groups when discussing MPs, as they can represent different types of materials to different groups of people (e.g., general public versus polymer scientists). For instance, in some instances tire and road wear particles are considered to be MPs, whereas other groups refer to tire and road wear particles as a separate group of particles from MPs. Similarly, how to handle the inclusion of fibres in the context of MPs can also prove problematic, with further challenges encountered when differentiating between nano-sized plastic particles and micro-sized particles. The classic definition of MPs, for example, often refers to plastic particles <5mm, which implies that even particles <1µm might be considered as MPs under this definition. However, several groups have also recently attempted to suggest the adoption of different terminology to differentiate between different sizes of particles, with reference to nano, micro, meso, macro, etc. In the context of developing a MP reference material repository, the group considered what a reasonable number of materials would be needed to include in the repository and mentioned that four different types of materials could be readily identified from HDPE. It might be most beneficial and efficient to possibly conduct a survey to better identify more accurately what the actual needs of potential users. This would help to ensure that the generation of MP reference materials are immediately impactful and would be fit-for-purpose. The group also discussed the advantages of providing MP reference materials in the form of kits, analogous to the polymer kit 1.0 distributed by HPU, but that perhaps the kits should be developed with the intended purpose in mind when assembling them. It was noted, for instance, that a polymer kit might not be the best option for all applications and that people will most likely appreciate opportunities to purchase reference materials individually. Some incentives for people to use the MP reference materials were discussed and included the quality of data and the ease of use, as well as cost, characterization, and fit-for-purpose application. They also discussed the challenges and limitations of generating heterogeneous mixtures of MPs, although having at least one mixture would likely prove beneficial to the research community, particularly if the properties of the particles within the mixture are well-characterized and affordable. Access to a standard mixture MP reference material would help ensure better comparability between research studies using the materials, as opposed to individual groups attempting to generate their own bespoke mixture. With an emphasis on considering the use of MP reference materials in effects testing, it was emphasized that there is a need to move away from the current trend of using spherical polystyrene particles. Consequently, the generation of reference materials consisting of a range of properties (e.g., size, shape, polymeric composition) would likely represent an important opportunity towards better understanding toxicological mechanisms of action. Finally, the group returned to the challenge of scale, whereby relatively large volumes of materials were likely needed to be produced and characterized, and how this might influence the cost of the materials. Large quantities, for instance, will be needed to support fate and transport studies, analytical method development, and the use of materials in the context of biological testing, such as with respect to effect studies as well as cellular translocation uptake, fate and elimination mechanisms. The group discussed access issues and mentioned subsidies.

X. Breakout Group 2 – Weathering and Ageing, Methods/Best Practices

Chair: Jennifer Lynch *(NiST)* Rapporteur: John Davis *(Consultant)*

Dr. Jennifer Lynch welcomed the group and stated that the purpose is to discuss methodologies and best practices for ageing and weathering of plastics, specifically aimed at supporting toxicity, fate and transport studies, and how to generate a group of associated reference materials. The group began by identifying the methods for weathering plastics which include the use of environmental chambers that subject plastic to varying humidity, UV/light (artificial lamp), temperature, and mechanical (shaking, rocking, etc.) aimed at speeding up the weathering and aging process; alternatively, outdoor chambers using mesocosms to examine the natural environment can also be applied; as well as acquiring weathered and aged MPs directly, through the collection of plastic debris from the environment (i.e. “real-world” plastics). The group noted whether it was necessary to distinguish between chemical and mechanical weathering. Tree main toxicology perspectives were also discussed,

which include the need to have reference materials as commercially available products, which could be obtained directly from a production company, generating weathered and aged MPs in the lab using standard methods, and the use of real-world plastic samples, obtained directly from the environment. It was questioned whether materials should be aged and weathered before making reference materials, to which the group agreed that being able to buy weathered material would be helpful, but also being able to weather the materials in-house may also be practicable, depending on the research question being raised. The generation of weathered and aged MPs should thus address fit-for-purpose needs. The group mentioned needing Technical Guidance Documents that would provide best practices on weathering, similar to the Organisation for Economic Cooperation and Development (OECD) or the Environmental Protection Agency (EPA) standard methods. It was also noted that when EPA makes weathered materials, there is a biofilm which would have to be added after the reference material was made. The group discussed this further and decided that in some cases it might not be needed, such as in cases of occupational exposure where the formation of a biofilm is unlikely to have had sufficient time to develop. The group also noted that biofilms are not uniform in the environment and there is no way to standardize and stabilize them within a reference material repository. Concern was thus raised in terms of making weathered materials in that only the outside layers may be weathered, and the inner layer may not be as damaged or oxidized, as might occur in the real world. Suggestions were raised about the possibility of grinding the material first and then weathering the smaller particles subsequently generated. Alternatively, the group questioned how weathered the materials need to be, as different layers will be at different levels of weathering making QA/QC difficult.

Taking a step back, the group began to question the feasibility of generating reference materials representative of environmentally relevant weathered plastics. Nevertheless, there was a growing consensus among those in attendance that while there is a high likelihood of groups using MP reference materials in the absence of weathering, and then performing their own bespoke weathering studies, not all researchers have capabilities to generate their own weathered materials and may therefore benefit from at least a minimal set of weathered reference materials. The group then discussed that the reason they would benefit from access to weathered MP reference materials would be to support research aimed at comparing between weathered and virgin materials. Additionally, the group discussed how the degree of weathering relates to how long the plastic stays in a relevant area (e.g., if it is buried in sediment or soil within a relatively short period, such as 6 months, it may not be as weathered to plastic that might be at the surface of the ocean for 5 years). The extent of weathering, is thus likely to be environment-specific, resulting in challenges towards characterizing the properties related to a standard environmentally relevant weathered plastic, since there exists a significant degree of heterogeneity. The practice of attempting to timestamp or date plastics that wash ashore was discussed, but that the science was not sufficient to accurately quantify the age of environmental plastic. The group also emphasized the importance of heterogeneity in the reference materials and simple parameters should be set up (e.g., up to 20 years). They mentioned using the ASTM method D1435-20 for outdoor weathering plastic and G154 for accelerated. It was questioned if NIST could characterize different naturally occurring materials to create a suite of polymers but acknowledged that attempting to perform such an activity would require a significant time and cost resource, with it being unclear the added value that such an activity might bring. Another idea was to create a Weathered Polymer Kit by starting with common weathering processes, and building onto it over time, possibly starting with polyethylene as it is already available. In other words, advocating the need to start simple. The group also mentioned that it would be beneficial to identify and prioritize which types/grades of polyethylene would represent the most environmentally relevant types of materials, and that there are environments other than water, such as fate in sludge and terrestrial systems, to also consider. One idea mentioned was to create a community of practice for sharing weathering and aging methods that could possibly tie into the ASTM method and create a catalogue of existing methods for subsequent evaluation at a later stage. It was also mentioned that having academic groups work toward this would be beneficial as they would have labs and could generate data with the methods. The overall recommendation decided upon is to develop protocols and to apply methods to polymers available in the Polymer Kit 1.0, in an effort towards generating an initial set of standard weathered

materials which could be replicated. Additionally, collating and evaluating existing methods for applicability and relevance should be performed, possibly as a parallel activity. The group then discussed initial recommendations such as for UV light using the Xenon lamp and making sure the energy/output of the lamp is measured and samples are compared across the world where there are different levels of UV radiation. For temperature specifications the group mentioned only using naturally occurring temperatures and weather events. One suggestion was to use temperatures found at 30 degrees latitude because of the accumulation of plastic debris in the largest gyres. The group also discussed the role of humidity, water (i.e., salt), mechanical, biological, ozone, pH, standardized carbonyl indexes and other important considerations.

The major takeaways of the group discussion include having a standard virgin resin where accelerated weathering could be done in a lab to match natural weathering to create a standard weathered reference material. Additionally, it would be good to have guidance in relation to characterizing the extent of weathering, such as variance in the carbonyl index, but even better to be able to purchase pre-weathered reference materials. Furthermore, there should be a common understanding of weathering, how to measure it and standardize it. A full characterization of what is being seen on the beach currently to get a sense of what properties are being seen in naturally occurring materials would be helpful as well as creating a team or consortium to review the options and develop guidance.

XI. Breakout Group 1 – Particle Generation, Methods/Best Practices

Chair: Denise Mitrano *(ETH Zurich)* Rapporteur: Erik Rushton *(LyondellBasell)*

The group discussed how making simple materials first and focusing on a few characteristics, for example, MP reference materials of specific sizes and shapes, and which should include fibers, would represent a good starting point – in other words, start simple. They noted that where groups physically alter the starting reference materials, then it is no longer a reference material. Consequently, input from potential users identifying their research needs with respect to reference materials would likely help to prioritize the particle sizes and shapes that are currently most desired, and therefore avoid the need for groups to manipulate the starting materials further. Based on current feedback from the Polymer Kit 1.0, it is clear that most groups are looking for smaller size particles, this should thus help to direct initial steps towards providing MP reference materials. The research question and how the MP reference materials will be used is also important to clarify, whereby materials should be fit-for-purpose. In the context of toxicity effect studies, for instance, it may be that MPs <10µm may be most useful towards progressing understanding of potential effects for particles that are at biologically relevant particle sizes. At the same time, however, MPs <10µm would also help to support analytical method development, since current methods appear to be inadequate for these smaller size particles, as well as helping to support environmental fate and transport studies. Access to variations in particle shape would also be beneficial. In particular, there is a need for microplastic fiber reference materials, for which there are currently no standards readily available. For environmental studies it was noted that having access to a continuous distribution of particle sizes would also be helpful, as this would better reflect environmental presence of MPs. The challenges with fibers are non-trivial and will require careful consideration regarding how to generate a reference material, for instance, with the influence of physical abrasion and the likelihood that fibers are likely characterized by considerable variability in size in the environment. It was mentioned that access to MP reference materials must be affordable, or else there is a potential that researchers will revert to making their own standards for use in their experiments. Intuitively, this would reduce the impact of a MP reference material, whereby the goal should be to ensure a high level of uptake by the research community in an effort to produce results that can be compared between a number of different studies. The group suggested that perhaps instead of developing additional kits, that the establishment of a repository with a catalogue of varying types of MPs (varying in shape, size and polymer composition), would potentially be more impactful, as this would likely help to maximize the number of people who can test a larger number of hypotheses. The group also suggested a need to consider the inclusion of a reference material whereby crystallinity is well characterized and for which there is some variability

between different types of particles available. When considering methods aimed at generating a MP reference material the group identified different methods and suggested that the use of milling may prove most effective, as it could be used to produce relatively large quantities of particles in a cost-efficient manner. It was also acknowledged that in order to generate the smaller size classes of MPs, that the application of smaller batches would be more appropriate, since there is a need to ensure relatively narrow particle size distributions and thus the quality of the production process will likely be more efficient when handling smaller quantities, thus always important to ensure that the generation of the MP reference materials are fit-for-purpose. The group also discussed detection methods and stated that there needs to be a sufficient quantity of particles to ensure that recorder measurements are above a method's detection limits, although this will depend on the analytical system and the rigour of the QA/QC practices of the lab. There also needs to be better understanding of environmental exposure to MPs in general, since having an awareness of which types of polymers, their shapes and sizes that occur in the environment, plays a critical role towards influencing which types of MPs should be generated to form a MP reference material repository. The group discussed needing reference materials to help in method development and to assess recovery efficiencies. They also discussed creating categories for the reference materials, such as one for human health, analytical method development, environmental fate and transport, etc. With this, they noted that initially they should just start with a basic reference material. The group then moved on to discuss shape. They stated that while fibers are important and the most abundant, they are difficult to work with. The group questioned how uniform a reference material needs to be and suggested that fundamentally it all comes down to the analytical method detection limit with respect to particle size. The group also noted that they want irregular shapes and not spheres. They discussed cryo-milling larger spheres and buying larger microbeads. Someone also mentioned that if it cannot be made in the lab then it cannot be a reference material. The group also discussed the relevance of using virgin fragments as reference materials, particularly if such a material would be characteristic of environmentally relevant MPs. Someone noted that one category of exposure for a fragmented virgin material may be via food packaging, which would essentially be a non-weathered particle. Consequently, having MP reference materials characteristic of food packaging may represent a unique category within a repository of reference materials. Finally, the group mentioned the need for heterogeneous mixtures of three to four polymer types, where you can do your own weathering based on best practice guidance. They also discussed creating two or three size classes as an initial starting point.

XII. Breakout Group 2 – Weathering and Ageing, Methods/Best Practices

Chair: Jennifer Lynch *(NIST)* Rapporteur: John Davis *(Consultant)*

The feedback from breakout group 2, began with a summary of the discussion from the first breakout group. The members discussed the importance of starting simple by looking at what scientists are producing and identifying the polymers and data that are most routinely detected in the environment, and to select polymer grades with the least number of confounding factors. Points that were generally consistent with the discussion from the first breakout group. They also summarized the importance of testing mixtures and that it would be beneficial to provide at least one reference MP mixture to start, and to add more with time. Additionally, the group mentioned it would be helpful to create a catalogue of the reference material parameters, as there are projects currently producing reference materials that are not standardized or well characterized with respect to their physical and chemical properties. The group also referenced the important need to have access to fiber as a reference material and ensuring that the fibers should also be thoroughly characterized. Overall, the topic of weathering and aging of MPs contains a multitude of questions and challenges to consider, consequently the consensus is a suggestion to start with things that can be done properly now, then introduce additional complexity as newer methods are standardized. The group mentioned that there should be more discussion on the generation of the particles because the transformation process that the particles experience can itself alter the properties of the generated particles from that of the original starting material – would this process be representative of how MPs are generated under environmentally relevant conditions? It was also emphasized that everything produced must be

generated following a standardized approach and must be appropriately characterized, which could limit the number of organizations capable of producing, storing and transporting MPs following a transparent and auditable system, important towards ensuring consistency.

Additionally, efforts currently being undertaken by other groups, such as ASTM and ISO, should be considered in the context of this activity, as there was an understanding that they are already creating guidance for producing reference materials. It would therefore be prudent to ensure alignment before starting something new – avoid duplicating effort, while at the same time ensure activities optimize complementarity. The group then went over the methods for weathering plastics which included Q-UV, an accelerated weather chamber; potassium hydroxide for chemical oxidation; outdoor or "real-world;" mesocosms; or UV exposure. They also mentioned relevant conditions to consider such as temperature, UV (model sun or actual sun), humidity, mechanical, biological (bacteria, enzymatic activity, etc.), aeration, ozone, pH, and salinity.

The group then discussed the need and steps to create best practices for generating weathered and aged MPs. Considerations include providing pre-weathered materials in the form of a reference material or alternatively to provide the raw materials for labs to weather themselves following best practice guidance. It was agreed that there is likely a need for both scenarios, as some research questions may wish to consider specific sets of questions to control for as part of their experiments (e.g., differences in weathering and aging associated with different environmental conditions, such as rainwater versus ocean water versus river water). Consequently, having a 'standard' weathered reference material to address all questions may prove to be impracticable and would thus reduce access to particles that are 'fit-for-purpose.' Another consideration related to a need to better understand how a particular plastic polymer might be normally weathered under various environmentally relevant conditions. Attempting to develop a comprehensive guide that addresses all conditions is perceived as representing a potentially unnecessary time and resource-intensive activity. Providing flexibility in form of best practices, may thus be more impactful. There should thus be guidelines to address different types of weathering processes, so that researchers follow a consistent standard when generating their own weathered materials. For example, if something is weathered by UV it should be done following a standard or technical guidance document. A number of additional shortcomings when considering generating weathered and aged MPs within a reference material repository were also summarized. These include the challenge of ensuring weathered and aged materials stored within a repository remain stable during storage and transport, which could be difficult to establish, since there may be a potential for the particles to continue the aging process while in storage. Additionally, the implication of attempting to include a biofilm as part of a weathered MP reference material would be represent a substantive challenge. It would likely be extremely difficult and problematic to create a stable biofilm on a reference material that would sufficiently satisfy all users with one or even a few biofilms. Alternatively, there may be various forms of guidance that could be provided in the context of generating a biofilm on MPs, such as artificially weathering the MPs in the environment of interest and then to allow a biofilm to naturally form and to provide technical guidance aimed at helping researchers to appropriately characterize the biofilm that might form under different conditions.

Developing and applying methods that could potentially measuring the age of plastic found in the environment was also considered as a discussion point. One suggestion was to consider standardizing methods for characterizing and quantifying aging, such as measuring the Carbonyl Index when considering the aging of polyolefins, such as polyethylene, but this could represent an activity that could be developed later and be included as part of best practice guidance used towards methods used for characterizing the degree of weathering plastic and MP may have been subjected to while in the environment. It was further suggested that the polymer kit, mentioned by the previous group, should be supplied with virgin MP reference materials, with the possibility of including a reference weathered material, which might represent a relatively short-term approach to allow groups to compare between both versions of the reference material. One suggestion was to create points on a curve that would go from pristine to weathered to use such a curve as a method for quantifying the level of weathering and aging that the material has undergone. Points used to populate a curve

could include the carbonyl index, with both the original and weathered materials compared accordingly, or crystallinity and glass transition temperature (Tg), surface topology, and surface area, which may represent other properties that may change as a result as weathering and aging processes. The type of chemical index that could be used to identify how old plastic and/or MP obtained from the environment might be, was also discussed. For instance, the group considered if the rate of loss of specific chemical additives might prove helpful, or to measure the oxidation potential of the particles. It was also suggested that there may be utility in measuring the surface charge/energy of larger plastic debris, which can provide output for the top ten micrometres (hydro affinity specifically). Additionally, the presence of endotoxins for toxicological testing was discussed. It was mentioned that there are efforts in the European Union (Operation Clean Sweep) to see if these efforts are effective and, which have also helped to reduce the extent of pre-production pellets being released into the environment. Additional information needed includes the effect that ageing has on the toxicological properties of MPs. For example, if the material becomes 'safer' over time because of weathering or if it becomes more hazardous. Examples of top applications were discussed and include the need to test weathered and aged MPs in both in-vitro and in-vivo testing, PhD toxicology mesocosms, analytical uses, recovery experiments, and metal development. The amount of materials that would be required for these applications ranged from 50 mg to around 20 kg. In summary, the group concluded that best practices are needed for characterization of weathering, how to weather something, biofilm preparation, accelerated weathering chambers, chemical weathering, real-world materials, outdoor weathering, leaching of materials, and determining environmentally relevant preparations of samples.

The main takeaways from this group include that there is a need to develop both pre-weathered materials and guidance on how to best weather materials in the lab. Additionally, the need for more research in general, starting with research that addresses relatively simple questions and then moving on to more complex ones, as well as academic lab requirements. Two big gaps are timestamping and the lack of methods on non-UV ageing. Furthermore, there is a need to determine which properties of plastics as a result of weathering and aging are of greatest concern. The field could benefit from one material that is weathered with a lot of characterization. Additionally, not focusing on reinventing the wheel but looking at what organizations are doing and vet them for purposes needed here.

XIII. Plenary Feedback

Breakout 1: Particle Generation Methods

Some feedback mentioned was that there should be a central repository. There should be a few initial target references and then one standard mixture of equal parts. For instance, begin with establishing MP reference materials for polymers of high environmental relevance and of discrete homogenous sizes (e.g. <10 µm), but to also create mixture of MPs with different polymers and sizes identified as potentially being of environmental relevance. Additionally, the materials depend on what will be done with them. For example, if it is basic effect testing then access to lower particle sizes (e.g. <10 µm) quality or a wider distribution of polymers and particle sizes and shapes could be used. It was also noted that fibers represent an important category of MPs that needs to be included in any MP reference material repository. The challenges associated with generating and characterizing fibers, however, should not be underestimated. Consequently, priority should be given towards supporting the appropriate level of research to help make progress on the generation of fibers as part of a MP reference material library. Having materials heterogeneous in some way (e.g., size, shape, etc.) would also help to ensure that the reference materials are relevant and immediately impactful.

Breakout 2: Weathering and Ageing

A question was raised about if weathered reference materials are necessary, and the answer depends on the research question and the polymer. The group decided that having simple reference materials would be helpful. Both groups named three weathering methods which were lab weathered, naturally weathered and controlled

outdoor weathering. Types of weathering methods mentioned were UV, water/humidity, chemical weathering, and mechanical weathering, with second tier options being pH, salinity, ozone, and biological activity. The big question the groups discussed were if pre-weathered material was necessary and the group decided that it is needed, but the methods on how to weather will also be important to take into consideration. They also decided that any "kits" created would include pristine, unweathered, and weathered materials. Additionally, creating best practices and standard operating procedures would be helpful. Both groups agreed that we should look to the standards organizations like ISO and ASTM to identify progress in this area. Other considerations mentioned include characterization of weathering, how to weather something, biofilm preparation, accelerated weathering chambers, chemical weathering, real-world materials, outdoor weathering, leaching of materials, and determining environmentally relevant preparations of weathered samples.

DAY TWO

XIV. Plenary and Introduction for Day 2

Dr. Todd Gouin provided an overview of the Day 1 discussions. He discussed particle generation and the need to generate particles representative of both discrete sizes, shapes, and polymer composition as well as a standard heterogenous mixture. He noted there is also a need to review the methods used for generating particles, with an emphasis on identifying strengths and weaknesses. The workshop goal is to identify logical and practical opportunities as to where and how to initiates these activities. He added there is a need to review methods used for weathering plastic. Dr. Gouin suggested to start with a simple weathered material and focus on determining best practices for groups to perform their own weathering experiments. He described the challenges when considering what researchers might do with weathered materials once they are available. In the effects group, he described the discussion will be about dosimetry *in-Vitro* and *in-vivo*. In terms of analytical methods, the use of reference materials to support method development will be discussed. Additionally, it was noted that problem formulation is important in the matrix of effects testing. The overall goal is to align all stakeholders in a way to effectively address risk assessment.

XV. Leveraging Environmental Testing Methods for Nanomaterials to Evaluate Microplastics

Elijah Petersen *(NIST)*

Dr. Elijah Petersen began by reviewing the need to systematically evaluate methods since there could be biases that could occur at every step of the process. He listed examples of potential artifacts that could occur during microplastic toxicity testing. For instance, they can occur during procurement of NPs, storage, dispersion, measurement of toxic endpoints, and characterization in tissues. Artifacts can lead to confounding results or inaccurate dosing. He then reviewed the OECD guidance document 317. Dr. Petersen described the sections in the guidance document: introduction, scope, background, analytical and measurement techniques, test dispersion preparation, conduct of the test, and data analysis and reporting. He highlighted key topics covered in OECD 317. He suggested three topics for further refinement in future versions of the guidance document. One, whether a single test media can be proposed for a specific test method to improve interlaboratory agreement of test results. Two, whether advances in analytical methods should lead to recommendation of alternative exposure metrics instead of, or in addition to, the mass concentration. Three, whether settled particles should be included in the exposure and dosimetry. Dr. Petersen recognized the advances of having microplastic test materials for effects testing and listed the ideal characteristics to complete this testing.

Discussion:

The group discussed that there are probably lessons to be learned from the study of surfactant in terms of test systems (e.g., testing in labs vs river water as well as the issue of dispersion and stability of test chemicals. Lesson learned with surfactant testing will likely inform the laboratory testing of microplastics.

XVI. Learning from the Development and Application of a Human Health NMP Toxicity Screening Assessment Tool for Quality Assurance and Quality Control (QA/QC) Criteria

Rob Ellis-Hutchings *(Dow)*

As an overview, Dr. Rob Ellis-Hutching discussed a screening tool that has been developed. He noted that a lot has been done for human health assessments of microplastic in Europe and Canada but there is a need for more quality data that could be used by all scientists and regulators. This includes needing data on relevant exposures, targeted tissues and thresholds, and well designed, quality-control study types. He noted that there is a need to proceed systematically through the process and described a tiered approach for screening and prioritizing effects studies but noted that it is a general approach and something more specific to microplastics is required. Dr. Ellis-Hutchings stated the presentation will focus on the development and application of a Tier 1 Human Health Screening Approach. He described Klimisch reliability code, ToxRTool (which came from Klimisch), and GUIDEnano (adapted the ToxRTool for nanomaterials), noting that for MPs and human health, there is minimal quality data available. For this reason, there was an attempt to develop a Tier 1 evaluation tool to screen in-vivo mammalian and in-vitro NMP hazard studies for use in human health risk assessments. The Nano/Microplastic Particle Toxicity Study Assessment Tool (NMP-TSAT) was applied to evaluate the reliability and relevance of studies based on how well data reporting and handling on particle characterization, applicability for risk assessment, and their experimental design were addressed. As an additional screen, only studies that addressed a minimal number of criteria would be prioritized for consideration within the context of human health risk assessment. In this presentation, Dr. Rob Ellis Hutching summarized the evaluation of *in vivo* oral effect studies, noting that most studies did not meet the minimum criteria and according to the scoring system of the NMP-TSAT tool there were no high-quality studies (medium at best) currently available. For those studies that did meet the minimum number of criteria, these were further subjected to an expert Tier 2 evaluation, whereby the positives and negatives of each of the studies could be more thoroughly considered.

Discussion:

A question was asked if all of these criteria should be of equal criticality. Dr. Gouin noted he agrees about all of the criteria being of equal relevance. However, the purpose of the evaluation was to screen studies for use in the context of potential human health risks. Consequently, certain criteria are likely more critical to the evaluation purpose, such as those related to providing a dose-response curve. Thus, the purpose of identifying a minimum set of criteria is to identify studies that are likely to be 'fit-for-purpose,' with a more thorough evaluation of such studies being conducted by study-area experts. This represents a reasonable approach towards handling a screening and prioritization tiered evaluation. Consequently, studies that were prioritized for Tier 2 should be appropriate, which was agreed within the SCCWRP workshop where the evaluation presented was performed. One individual stated critical pieces are necessary to identify to have studies move forward and you still have to determine where you will set the bar in regard to low-quality studies that passed the critical components. Dr. Gouin stated when we took this to experts and they looked in more detail, they found a large number of technical aspects that were of concern. There were significant failings that were not being considered as part of the screening tool, which given the level of technical detail required to judge these aspects of the studies would be inappropriate to include as part of Tier 1 screening tool. Consequently, a tiered approach represents a more efficient use of resources. A question was asked about the progress or evaluation accuracy of dosing for in-vitro, what actually reaches the cell membrane in vitro, and how that information is further evaluated. Dr. Gouin noted that while in vitro studies were evaluated using the ToxRTool, none of the available studies are deemed appropriate for use in evaluating human health risks. This due to various concerns regarding the in vitro studies, such as in relation to challenges associated with dosing, but also due to a lack of availability of in vitro-to-in vivo extrapolation methods for MPs. The group discussed efforts to go out and engage with the regulators and try to get them to adopt these ideas, and the communication, engagement, and buy-in aspects.

Executive Summary of Environmental Measurements:
Both breakout groups discussed the goals they aim to accomplish which include effectively understanding an exposure, discussing long-term monitoring, having environmental and human health relevance as applicable, and having higher quality data acquired following standardized analytical methods. The groups also discussed using different matrices and how to generate a realistic matrix. Improvements in fiber detection were also mentioned. As a roadmap forward, the groups discussed having surveys, discussing what they can do, want to do, and long-term goals as well as possibly creating a working group from this group of experts.

Executive Summary of Effects Testing:
The effect testing breakout groups focused on the steps in relation to the particles and reference materials. The group discussed the creation of data sheets for material characterization and labels, which might be more long-term. In the short-term using research grade materials with guidance from the European Centre for Ecotoxicology and Toxicology of Chemicals. The groups also discussed OECD 36 characteristics for nanomaterials. There was discussion of characterizing materials to test methods and compare outcomes with the manufacturer's information. The groups also discussed controls – positive and negative experimental, vehicle, and particle.

XVII. Breakout Group 1 – Environmental Measurements

Chair: Jeanne Hankett *(BASF)*

The first breakout group on Day 2 was related to the potential use of MP reference materials to support the analysis of environmental samples. Responses included the use of materials to support quality assurance/quality control practices, the development of analytical methods to support long-term monitoring needed to evaluate responses to societal or production changes of plastic, exposure, fluxes of pathways, disruption in biomass, discovering hotspots, characterizing and quantifying human and environmental exposure, understanding the relevance of test systems, quantification of use and release patterns, including the generation of quantitative emission factors, material flow analysis, fate and transport, remediation, and mitigation effects. The major goal discussed is towards strengthening the quality, reliability and relevance of data generated from environmental monitoring studies. To achieve this goal the group discussed the need for accuracy, precision, ease of use, access to reasonably priced materials, harmonization methodologies, and to define best practices towards the generation of comparable data. The group then discussed what is needed from reference materials. It was stated that they will not standardize the sample, as it was generally perceived that it is highly unlikely there will be a standard method for microplastics applicable to all environmental matrices. Nevertheless, there is a need to access materials with known and well-characterized sizes, shapes, and which are known to be stable, for use as recovery standards. Additionally, reference materials must be appropriate for analytical use, have intra-lab and inter-lab consistency, and quality of analysis should be monitored over time. Considering their potential use as recovery standards, there is a need for guidance on how to best add the materials to sample matrices, with a need for the reference materials to consist of both heterogeneous and homogeneous types of particles with respect to size, shape and polymeric composition. Having reference materials of various polymeric composition would also prove beneficial, as these would help support the calibration of analytical instruments and methods. Factors that would be important to consider for reference materials include their environmental relevance, access to smaller size particles, varying color was identified as also being potentially important to consider. With the availability of MP reference materials, there was a recognition that the ability to accelerate advances in analytical method development should occur. It was noted that the Polymer Kits were likely being largely used by start-up labs towards helping them in their own method development activities, but it would be assumed more established labs would want to ensure that their data sufficiently addresses all QA/QC criteria. The question was raised as to whether guidance should be established for more established labs to use reference materials, and it was noted that they cannot force everyone to use the reference materials. The group also discussed the role of

MP reference materials towards supporting the accreditation of labs who wish to specialize in the analysis of microplastic, and it was noted that NIST does have an established system that anyone using SRMs can access, and which support accreditation activities. Finally, intended output was discussed which mentioned accreditation, protocols, improved laboratory methods, controlled databases, and a community of practice. The take-home messages were that there is a clear need for reference materials and there will not be a one-size-fits-all. These reference materials also must come from a place of biological and environmental relevance, and therefore need to address a variety of particle size, shape and polymer composition classes. They discussed some limitations which include resource availability, time, money, and scope. They also discussed creating working groups from this group of experts and creating a roadmap of what they can do, want to do, and long-term goals.

XVIII. Breakout Group 2 – Effects Testing

Chair: Stephanie Wright *(Imperial College London)* Rapporteur: Rob Ellis-Hutchings *(Dow)*

Stephanie Wright started the discussion and noted that the discussion today would be about sample preparation in the context of effects testing and the steps to take to prepare a sample, introduce the MP reference materials into the test systems (whether is ecotoxicology, sediment, in-vitro cells, etc.), and the various considerations that need to be made. There was heavy reference to OECD guidance No. 36 in this preface.

The group started by discussing how it is important to start with the characterization of the test material and what needs to be done by the manufacturer versus the lab site. Aspects that should be characterized include storage conditions, other items from the manufacturer, what the material is, where it was made or collected, class type, appropriate metadata for the sample, weathering information, and essentially all the readily available information. Additionally, polymer size, size distribution, type, shape, known hazards, solubility, surface chemistry, sample preparation, dosimetry, and dose characterization. Some challenges mentioned include the fact that these materials are often hydrophobic, therefore acquiring the required measurements can be difficult. Surface charge, size and shape distribution will require standardized and centralized activities, and which could be provided by a central group, such as NIST. Additional considerations mentioned were if the manufacturer should report if any contaminants might be present, therefore which contaminants should be characterized and what levels of contamination are acceptable/unacceptable? The group mentioned that there are two categories: commercially prepared and self-generated particles. Ideas of methods were mentioned including sonification and electric, which has issues with probe contamination. In regard to guidelines, it was mentioned that OECD guidelines for plastics could be wrong and nanomaterial guidelines might not be applicable to larger particles, so how to use these guidelines in this context needs to be reviewed. Additional guideline thoughts include built in aseptic conditions and good handling practices as a minimum guideline to prevent contamination and not using plastic materials at any step of testing.

Once characterization has been completed the next question is what to test in the lab. Density, polymer constitution, surface charge, additives, surface area, volume, aspect ratio, and what it is suspended in were all mentioned. It was also noted that introducing surfactants to extract particles makes a new artifact that can cause its own health effects, so delivering the material in multiple formats could be helpful to test the differences (and then characterize the mediums of delivery). The material also needs to be characterized as it is administered and after being in the system for a period of time and these tests must be fast, cheap, and simple. It is also important to use positive and negative controls and testing the full extract of the additive to characterize any potential adverse effects due to the additive relative to the particle itself. It is also important to have hazard identification and then hazard characterization to see if there is an environmentally or biologically relevant amount of effects. The group discussed biomolecular coating characterization as well and for biofilms, they decided environmental and ecological was more important than animal and cell.

Dosing and dosimetry were also discussed and the need to determine the dose that is actually reaching the cells/target of the effects. They noted that if the material is agglomerating it may have less exposure potential.

Other concerns relating to that include having some knowledge pertaining to the physiological and biological traits of the test species (e.g., in fish plastic could clog gills), characterization and quantification of the internal dose as opposed to relying solely on external dose concentrations, since the approach frequently misses what is internalized in tissue. Additionally, exposure pathways via food, water, and air are identified as being the most relevant for purposes of assessing risks, although the use of other pathways, such as through an injection may support mechanistic understanding. The exposure dosimetry should thus be fit-for-purpose. There should also be a recommended minimum number of particle counts and cell counts for standards. Concerns relating to characterization include a need to focus on the most important and feasible aspects, since there are so many aspects that could be characterized and therefore, measurements performed should be fit-for-purpose. Additionally, there are many different testing methods that could be used with their own strengths and weaknesses, including Raman/FTIR, TEM, DLS, SNM filters and ML with microscopy and multiple methods might be needed to characterize a single parameter.

The group then went over a list of recommendations from OCED for Particle Characterizations to discuss their relevancy to MPs. Particle size, shape and mean distribution, chemical description (including composition and ID), surface chemistry, surface charge, crystallinity, porosity (pore density), and polymer ID were identified as representing the potential minimum requirements. Aggregation and agglomeration, specific surface area, interfacial tension, dustiness, crystallite size, electron microscopy, radical formation potential (reactivity), and pour density fluidity (if suspended material) were also identified as being potentially relevant. Octanol-water partitioning coefficient was removed from consideration, and photocatalytic activity was found only to be relevant in a few cases and in terms of prioritization should be removed. Solvent compatibility, density, volume, polymer ID, weathering status, additives, impurities, solvent, and endotoxins were also included as other key properties requiring some level of characterization.

The group also discussed appropriate labelling of microplastics, which they decided depends on the intended use (are they being tested on the surface or internally). For manufactured MPs the manufacturer, technical specifications, and intended and foreseeable uses (e.g., instrument calibration or other use) should be reported. For natural materials, at minimum it should have the source or origin. Issues with storage were also discussed. MSDS labels should have storage, shelf life, incompatibilities, and disposal methods. Those with a shelf life of one year need to be recharacterized if used beyond that. The manufacturer would need to specify the storage conditions and it was noted that storing in liquid nitrogen could damage some materials and they should also be kept in the dark. Additional considerations include possible microbial growth, opening materials at room temperature would make them nonsterile, and if something needs to be cleaned how should that be done. Some members mentioned they have done effect studies in the lab recently and oral or inhalation exposure studies are currently being conducted by Dow.

XIX. Breakout Group 1 – Environmental Measurements

Chair: Jeanne Hankett *(BASF)*

Jeanne Hankett summarized the discussions. The group discussed the primary goals, which include having environmental relevance, human health relevance, increased quality, accurate percent recoveries for mixtures, inclusion of human exposures such as diet and air, having best practices, and performance standards. The group also discussed the issue of homogeneity within a sample matrix when spiking with MPs for the purposes of method development and/or evaluation of recovery efficiency, and a need to clarify the appropriate level (sample-wise) where homogeneity reached. They also identified a need to consider developing and providing a matrix-based standard that would likely prove beneficial towards providing guidance for labs that generate their own matrices (and matrix standards within that). Additional goals mentioned include teaching AI algorithms including matrix learning, having a standard to be able to bin shapes so that there is consistency across researchers, and labelling of particles. The group also discussed the most important shapes which included fibers, spheres, fragments, and films. Furthermore, they discussed differences between establishing a test material versus reference material, noting that reference materials are generally much more expensive, which may impede their uptake by individual research groups.

XX. Breakout Group 2 – Effects Testing

Chair: Stephanie Wright *(Imperial College London)* Rapporteur: Rob Ellis-Hutchings *(Dow)*

The breakout group focused on discussing sample prep and characterization, assuming there are already reference materials. The group raised the question of whether materials need to be characterized again if already done by the manufacturer, which the group concluded that it is beneficial to characterize again to make sure the methods being used work and the machines are calibrated. Additionally, manufacturer's measurements may lack reliability instruments used for measurements and can often differ increasing the uncertainty in the characterization. It was also noted that most methods are for spherical materials and there needs to be other methods available. For example, DLS only works on nanoplastics as microplastics might be too large. Some questions were then raised, including if an entity like NIST would create standards to characterize materials, solutions, and systems. Additionally, the group discussed what the expectation for sample analysis as part of the matrix is. It was noted that if reference material is sent by a group like NIST, then there is an expectation that it would be reliable – but assuming NIST or other groups could produce scientific research material (SRM) in a short time frame, however, is unlikely a reasonable path forward. Furthermore, it was stated that materials should not have to be re-tested upon being received, only re-characterized. It should also be assumed that the reference material be suitable for application to toxicity, weathering, and cellular uptake and elimination experiments. The group also discussed what the reference material would be used for and came to the conclusion that they could be used for positive controls and calibrations, but also as an accreditation scheme to see if labs are operating properly. It was also mentioned that the reference material should have a centralized source to reduce any variables influencing results.

The group then discussed proper lab techniques and stated that the media depends on the final needs to see if it is possible. They gave an example using a powder put in phosphate buffer saline (PBS) at four times the desired solution. The issue is that if you dilute this, then it can be difficult to determine if there is a uniform and homogeneous dispersion of the particles. Standard practice is that there also needs to be a control in addition to the test particle, which can simply be the delivery vehicle. They noted that whatever the solution is, it needs to have similar viscosity as the material to produce a uniform dispersion. The question of whether there needs to be a reference material in an aqueous solution was brought up, which has been a request of regulatory agencies such as California State Water Resources Board (SWRCB). It was noted that for analytical reference standards, a stable, evenly dispersed reference standard would be beneficial to a wide audience.

Methods were then discussed, which included sonication in water, baths, and probes. It was noted that sonication of plastics will almost always produce nanoplastics and that if the material is already dispersed out, there does not need to be sonication. The fundamental point was made that modifying a substance to make it suspend properly in a solution is not the goal, as that would fundamentally change the substance. There should be testing of the individual product to characterize any associated effects, then test each part to see what they do. Some guidelines from OECD include shelf-life, how long materials take to settle out of a dispersion, and it was noted that the stability of the particles in chronic studies are important to consider, particularly with respect to the duration of the experiment and how this might impact the dispersion and therefore the exposure dose, which may change during the experiment. Furthermore, it was mentioned that the stability of dry powders can be more effectively controlled, at least short term. There is also a need for guidance on how to best suspend and/or dose powders otherwise there is a high likelihood for significant variability associated with the exposure dose. The grades of material were also discussed (RGTM (Research Grade Test Material) being the lowest and the SI-traceable SRM (Standard Reference Material) being the highest). Additionally, the method used to confirm concentrations in toxicity testing was questioned. There is some testing on microbes in sediment, but it is not possible to get the polystyrene out of the sediment. It was also noted that confirming the nominal concentration is key.

Positive and negative controls were also discussed. Part of the issue is the vehicle and the form, so a known toxic form of the same chemical could be a positive control. For example, a particle control could be a 1-micron micro cellulose particle. It was so noted that natural fibers and glass fibers do not have the same properties as plastic fibers. Additionally, particle controls are less relevant for sediment and soil studies. One idea mentioned was that if you can get a level that most polymers are toxic at, then you can use that as a comparison and if a material is more damaging than that group then you know it is not due to overwhelming the system with any particle, it is that there is a method of analysis involved.

XXI. Plenary Feedback

Environmental Measurements

Both groups discussed major goals and aims for environmental research projects and discussed what they hope to get from the projects. Important goals mentioned were to increase our overall understanding of exposure through the acquisition of high-quality data, and the importance of conducting relevant and reliable fate and transport studies, and thereby better understanding the life cycle of MPs. With specific guidance to the use of MP reference materials in the lab, the overall goal is to utilize well-characterized reference materials to strengthen the development of analytical methods and help to reduce and/or better quantify uncertainties that may occur due to human or system errors through the establishment of best practices. The groups also discussed improving applicability and detailed relatability, which they mentioned could be done by reducing uncertainty, generating relatable bins, identifying specific applications, and the matrix impact. Reproducibility and best practices were discussed, including the challenges when considering different environmental sample matrices, and how to generate a realistic matrix. Finally, a need to develop materials that would support improvements towards strengthening the analysis of fibers. A potential roadmap forward was identified discussing the use of surveys to clarify and prioritize next steps based on an improved understanding of the needs of researchers, and to utilize internal information from industry trade associations and manufacturers to identify the most market relevant materials. Finally, it was noted that labs are going to need guidance which will be helpful for analytical purposes, choice of reference materials, and what types of QA/QC will be necessary.

Effects Testing

The effects testing breakout groups focused on the steps in relation to the particles and reference materials, not the test systems themselves. Furthermore, only a standard or reference material generated for effects testing was discussed, not the testing of commercial products. The discussion focused on identifying the data necessary for characterizing and labelling reference materials (information which may possibly be generated and/or distributed by NIST), which includes shelf life, expiration date, storage conditions, and handling and guidance (alternatively this level of detail may come from groups, such as OECD or ISO). It was noted that the characterization of reference materials represents a time intensive activity and could thus take several years before the necessary data are generated, consequently, research grade material with appropriate guidance may prove impactful in the short-term. The groups also reviewed OECD 36, which provides guidance with respect to nanomaterials, and acknowledged that there were aspects relevant to MPs, but some aspects may also not be relevant. Additional work may be required to clarify fit-for-purpose aspects. The groups also discussed doing baseline characterization of the MP reference materials when they are received by research groups as an approach that should be advocated, as the information obtained would be useful to test the efficacy of the methods employed and to enable the opportunities to appropriately compare results with those provided by an individual manufacturer, for instance. With respect to the role of controls – positive and negative experimental, vehicle, and particle controls were all considered, and the strengths and weaknesses considered, accordingly. Additionally, regarding bio corona, several challenges were considered, although it was recognized that a corona forming on the particle could potentially play an important role towards influencing test results, consequently, the ability to characterize the presence of a corona is suggested as representing an important piece of information to obtain.

Final Wrap Up:

Having a roadmap to move forward is a good idea so that the appropriate connections needed to get the particle creation started can be done, such as in collaboration with NIST, JRC, etc. For next steps, various outreach activities will be planned over the next several months in an attempt to further engage all potential stakeholders, such as through individual one-on-one meetings or through the use of webinars or other workshops. In the short-term, a summary of the reference material workshop will be presented at the SETAC North America meeting in Pittsburgh, PA, November 2022. Material included in this workshop report will also be used as a basis for a manuscript that will further summarize and communicate the importance of advancing a MP reference material repository, and which is tentatively planned for submission end of 2022. Additional suggestions for outreach and communication include establishing a community of practice, with those who attended the workshop representing the initial group of members, with opportunities for expanding the community to those who could not attend but are still interested in ongoing activities to be readily added.

APPENDIX A: LIST OF PARTICIPANTS

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Camilla Carteny, *Plastics Europe*
Emily Clark, *Eastman Chemical Company*
Richard Czarnecki, *Micro Powders*
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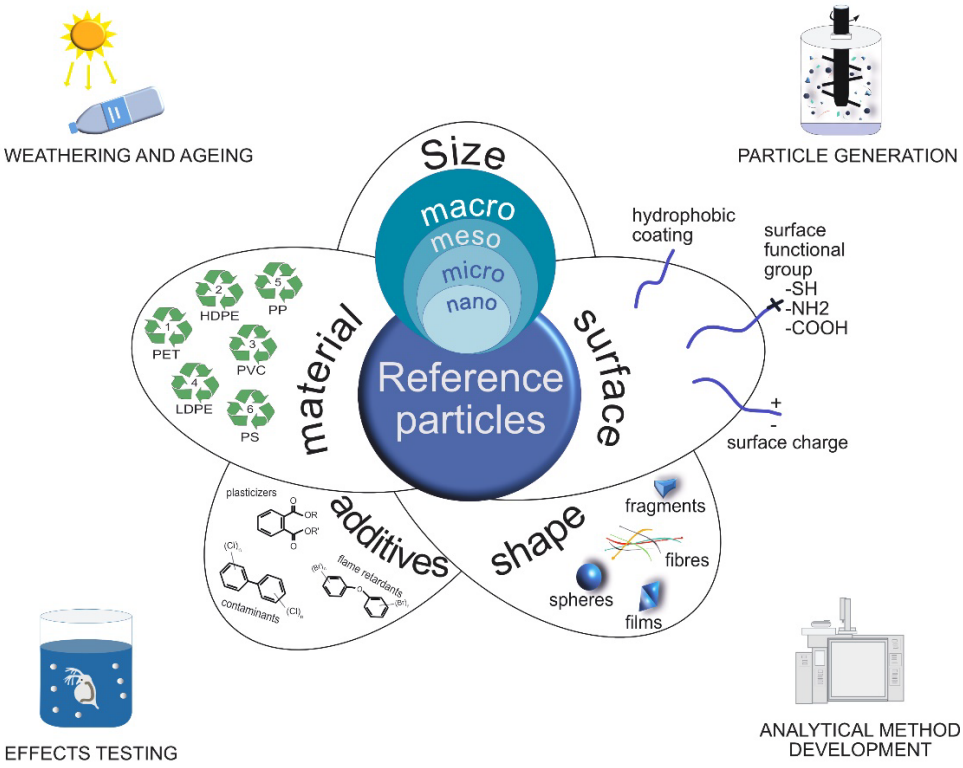
APPENDIX B: WORKSHOP PROGRAMME

Microplastic Reference Materials
– Invited Expert Workshop –



25-26 May 2022
The American Hotel, Atlanta, Georgia

BACKGROUND

Microplastic research is at a nascent stage, with numerous studies observing a need for stronger adoption of robust quality assurance / quality control (QA/QC) practices regarding sample collection, analysis and effects testing. An improvement regarding the adoption of QA/QC practices represents a critically important component to microplastic research. The ability to demonstrate good QA/QC is needed to help demonstrate the reliability and relevance of data, which further supports comparability across studies, and which can be used within a risk assessment context. It is generally understood that an important element of QA/QC protocol relates to a demonstrated understanding of the characteristics of the stressor under investigation. The development and application of sampling and analytical methods, for instance, relies on the use of analytical standards, which are used to quantify the efficacy of the sampling and analytical method, such as in the reporting of recovery efficiencies or in the use of quantifying calibration curves. In the instance of effects testing, standards that report and/or provide certification of the properties of the stressor under investigation are critical to ensure that any observed effects are entirely due to the stressor itself, and not influenced by a potential contaminant that may be associated with the test material, as well as for use towards better mechanistic understanding that might relate the properties of the stressor to various toxicological endpoints. At present, there are no readily available standardized MP materials or methods. Consequently, it is difficult to ascertain the environmental and human health risks from existing studies because the data may be of varying quality and reproducibility. Variance in the relative quality of data and its reproducibility is influenced by a variety of factors, with limitations related to the lack of availability to standard reference materials representing an important barrier towards strengthening the quality of microplastic research.

To address these challenges, ACC and its member companies are exploring opportunities to support the generation of a suite of environmentally relevant standard reference MP materials that could be used to support effects testing as well as for use to support the validation of sampling, preparation, and analytical protocols. These MP reference materials would encompass different resins, morphologies, and sizes to represent in some degree the particle variability present in the environment. Standard materials would serve a variety of needs but would be particularly valuable in supporting the adopting of good QA/QC practices for both environmental monitoring and effects testing, thus helping to strengthen the quality and reliability of data to support risk-based decisions.

AIM

There are two main objectives of bringing together a multi-stakeholder group of experts to discuss the development of MP reference materials.

1. How to generate the reference materials, including both monodisperse and polydisperse particles of environmental relevance.
    a. Stakeholders will be asked to consider varying approaches that could be used to generate particles of varying size and shape, with an emphasis on polymers typically detected in the environment, as well as methods that might be employed to enable access to MPs that have undergone weathering and aging.
2. How to provide guidance related to the use of reference materials to support either the acquisition of environmental monitoring data or in effects studies.

The structure of the two-day in-person workshop will include both plenary and break-out group discussions. Primary output from the workshop will be in the form of guidance and/or best practices, complemented by the development of a framework strategy for generating a reference MP repository, based on input received from expert participants. Due to continuing challenges related to international travel, it is understood that not all key stakeholders are able to attend the in-person workshop. Consequently, output generated from the workshop will be used as part of an outreach communications strategy, aimed at providing additional opportunities for all stakeholders to provide input to the overall process. The workshop's final report will thus serve as a "state of the science" for reference materials and provide a blueprint for advancing the assessment of adverse effects associated with exposure to MPs.

INTRODUCTION

In a number of recent publications, the continuing challenge regarding the lack of reference materials to support the hazard assessment of environmentally relevant microplastic particles (MPs) is identified as representing a critical research need in the context of supporting both the environmental and human health risk assessment. In their review related to the common use of synthesized polymeric micro-beads, Rubin et al. (1), for instance, demonstrate that differences between particle size, morphology, surface chemistry, and polymer type are sufficient to limit our overall ability to extrapolate lab-based observations of toxicity, fate and transport to real world environments. With a predominance of MP studies reporting results from ecotoxicological model systems (74%), Rozman and Kalčíková (2) report that the vast majority of studies have relied on the use of polystyrene (PS), ranging in size from 1 to 50 µm, with 63% of all studies using spheres or pellets. This is in contrast with the most commonly found plastic polymers, which are typically dominated by polyethylene (PE) and polypropylene (PP), followed by polyethylene terephthalate (PET) > polyvinyl chloride (PVC) > PS. Fibers, fragments and films are also routinely observed in the environment, yet the number of studies evaluating the influence of shape are limited, with 25% of all studies using fragments, 6% fibers, and 3% with films (2). Only 1% of all studies included a mix of MPs.

The observations of Rozman and Kalčíková (2) are consistent with Gouin et al. (3), who reviewed studies with relevance to human health, and observed a predominance of studies using spheres (≈60%), consisting primarily of polystyrene (≈46%). Furthermore, an observation that the source of MPs used in human health effects studies is limited to a relatively small number of suppliers, with approximately 45% of studies reporting particles obtained from five companies; BaseLine Chromtech Research Centre (China), Sigma-Aldrich (USA), Cospheric (USA), Kisker Biotech (Germany) and Microspheres-Nanospheres (USA) (3). Information obtained from the product data sheets for the particles used in the studies suggests that the particles have been produced for purposes other than for use in testing potential human health effects of environmental exposure. For instance, some particles are described by suppliers as being monodisperse for the purposes of use in immunodiagnostic assays as size standards for calibrating analytical equipment or as substrates or supports for immunologically based reactions, tests and assays. Additionally, particles may be used to support cellular biology applications, typically by providing a substrate for binding protein ligands. In some instances, particles can be obtained in powder form, but in most cases, the particles are obtained as a liquid suspension. BaseLine Chromtech Research Centre, for example, supply their polystyrene particles in a 1:1 ethanol/water solution, with other suppliers describing the particles as being dispersed in an aqueous solution of undefined specifications. Although data obtained from the product data sheets might be used to evaluate the particle size distribution for the particles, details related to the purity of the particles themselves are less well understood. Of particular concern is the lack of information reported on the levels of unreacted monomer or impurities that may be present in the polymer, whereby residual levels of styrene in polystyrene may represent a potential chemical contaminant that may influence toxicity test results (4). The extent of surfactants, antimicrobials and/or dispersants present in the product, or which may have been used during their manufacture, as well as the production date and/or batch number of the particles, is also typically unknown. The observation that some particles are supplied in an ethanol/water solution is illustrative of a scenario whereby the particle product matrix or residual chemicals, such as ethanol, surfactants and/or dispersants may potentially confound interpretation of

observed adverse effects (5-7). Nevertheless, in their evaluation of seven quality assurance and quality control (QA/QC) criteria for particle characterization, Gouin et. al (3) observed a limited number of studies reporting details related to particle surface chemistry or evaluated the potential for chemicals and/or endotoxins to potentially influence test results. Finally, while there are several examples of groups verifying the size and shape of particles, few studies analytically verify the polymer composition of the particles or provide any details regarding the generation of the particles by manufacturers, with reporting typically limited to the supplier name.

While there have been some efforts towards characterizing and quantifying the distributions of MPs in the environment with respect to size, shape and density, as well as providing guidance that can potentially help to extrapolate results between studies to characterize risk (8-11), the availability of a suite of MPs representative of environmentally relevant exposure is generally understood as a critical need for reducing associated uncertainties and strengthening our overall understanding of risk (1-3, 12).

The development and application of methods to generate MPs that could simulate weathering and aging mechanisms of plastic debris have been explored by various groups. These include methods to generate MPs in the lab using various milling approaches, laser ablation, ultrasound treatment and the use of solvents (13-22). Additionally, there have also been various approaches aimed at attempting to simulate aging in the environment, which include Fenton and persulfate oxidation, exposure to UV light, electrical discharge plasma, as well as approaches to simulate weathering in the marine environment including mechanical agitation in salt water (23-29). Generating and aging MPs that are representative of environmental exposure is understood to be important, since the weathering and aging processes can result in important changes to the melting point, glass-phase transition temperature, crystallinity, surface functional groups, surface area, density and porosity and the formation of a biofilm on the surface of particles. Changes to the physicochemical properties of the particles as a result of environmentally relevant weathering and aging processes have been shown to influence toxicity and environmental fate and transport and can also interfere with analytical verification. Consequently, methods aimed at generating a suite of reference materials would need to consider a number of factors – a key objective of the MP reference workshop.

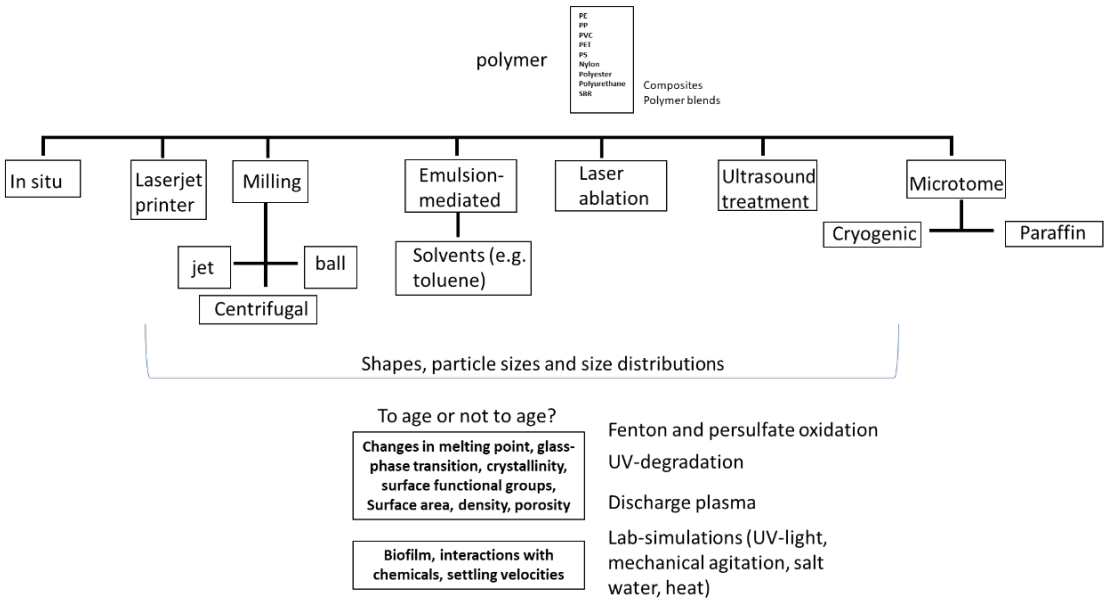


Figure 1: Summary overview of various methods that have been used to generate MPs.

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PROGRAMME: WEDNESDAY 25 MAY 2022

07:30 – 08:00	*Registration and coffee*
Main session room – Apollo A & B	
08:00 - 08:25	**Welcome and introductory remarks Organising Committee**
08:30 - 08:50	**Title: Reference materials for micro and nanoplastic Research: Efforts by NIST and HPU Center for Marine Debris Research – Jennifer Lynch (NIST)**
08:50- 09:10	**Title: Nanoplastic standards – known unknowns and new order – Samuel Stavis (NIST)**
09:10 – 09:30	**Title: To properly address the multidimensionality of microplastic in risk assessment, your reference material must also be multidimensional – Bart Koelmans (Wageningen University)**
09:30 - 09:50	**Title: Industrial Microplastic Powders; Chemistry, Manufacturing and Characterization – Richard Czarnecki (Micro Powders)**
10:00 - 10:30	*Coffee break*
10:30 - 10:50	**Title: Applying lessons in nano standard materials generation and microplastics quantitation towards future standard reference materials for hazard assessment – Jeanne Hankett (BASF)**
10:50 – 11:10	**Title: MNP Toxicology - What can we learn from that which has preceded it? – Martin Clift (University of Swansea)**
11:10 - 11:30	**Title: Degradation and Fragmentation of Plastics – Anthony Andrady (North Carolina State University)**
12:00 - 13:00	*Lunch*
13:00 – 14:30	**break-out groups**
Workgroup 1 Particle Generation – Methods/Best Practices	

Chair: **Denise Mitrano** (ETH Zurich) *Rapporteur*: **Erik Rushton** (LyondellBasell)

Workgroup 2 Weathering and Ageing – Methods/Best Practices

Chair: **Jennifer Lynch** (NIST) *Rapporteur*: **John Davis** (Consultant)

14:30 - 15:00 *Coffee break*

15:00 – 16:30 **break-out groups**

Workgroup 1 Particle Generation – Methods/Best Practices

Chair: **Denise Mitrano** (ETH Zurich) *Rapporteur*: **Erik Rushton** (LyondellBasell)

Workgroup 2 Weathering and Ageing – Methods/Best Practices

Chair: **Jennifer Lynch** (NIST) *Rapporteur*: **John Davis** (Consultant)

16:30 - 17:00 **Plenary feedback**

19:00 *Dinner (details to follow)*

PROGRAMME: THURSDAY 26 MAY 2022

07:30 – 08:00	*Registration and coffee*	
Main session room – Apollo A & B		
08:00 - 08:25	**Plenary and Introduction for Day 2**	**Organising Committee**
08:30 - 08:50	**Title: Leveraging Environmental Testing Methods for Nanomaterials to Evaluate Microplastics – Elijah Petersen (NIST)**	
08:50- 09:10	**Title: Microplastic Risk Assessment / Lessons to be Learnt from Nano – Olivia Osborne (UK Food Standards Agency)**	
09:10 – 09:30	**Title: Learnings from the development and application of a human health NMP toxicity screening assessment tool for quality assurance and quality control (QA/QC) criteria – Rob Ellis-Hutchings (Dow)**	
09:30 - 09:50	*Coffee break*	
10:00 – 12:00	**break-out groups**	
Workgroup 1 Environmental measurements		
Chair: **Jeanne Hankett** (BASF) *Rapporteur*: **tbc** ()		
Workgroup 2 Effects testing		
Chair: **Stephanie Wright** (Imperial College London)		
Rapporteur: **Rob Ellis-Hutchings** (Dow)		
12:00 - 13:00	*Lunch*	
13:00 – 15:00	**break-out groups**	
Workgroup 1 Environmental measurements		
Chair: **Jeanne Hankett** (BASF) *Rapporteur*: **tbc** ()		
Workgroup 2 Effects testing		
Chair: **Stephanie Wright** (Imperial College London)		
Rapporteur: **Rob Ellis-Hutchings** (Dow)		
15:00 - 15:30	*Coffee break*	
15:30 - 17:00	**Plenary feedback**	
17:00	**Close Workshop**	

WORKGROUPS

DAY 1

Key considerations for both Day 1 breakout groups:

- Clarify expectations.
    - What is envisioned by a suite of microplastic particle reference materials?
        - Size?
        - Shape?
        - Polymer composition?
    - Who will be responsible for generating the reference materials?
        - Central organization(s) (industry, government, academic/research center)?
        - Individual researchers supported by best practices guidance documents?
    - Should reference materials include chemical additives, monomers, chemical residuals, other contaminants?
        - If so, which ones with which types of polymers?
        - If not, why not?
    - In the context of the above, also need to consider implications related to expectations regarding how the particles will be used, and if there is a need to generate particles specific to supporting the development of sampling and analytical methods as opposed to the use of MPs to support effects testing.
- Additionally, background information contained in OECD No. 90 (Physical-chemical decision framework to inform decisions for risk assessment of manufactured nanomaterials) and OECD No. 91 (Guiding principles for measurements and reporting for nanomaterials: Physical-chemical parameters), may provide a foundation upon which to develop a potential guiding framework for MPs. Consequently, it would be appropriate to consider similarities and differences and/or strengths and weaknesses when applying approaches that have been developed for nanomaterials to MPs.

1. *GENERATION METHODS*

Chair: Denise Mitrano **Rapporteur: Erik Rushton**

Figure 1 provides a schematic illustration of the various methods that have been proposed related to the generation of MPs in the lab. In many instances, the methods proposed attempt to address a need towards generating MPs using relatively simple techniques that include the ability for high reproducibility. While some methods might be appropriate for generating MPs for a specific particle size range, such as the use of microtomy for generating a relatively narrow particle size distribution of fibers, others may produce a wider particle size distribution. Importantly, most methods typically limit their evaluation to a single or small number of polymer types, however, it may not always be necessarily clear if the method would produce similar results for a different type of polymer. Additional challenges may also require consideration, such as the generation of MPs from polymer composites and blends (29, 30).

Questions to consider within this group, therefore, relate to soliciting experts regarding the most appropriate method that might be recommended for generating MPs.

- Identify strengths and weaknesses of the various methods that the group are aware of.
    - Ease of use
    - Cost
    - Reproducibility
- Where possible, characterize the applicability domain of the methods with respect to particle size distribution, morphological considerations and relevance towards specific polymers.
- Summarize methods needed for particle characterization

First Name	Name	Role
Denise	**Mitrano**	Chair
Erik	**Rushton**	*Rapporteur*
Jeanne	**Hankett**	
Bart	**Koelmans**	
Richard	**Czarnecki**	
Samuel	**Stavis**	
Samantha	**Romanick**	
Stephanie	**Wright**	
Emily	**Clark**	
Mark	**Pemberton**	
Yash	**Parulekar**	
John	**Norman**	
Camilla	**Carteny**	

2. *AGING and WEATHERING METHODS*

Chair: Jennifer Lynch **Rapporteur: John Davis**

To enable groups to evaluate the toxicity, environmental fate and transport of environmentally relevant MPs an important consideration relates to the application of methods that could simulate aging and weathering. Questions to consider within this group include:

- Identify strengths and weaknesses of the various methods that the group are aware of.
    - Ease of use
    - Cost

    - Reproducibility
- Should the reference materials include opportunities for individual groups to perform their own in-house aging and weathering or should standard protocol be recommended for groups to follow in their own labs prior to testing MPs or both? Identify strengths and weaknesses.
- Summarize methods needed to support the characterization of particles, specific to properties related to weathering and aging
- Analytical considerations in relation to weathered and aged plastic – interferences/differences in spectroscopic detection, how serious is this concern?

First Name	Name	Role
Jennifer	**Lynch**	Chair
John	**Davis**	*Rapporteur*
Anthony	**Andrady**	
Kay	**Ho**	
Kevin	**Thomas**	
Christie	**Sayes**	
Alan	**Owens**	
Craig	**Davis**	
Gaurav	**Amarpuri**	
Phil	**Brondsema**	
Rob	**Ellis-Hutchings**	
William	**Robertson**	
John	**Kucklick**	

DAY 2

There exists a number of standard protocols for the analysis and effects testing of various pollutants, both chemical and non-chemical (e.g., engineered nanomaterials), where various criteria related to QA/QC considerations are documented. Given the potential availability of a suite of standard MP reference materials in the future, it is perceived as important to consider how they might be used to support either the analysis of environmental monitoring data or in effects test systems. In an effort to ensure that researchers are fully supported in their research activities, the ability to provide documentation that summarizes best practices related to the use of standard reference materials is seen as an important contribution to the research community.

1. *Sampling and analytical method development: Best practices*

Chair: Jeanne Hankett (BASF) **Rapporteur: John Norman**

The availability of environmentally relevant standard MP reference materials can be perceived as important towards supporting the development and application of sampling and analytical methods for use in strengthening the quality and reliability of environmental monitoring data. Standard MP reference materials, for instance, can be used to help support the quantification of the sample method recoveries, whereby reference materials are spiked into sampling media prior to extraction, clean-up and isolation. Following from Day 1 discussions aimed at providing greater clarity with respect to the properties that a standard reference MP might reflect, it would be useful to consider providing guidance regarding the use of the reference materials in monitoring studies.

- Consider the various steps during sampling and analysis of environmental samples and summarize best practices for using reference materials.

- Sample method
- Filtering and extraction steps
- Digestion methods
- Analytical verification
- Inter- and Intra-laboratory performance evaluations
- Other considerations

First Name	Name	Role
Jeanne	**Hankett**	Chair
John	**Norman**	*Rapporteur*
Denise	**Mitrano**	
Jennifer	**Lynch**	
Kevin	**Thomas**	
Anthony	**Andrady**	
John	**Davis**	
Alan	**Owens**	
Craig	**Davis**	
Gaurav	**Amarpuri**	
Richard	**Czarnecki**	
William	**Robberson**	
Kay	**Ho**	

2. *Effects testing and exposure considerations: Best practices*

Chair: Stephanie Wright **Rapporteur: Rob Ellis-Hutchings**

Over the last several years OECD has published guidance documents aimed at helping to point researchers in directions that, at present, appear to represent the most promising methods for producing meaningful and reproducible test results (see for instance OECD No. 36: Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials) – What is the applicability of such guidance to MPs?

It can be anticipated that a number of similarities exist between engineered nanomaterials (ENMs) and MPs, particularly with respect to sample preparation, dosimetry and in the identification of an appropriate dose-metric. Consequently, the OECD No. 36 Guidance document should represent a primary source of information in helping to frame discussions within this workgroup. It will be important to consider, for instance, the preparation of stock solutions of MPs, how are they to be stored, the influence of the test medium on the dispersal of MPs, the influence of methods that might be used to disperse particles in the test medium (e.g. ultrasonication versus the use of chemical dispersants), methods that might be used to confirm exposure concentrations in test medium and in the test system. Are there any guidance recommendations made for ENMs that are not appropriate for MPs? Can guidance be suggested that is unique to MPs? As a further consideration, can recommendations be made with respect to the use of appropriate labelling for MPs and the potential use of positive and negative controls.

Session Room – tbc

First Name	Name	Role

Stephanie	**Wright**	Chair
Rob	**Ellis-Hutchings**	*Rapporteur*
Samuel	**Stavis**	
Samantha	**Romanick**	
Christie	**Sayes**	
Erik	**Rushton**	
Mark	**Pemberton**	
Camilla	**Carteny**	
Phil	**Brondsema**	
Yash	**Parulekar**	
John	**Kucklick**	
Emily	**Clark**	
Bart	**Koelmans**	

SPEAKERS AND BIOGRAPHIES

Dr. Jennifer Lynch

Jennifer Lynch's research interests are to improve measurement methods to study ocean plastic pollution. She has worked for the National Institute of Standards and Technology since 2003. She founded and became the Co-Director of the Hawai'i Pacific University Center for Marine Debris Research in 2019, purposefully established in Hawai'i at one of the most plastic polluted marine environments. Dr. Lynch's research focuses on developing optimal methods to quantify and chemically characterize nanoplastic to megaplastic pollution, to answer questions about quantities, sources, transport, fate, effects, and reuse. She has authored 61 peer-reviewed publications, four book chapters, and graduated 39 graduate students.

Dr. Samuel Stavis

Samuel M. Stavis is the Leader of the Nanostructure Fabrication and Measurement Group at the National Institute of Standards and Technology (NIST). He received a B.S.E. in Engineering Physics from the University of Michigan and a M.S. and Ph.D. in Applied Physics from Cornell University, where he was also a Postdoctoral Research Associate in Biological and Environmental Engineering. At Cornell, he performed early research in measuring fluorescence from single molecules in nanofluidic devices. Sam joined the NIST staff through a National Research Council Postdoctoral Research Associateship award. At NIST, he has advanced what is possible to make and measure at small scales. By developing and combining fabrication processes, device technologies, and microscopy methods, he has established new ways and limits of controlling and quantifying nanoscale systems. His research has diverse applications in manufacturing, healthcare, and the environment. Sam has received a Bronze Medal award, two Innovations in Measurement Science awards, a Strategic and Emerging Research Initiative award in support of the Circular Economy Program, and an Outstanding Authorship award from NIST.

Dr. Bart Koelmans

Dr. Bart Koelmans is an environmental chemist and ecotoxicologist by training who heads the Aquatic Ecology and Water Quality Department at Wageningen University. In the field of plastic research, his group aims to bridge the gap between conceptual and empirical approaches to obtain a mechanistic understanding of the risks of microplastic for human health and the environment. Bart is a global highly cited researcher (Clarivate analytics), advises international organizations like the World Health Organization, led international working groups about risks of plastic pollution, such as the European Commission's Science Advice for Policy by European Academies (SAPEA) expert group on Microplastics in Nature and Society, and is Editor-in-Chief of the new journal Microplastics and Nanoplastics.

Mr. Richard Czarnecki

Rich joined Micro Powders in 2011 as Technical Director. He began his career in the printing inks industry in 1983 and has held of number of leadership roles in polymer and formulation development, technical services and regulatory affairs before joining Micro Powders. He holds a BA in Chemistry from Rutgers University and as MS in Polymer Science from NJIT.

Dr. Jeanne Hankett

Dr. Jeanne Hankett is a Senior Scientist and the Microplastics Research Liaison for North America at the BASF Corporation. She manages BASF's regional Microplastics Research Program, working with the global internal network and members of academia, industry, and government to tackle the microplastics topic and develop next generation solutions supporting the circular economy. In addition to microplastics, she is responsible for the development and growth of select sustainability technical platforms in the region. She is based in Corporate Analytics North America in Wyandotte, MI and has been with BASF since 2017. Prior to BASF, Jeanne earned her BS in Chemistry from the University of Illinois at Urbana-Champaign and a PhD in Chemistry from the University of Michigan where she also held a teaching & research post-doctoral appointment. Jeanne has published more than a dozen peer-reviewed articles in sustainability and her academic and industrial research has spanned the topics of fuel cells, solar energy generation, sustainable plastics & polymers, molecular environmental interfaces, recycling, and of course, microplastics.

Dr. Martin Clift

Martin Clift is a Professor of in vitro systems/particle and fibre toxicology at Swansea University Medical School. He is globally recognised for his research of using advanced in vitro lung models to assess the inhalation toxicology of varying particulates and fibres. He focusses upon deducing the particle-lung cell interaction and relating this towards understanding the particles' mechanistic toxicology, as well as immune response, both in healthy and diseased models. More recently, he has focused his efforts on establishing advanced 3D, dynamic moving in vitro models that better represent the complexity of the lower airways. Using such models, combined with next-level testing strategies and exposure approaches, Clift has further initiated investigation of combined exposure events to better consider real world exposure patterns and effects. Clift has received research income of >£4.8 million as principal investigator, and >£3.2million as co-investigator since 2010. Clift has >155 publications (h-index 34 (i10-index 70); citations >5180) within the field of particle/fibre toxicology and the particle-cell interaction in vitro and is currently supervising eight post-graduate students (with 14 graduates since 2011) in this field of study. Clift is Editor-in-Chief for Fibres, an Associate Editor for Journal of Nanobiotechnology and Particle and Fibre Toxicology, as well as an editorial board member for Mutagenesis, Food and Chemical Toxicology, as well as In Vitro Methods. Clift has been an expert panel member of the British NC3Rs working group upon Nanotoxicology and is currently the Chair of the UK Animal Alternative Technologies (UKAAT) society. Clift is a member of the British Toxicology Society (BTS) Sub-Scientific Committee, as well as the Scientific Board of Animal Free Research (AfR). In addition, Clift is the Chair if 'Alternative Models' for the EU NanoSafety Cluster. In addition to his activities within current H2020 EU projects, Dr. Clift has assisted in a work-package (WP) lead of the EU FP7 project NanoImpactNet (2008-2012) and was deputy WP lead in the EU H2020 project PATROLS (2017-2021). Currently Clift is co-PI on a UKRI (NERC)

funding project assessing the impact of both indoor and outdoor pollutants upon pregnancy and developing organs in vitro. Clift has further developed his research management experience by being the co-Director for the Post-Graduate Research programme at Swansea University Medical School since 2017. Clift has also recently been elected as Full Member to the UK Government Committee of Medical Effects of Air Pollutants (COMEAP) and is the Scientific Chair of the next BOHS sponsored Inhaled Particles Conference (in 2022).

Dr. Anthony Andrady

Based at North Carolina State University, Dr. Andrady will present on the degradation and fragmentation of microplastic. Secondary microplastics are derived from various weathering degradation processes of common polymers in the environment, causing them to become morphologically and chemically modified in the process. These changes result from the oxidative reactions and scission/crosslinking reactions that accompany the weathering process. They are likely to influence processes such as surface fouling, partition of organic pollutants, propensity for further oxidation and the generation of nanoscale plastics. In designing reference materials for microplastics these polymer-specific changes need to be taken into account.

Dr. Elijah Petersen

Elijah J. Petersen completed his PhD at the University of Michigan in Environmental Engineering in 2007. Then, he completed postdocs at the University of Joensuu (Finland) on a Fulbright scholarship and then the University of Michigan before joining NIST as a National Research Council postdoctoral fellow. He became a staff scientist at NIST in 2010 and works in the Cell Systems Science group in the Biosystems and Biomaterials division. His research currently focuses on the development of robust, reproducible in vitro test methods and methods for plastic toxicity testing. He is an associate editor for Nanotoxicology, Environmental Toxicology and Chemistry, and Nanoimpact and on the editorial board of Environmental Pollution and Nanomaterials. He recently was honored with the 2020 Chemical Research in Toxicology Young Investigator Award and the Presidential Early Career Award for Scientists and Engineers (PECASE) in 2019.

Dr. Olivia Osborne

Dr. Olivia Osborne is a multidisciplinary toxicological chemical risk assessment scientist. She has specialized in the research and development of chemical compounds and nanoparticle risk assessment via high throughput screening platforms in the fields of human health and the environment. She has worked on a wide variety of risk assessment projects including but not limited to nanotechnology, food, consumer products, biomedical applications, pesticides, anti-fouling paints, plastics, water systems and semiconductor technologies (including e-waste). She has collaborated and worked with numerous multi-stakeholders, consortiums, policy makers and working groups in the UK, Europe, USA as well as other international collaborators. She is author of peer reviewed publications and has written for a number of outlets including books. She graduated from the University of Exeter with a BSc Hons and a PhD in (eco) toxicology, nanotechnology and developmental biology. Following thereafter, she was a postdoctoral research scholar at the multidisciplinary California NanoSystems Institute (CNSI) and Center for Environmental Implications of Nanotechnology (CEIN) in the University of California Los Angeles (UCLA). During her time there, she established high throughput screening platforms for nanoparticles and compounds using novel approach methodologies and her research was highlighted by the American Chemical Society. She also worked on predictive toxicology and integrated approaches to testing and assessment towards nanoparticle hazard assessment. She is currently working at the Food Standards Agency in the Science Evidence and Research Division working on chemical risk assessment and new approach methodologies. She is a Member of Institute of Food Science and Technology (MIFST), UK and European Registered Toxicologist (UKRT ERT) ; member of the Organisation for Economic Co-operation and Development (OECD) Working Party for Nanomaterials, and member of the British Standard Institute (BSI).

Dr. Robert Ellis-Hutchings

As a Toxicologist for Dow, Dr. Ellis-Hutchings provides leadership and guidance on the health, safety, and sustainability of new and existing Dow products. He leads active efforts within Dow and the industry to understand and address scientific gaps relating to the potential risk of microplastic hazards to humans. He is involved with several multi-stakeholder microplastic committees including PlasticEurope's microplastic science team, which he chairs, and microplastic task forces within the American Chemistry Council (ACC), the European Chemical Industry Council (Cefic), and the International Council of Chemical Associations (ICCA). He is also a member of the International Life Sciences Institute (ILSI) Europe's dietary microplastic initiative.