Review

総説

Microplastics and Nanoplastics: Analysis and Method Development and the Relationship with HORIBA Instruments Incorporated (HII)

米国HORIBAグループのマイクロプラスチック分析への取り組み

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The ecological, human and marine health threat of Microplastics (MP's) and Nanoplastics (NP's) is huge and very real. In order for MP's and NP's to be accurately monitored, understood, legislated and reduced, there remains a significant amount of collaborative work needed between scientists, managers, policy makers and instrument providers such as HORIBA. Harmonized scientific method is required in order to allow legislators and agency managers to determine which issues to prioritize. In North America, HORIBA Instruments Incorporated (HII) is working closely with both scientists and federal and state government agencies. These collaborations are intended to support and develop the science and instrumentation to allow scientists and managers to achieve the directives and advances necessary to apply legislation and reduce the risks caused by MP's and NP's. This review paper explains HII's approach, activities and role in North America to support MP's and NP's analysis and method development towards eventual field monitoring devices and actionable legislation.

近年，マイクロプラスチック(MP'ｓ)やナノプラスチック(NP'ｓ)の人や海洋生物をはじめとした生体に対する影響が懸念されている。MP'ｓやNP'ｓを削減するために正しく測定し，その懸念を理解して必要な規制を制定することが必要である。そのためにはさらに産学官の枠組みでの連携に，HORIBAの様な分析装置メーカーが関与してゆくことがますます重要となる。行政関係者がどの課題を優先的に対処すべきかを決定する上で，このような連携は非常に重要である。我々，HORIBA Instruments Incorporated(HII)は，北米において科学者及び連邦機関や州政府機関と密な連携を図ってきた。我々は，科学技術や測定手法の開発を通じ，科学者や対策を主導する人々の方針立案や法整備に寄与することで，MP'ｓやNP'ｓによる環境リスクを低減することを目指している。本総説では，実用的な測定手法の開発と実行可能な規制を検討のために行ってきたMP'ｓの解析や測定手法の実現に対しての我々のアプローチや具体的な活動をはじめ，北米で果たした役割について報告する。

Introduction

Ever since the very beginning of HORIBA, including the early development work at the end of 1945 and through the very first glass electrode pH meter products in the early 1950's, HORIBA products have been applied to environmental applications to protect our planet. HORIBA found early success at the beginning of the 1960's through the automotive emission analyzer MEXA-1. Since then many of our products have been developed for environmental or related studies and applications. Today our corporate activity towards social responsibility is focused on energy, health, the environment and safety. Some key examples of these environmentally conscious products include decades of continuing innovation in FT-IR exhaust gas analyzers; our range of XRF analyzers that were applied towards the waste electrical and electronic equipment (WEEE) and the recycling of hazardous substance (RoHS) directives; and our AquaLog fluorescence Absorbance Transmission Excitation Emission Matrices (A-TEEM) spectrometer that was developed for rapid analysis of dissolved organic matter in water to allow environmental and water treatment monitoring. It was only natural therefore, based on the ever increasing concern over microplastics (MP's) and nanoplastics (NP's) in our environment, that HORIBA would be closely involved in leading the development and standardization of analysis methodologies for this pervading pollutant.

The ecological, human and marine health threat of MP's is huge and very real. It is estimated[1] that every year 4.8 to 12.7 million metric tons of plastic waste enter our oceans. One report[2] estimated that up to the year 2014 there were an accumulated number of MP particles, located as a global standing stock of small floating plastic debris, ranging from 15 to 51 trillion particles, weighing between 93 and 236 thousand metric tons, which is only approximately 1% of global plastic waste estimated to enter the ocean in each year. Presumably the remaining 99% of plastic waste ends up in sediment on the ocean floor with some washing up on beaches around the world, and some amount recovered in cleaning exercises.

HORIBA Scientific's North American involvement with MP's began with the development of a close collaboration with Dr. Chelsea Rochman and her research group in 2015 at the Freshwater and Marine Ecology Department at the University of Toronto. At that time Dr. Rochman acquired the XploRA Raman microscope for her groups MP research. In 2019, a second XploRA Raman microscope was delivered to the University and installed in the laboratory of Chelsea's colleague Dr. Robert Andrews in the Institute for Water Innovation. Dr. Rochman is a leading researcher and innovator[3] in the field of MP's, the work of Dr. Rochman's laboratory will be outlined in detail in an article by Dr. Bridget O'Donnell later in this issue of Readout.

In September 2018 the California legislature responded to the increasing threat and public concern towards MP's by enacting two new bills, as outlined below, that require quantification of MP's in various media and development of new management strategies.

Senate Bill 1422: California Safe Drinking Water Act–Microplastics[4]

Senate Bill 1422 (Portantino, Chapter 902, California Statutes of 2018) charges the California State Water Resources Control Board (SWRCB) with developing methodologies and a strategy for monitoring and tracking the concentration of MP's in drinking water. This includes, adopting a standard definition of MP's in drinking water by July, 2020; adopting a standard methodology to test drinking water for MP's by July 2021; adopting requirements for testing and public reporting of MP's in drinking water; and accrediting laboratories to analyze MP's.

Senate Bill 1263: Ocean Protection Council – Statewide Microplastics Strategy[5]

Senate Bill 1263 (Portantino, Chapter 609, California Statutes of 2018) requires the California Ocean protection Council (OPC) to adopt a Statewide MP's ocean and waterways strategy and report to the legislature on implementation by 2025. The bill also requires OPC to develop a prioritized plan to research and support the development of risk assessments in marine habitat by 2021. This includes, development of standardized methodologies for extracting, sampling, counting, and characterizing MP's in the environment; moving forward to characterize ambient concentrations, impacts, sources and pathways of MP's in California waterways; and developing approaches to reduce the introduction of MP's into marine environments, including source control.

With the announcement of these bills the HORIBA Scientific Business Development team, led by Dr. Kentaro Nishikata and Dr. Andrew Whitley proposed a working group meeting to review the analytical instruments and field monitoring required by these bills. We approached Dr. Rochman to discuss planning such a meeting. Dr. Rochman suggested that we collaborate with and hold the meeting at SCCWRP in Costa Mesa, CA, which happens to be just 13 miles from the North American headquarters of HII in Irvine. Chelsea introduced us to Dr. Steve Weisberg, Executive Director of the Southern California Coastal Water Research Project Authority (SCCWRP), and together we proceeded to discuss what was required to create a successful working group meeting. It was agreed that at the meeting it would be necessary to perform a gap analysis between existing methods and summarize the necessary actions to bridge these gaps. From an analytical instrument and environmental monitoring device manufacturer point of view, HORIBA needs

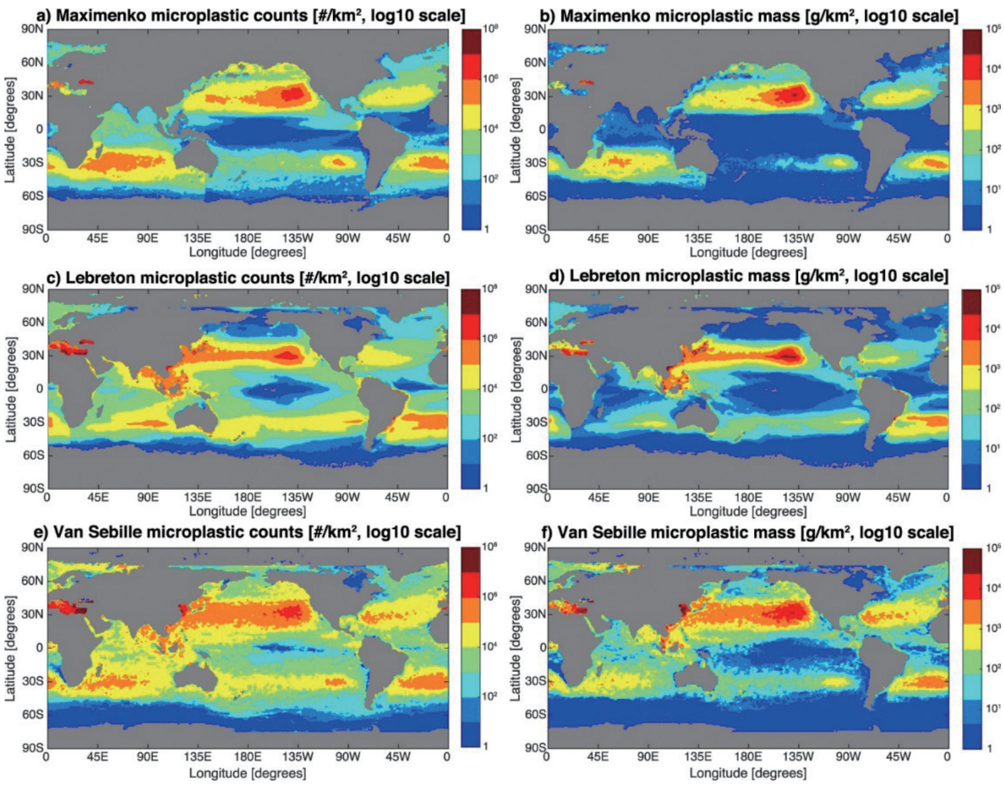

Figure 1 Maps of the solutions of MP's count (left column) and mass (right column) distribution for the three different ocean circulation models. Because fits are done on a per-basin level, there are a few discontinuities visible (e.g. South of Tasmania in the Maximenko solution, panel a)). Figure 3 from "A global inventory of small floating plastic debris". Erik van Sebille et al 2015 Environ. Res. Lett. 10 124006 doi:10.1088/1748-9326/10/12/124006[6]

to work closely with the scientists and managers that are tasked with advising policy makers what instrument and method developments are needed to meet legislation, in this case the two CA Senate bills. HORIBA and other manufacturers require the measurement requirement to be strictly stated so that we can collaborate and advise on possible solutions. Where possible collaborating on method development using existing instruments, but when needed adapting hardware, including sample handling and automation, and software, to meet the measurement requirements. In some cases, where the need is extensive and fully understood, the development of new instrumentation will be done, in the case of MP's, as an example, for in field or treatment plant monitoring.

On April 4-5, 2019 our workshop: "Measuring Microplastics: Building Best Practices for Sampling, Extraction and Analysis", hosted by HORIBA, SCCWRP and the University of Toronto, in coordination with the State of California Water Resources Control Board (SWRCB) and the California Ocean Protection Council (OPC) was held at SCCWRP in Costa Mesa. The main meeting objectives were:

1. Understand policy-maker needs in regards to microplastics methods.
2. Agree on the state of the science and determine the research necessary to reach shared goals.
3. Co-develop a manuscript on best practices for microplastics analyses.
4. Design a study plan to develop harmonized methods, including collection, laboratory and data management, for microplastics analysis.

There were 14 presentations from regulatory and legislation representatives, and scientists and managers from around the world. The meeting presentations were recorded and can be viewed via the link in this reviews references.[7] To begin the day, we heard from Deborah Halberstadt, the Executive Director of the OPC, and Darrin Polhemus, the Deputy Director for Drinking Water at the SWRCP. They shared their perspective and the targets they need to meet which were mandated by SB1263 and SB1422. The scientific presentations

Figure 2 Ann-Marie Cook of the EPA presenting at the "Measuring Microplastics: Building Best Practices for Sampling, Extraction and Analysis" workshop at SCCWRP in Costa Mesa, CA

were separated in to four main topics–extraction, sampling, analytical methods and data analysis. At the end of the first day, we asked everyone to think about what they had learned and what they needed moving forward in relation to their own research, monitoring or management. We asked everyone to answer four questions:

What is your most urgent need at this moment?
What would you like to see in a best practices report?
What types of methods would you like to see developed?
What are some of the key concerns that should be taken into consideration when developing/choosing best methods and practices?

The answers to these questions by all stakeholders were summarized in the final meeting report, they were used to guide day 2 of the meeting and will continue to be used to guide future work. On day 2 of the meeting our goals were to:

1. Create scientific journal review articles (for *Applied Spectroscopy*) to summarize the state of the science towards standardized MP's analysis.
2. Develop a study plan that addresses issues necessary to achieve method standardization.

First we worked on the articles for the special issue of *Applied Spectroscopy*. We spent the morning beginning drafts of each review paper that we were planning to write together and agreeing upon a general outline for the special issue. The special issue will be wrapped up in July, 2020 and come out in early Fall, 2020. Details of this special issue of Applied Spectroscopy can be

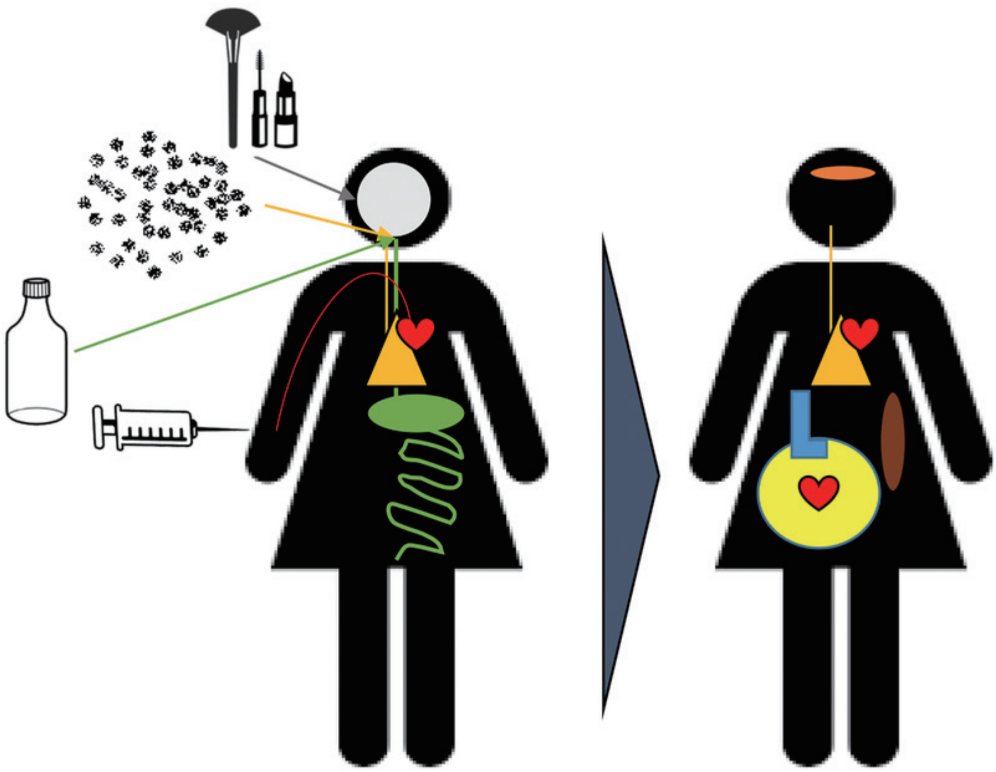

Figure 3 Diagram depicting the routes of NP's exposure (i.e., ingestion, inhalation, dermal, and injection), potential primary systems of impact, and potential secondary toxicity associated with particle deposition. *Reproduced by permission from PA Stapleton, AIMS Environ Sci. 2019; 6(5): 367–378.*[12] *Published online 2019 Oct 22. doi: 10.3934/environsci.2019.5.367*

found later in this review. The afternoon session was spent discussing the format for a multi-lab MP's methods evaluation study plan, this is detailed later in this review paper. The final results and actions of the meeting were then summarized, these were the final report detail to be shared after the meeting with all participants, the collaborative method evaluation study, a special issue in the journal of *Applied Spectroscopy*, and two scientific sessions on MP's analysis (organized by Andrew Whitley, HORIBA and Shelly Moore, SCCWRP) at the October, 2019, SciX conference in Palm Springs. There will also be a follow-up workshop at SCCWRP to share the results of the study plan and discuss how MP's may impact human health.

Toxicological considerations of Microplastics and Nanoplastics

The question of nanotoxicology and which types and sizes of MP's and NP's are most dangerous to human and animal health is still a very underserved research area and significantly more work is required here. It is certainly the case that certain size classifications of MP's down to a few tens of microns, whilst dangerous for wildlife and a pervading concern regarding pollution, do not offer as significant a health risk to humans as the smaller size classifications. Long term this larger size classifications, if allowed to go unchecked, could disrupt and damage marine populations with potentially huge cascading effects further up the marine food chain and on to humans. These larger particles if they are ingested and do not pass through the body can have long term health effects, in particular as they degrade they can leach hazardous chemicals in to the body of marine animals and ultimately humans. These chemicals have been shown to disrupt immune systems and negatively impact growth and reproduction. As a secondary effect MP's can also adsorb chemical contaminants on to their surface, transporting them within the environment or through a biological system. These differential surface absorbents, including biofilms, and particle transformations will impact MP and NP transport and toxicity. This subject is covered in more chemical detail by Dr. Bridget O'Donnell later in this issue of Readout.

The effects of MP's become much more pronounced the smaller the particles are, as they are more likely to pass from the gut and stomach to the bloodstream and other organs. As they become smaller in size these particles also can become airborne. It is known that MP's smaller than 25 microns can enter the human body through the nose or mouth and those less than five microns can end up in lung tissue.[8] Increasing the urgency to understand the impact of airborne MP's and NP's is critical. There is already a great deal of concern, research and attempts at legislating fine particulates in the air formed by burning fossil fuels, including black carbon or soot. These particles have been linked to a number of health impacts including respiratory issues, heart attacks and the impairment of neurological function.[9] Most countries have air pollution standards to limit the volumes of particles less than 10 microns, and especially those below 2.5 microns, respectively known as PM 10 and PM 2.5 standards.[10] However little has yet been done to understand the toxicity or to monitor and legislate the potentially more harmful airborne MP's and NP's pollution.[11] The size of a particle directly relates to the surface area-to-mass ratios. The surface area-to-mass corresponds to the amount of surface area of an object (particle) within a given volume or collection of particles. The fact that NP's have a larger surface area-to-mass than MP's therefore provides a greater surface for biological contact or chemical adsorption. NP's are also more likely to become surface charged, functionalized and therefore have a further likelihood to have species chemisorbed on their surface. Such surface modifications can aid transport across organ membranes. It is known that surface modification, if cationic, can aid bonding to the brains endothelial cells and therefore become a mechanism to endocytosis and transport across the blood brain barrier. It is established that NP particles can cross biological membranes and influence cellular signaling, however, the cellular and toxic effects of these exposures have yet to be evaluated. Future studies must also identify environmentally and health risk relevant concentrations and take into account the NP physicochemical properties of each NP type analyzed. It is critical that these studies take place rapidly to help guide the necessary development of monitoring and analysis methods to target the most critical size range and MP and NP types that requires the strongest and most urgent legislation.

Analytical Methods for the Analysis of Microplastics

As part of the Applied Spectroscopy special issue on MP's there is an excellent review paper that compares the various analytical techniques used to identify MP's. This review paper[13] "Critical Assessment of Analytical Methods for the Harmonized and Cost-Efficient Analysis of MP's" by Primpke et al includes a contribution by HORIBA's Dr. Bridget O'Donnell. The main focus of this comprehensive review paper is the currently applied identification and quantification tools for MP's. The authors evaluate these techniques and the need to provide a harmonized guideline for future SOPs to cover legislation like the two recent California Senate bills discussed above. The main techniques used for MP's are covered in this paper, these are naked eye detection, general optical microscopy, the application of dye staining–typically Nile Red, flow cytometry, Fourier transform infrared (FT-IR) spectroscopy and microsopy, Raman spectroscopy and microscopy and thermal degradation by pyrolysis–gas chromatography–mass spectrometry (py-GC-MS) as well as thermo-extraction and desorption gas chromatography–mass spectrometry (TED-GC-MS). A guideline to provide the necessary method harmonization in the time frames necessary to support legislation is provided. This includes an analysis of the cost of each method ranging from low cost towards higher analytical demands to measure MP's in an effective way by field laboratories and governmental institutions while maximizing information for risk assessment. It is important to achieve the goals of the California Senate Bills that we create analysis methods that are not only achievable by the most proficient experts, but ones that are transferable and repeatable among a wide array of laboratories, some of which will be introduced to MP's for the first time as a result of new legislation. Methods must also fit in to the requirements of any laboratory accreditation program to ensure the data generated are correct, consistent and traceable. The ability of the current analysis methods to meet all these requirements are being tested through the SCCWRP study plan discussed below. At HORIBA we will use the results of this study plan, our many ongoing MP collaborations and discussions with other government institutions like the EPA, NIST and ASTM to develop rugged, reproducible automated instrumentation and methods.

In Dr. O'Donnell's review of the research work of Dr. Rochman's laboratory she notes that in the characterization of MP's no single technique works for all samples encountered. It is important to use multiple tools to be able to confidently identify all or most collected particulates. This being said it has been shown and reviewed in the paper by Primpke et al that Raman microscopy does offer a number of significant advantages over other techniques. One of the most important advantages of Raman microscopy is that the spatial resolution is excellent, down to one micron or less. FT-IR microscopy typically has a spatial resolution of between 10-20 microns, and as we discussed above it is the smaller MP's that provide the largest threats to human health. In Dr. Lee's paper, in this issue of Readout, she discusses the optimum instrument and experimental configuration for MP's analysis. Dr. Lee also reviews some of our North American collaborations on MP's. It is clear from these collaborations in North America, and globally, that in order for there to be statistical relevance in studies of the number and distribution of MP's a huge amount of samples need to be analyzed. A single filtered sample of 5 liters of water can capture 1000's of particles. There is clearly a need for automated analysis. Dr. O'Donnell and Dr. Lee show in their papers how HORIBA has worked with researchers to extend development of our ParticleFinder software to start to provide a fully automated analysis of filters containing MP's. Using the XploRA Raman microscope and ParticleFinder software from HORIBA, researchers can automate the location, particle characterization and identification of MP's of their filtered samples.

Now that the measurement hardware and software is starting to be available it is critical to develop standardized measurement methods. MP management strategy requires monitoring to assess the relative contributions of multiple MP sources and assess the progress toward source reduction. Such assessments are of little value if they are confounded by incomparability of measurements among different groups, sample types or over time.[14] Placing results from North America into context of other locations is critical, but only if methods across geographies are consistent enough to warrant such comparisons. It is with this challenge of method harmonization in mind that, at the MP workshop at SCCWRP, see above, it was agreed to undertake an ambitious study plan called "Microplastic Measurment Methods Evaluation Study". The purpose of the study is to assess the consistency of a measurement, characterization and identification of MP's in a number of sample types. The study plan will assess the repeatability of results across a large number of laboratories. The study includes evaluation of five methods (stereomicroscope, staining with Nile Red, FT-IR, Raman and Pyrolysis GC/MS) applied to drinking water, wastewater, sediment and fish tissue matrices. Extraction methods to be assessed include filtration for clean water, peroxide oxidation for wastewater, density separation for sediments and KOH digestion for fish tissue. Each participating laboratory will be given a sample with known blind materials and a standard operating procedure (SOP) for the methods they have agreed and signed up to perform. Most of the expert speakers at the workshop agreed to participate in the study, but other groups have been invited across a range of laboratories, from novice to professional. The proposed timelines for the study plan were pushed back to late 2020 due to initial challenges to prepare the samples and then because of the COVID-19 outbreak in the Spring of 2020. The HORIBA, NJ Lab will participate in this study, along with 35 other laboratories around the world.

Thanks to the high spatial resolution of Raman microscopy it can be used to study MP's and NP's across a wide size range from around 0.5 microns, an order of magnitude less than the lower size definition of MP's, up to, and beyond, the 5 mm upper size limit definition of MP's. Dr. O'Donnell and Dr. Lee recommend, and have developed, a varied menu of Raman applications

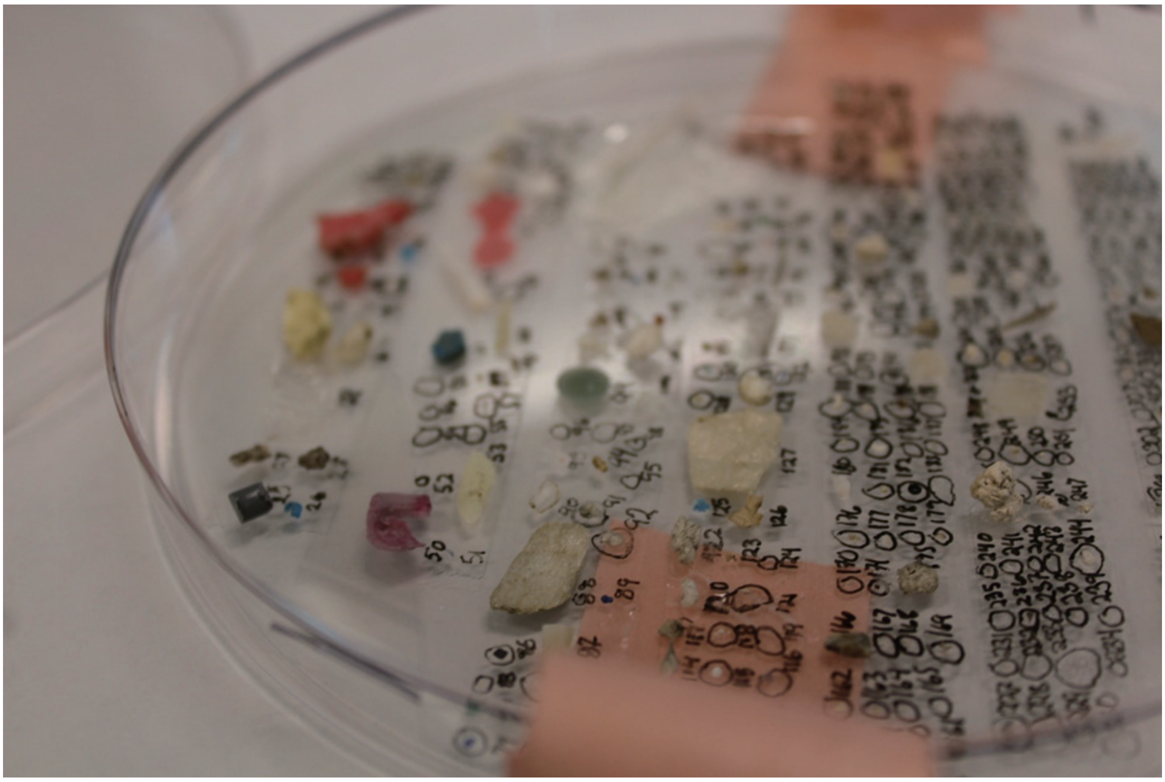

Figure 4 Examples of picked MP particles from a subset of a single experiment showing the large quantity and variation of particles that can be generated in MP analysis studies. Image reproduced by permission from the Rochman group.

methods to be used for the various size classifications of MP's and NP's and for the different morphologies of these particles. It has also been found that in order to maximize the number of particles that can be identified with Raman spectroscopy it is important to have both a 532 nm and 785 nm excitation laser for the analysis. Some particles will also burn under a focused laser, as used in Raman spectroscopy, in these cases it is important to be able to lower the laser power by accessing the laser control through the Raman software or by using neutral density filters to control the laser power reaching the sample.

Future Microplastics business opportunities for HORIBA

There are many institutes and industries that will likely need to monitor MP's and NP's due to legislation in the near future. Apart from the more obvious monitoring requirements for environmental water monitoring, waste water treatment plants and public water plants, there are other industries that will likely be subjected to regulations. These industries include packaged water, beverages and food. Even though there have been reports on MP's in drinking water, there have been no large scale studies on quality control of packaged water and beverages for MP's. It will become, however, most likely mandatory once regulations and reproducible methods are in place. Many companies will have to acquire an analytical instrument or use an accredited analytical service. Proactive companies such as Pepsi and CocaCola have already participated in MP's workshops, and expressed interests in having access to MP counting, characterization and identification capabilities.

The challenge and opportunities long term will be to monitor MP's in flow. One such method that could be adapted and applied to this challenge is flow cytometry, this technique was originally utilized for counting and characterizing cells to monitor growth, degradation, or aggregation processes, for example protein aggregation. The sample is typically diluted by a sheath fluid and transported into a flow cell. Once in the flow cell the cells or particles scatter light from a laser beam and are counted based on changes in the optical signal in a forward or side scattering angle. Utilizing different lasers and dye staining methodologies allow for size, quantity, and distribution to be quantified, especially when combined with a strong camera for imaging. Typical size ranges of analyzed objects are between 0.5 and 50 microns.

Another approach for analyzing particles in the range of 2 microns to 1 mm in flowing solution is flow imaging. Here the diluted sample is monitored by a camera system combined with a microscope unit and each particle passing the camera is digitally imaged. The advantage of this technique over flow cytometry are that it visualizes and counts single particles with the options to later validate the counts, removing outliers like bubbles. There are also field deployable units that can be put in the field or treatment plants. In all case of in flow monitoring of MP's pre-filtration and purification will most likely be necessary prior to analysis.

In the case of NP's there is promise that the HORIBA ViewSizer could be used to characterize and count these particles. The ViewSizer tracks scattering from individual particles to determine particle size distribution and concentration. This technique commonly known as nanoparticle tracking analysis (NTA) or particle tracking analysis (PTA). The instrument uses three lasers to simultaneously illuminate the sample and a color video camera for detection, allowing it to analyze the broad size distributions encountered with plastic[15] NP's. Such broad size distributions cannot be analyzed by other single laser systems on the market. Furthermore, there is the potential to discern plastic NP's from other NP's with the use of an appropriate dye such as Nile red. The ViewSizer can be configured to monitor only fluorescent particles and thereby analysis specificity is limited only by the selectivity of the dye. Interest in this technique will grow as concerns about plastic NP's in the environment and NP toxicity converge.

Conclusion

In order for MP's and NP's to be accurately monitored, understood, legislated and reduced, there remains a significant amount of collaborative work needed between scientists, managers, policy makers and instrument providers such as HORIBA. Harmonized scientific method is required in order to allow legislators and agency managers to determine which issues to prioritize. Legislators have great interest in ensuring that there are measurement methods and programs that characterize risk, however it is up to the scientists and managers to determine the specific techniques that are used to achieve the risk assessment and drive policy. HORIBA has an important role to play to develop laboratory instrumentation and methods that allow scientists and managers to achieve the directives of the legislative. Eventually HORIBA's experience and expertise in environmental monitoring systems can help drive and provide for the provision of field deployable monitoring devices for MP's and NP's in liquid and air. Ultimately these tools will be able to support strategies aimed at removal of MP's and NP's at the source, removal in the transport system and ways to remove materials from the ambient environment. Finally it is likely that such tools will be used to monitor imposed limitations on producers that would affect the chemical nature of the source material. HORIBA's intent in North America is to continue collaborations with scientists and management groups to understand the most urgent laboratory instrument and field monitoring system needs to enable harmonized method development. HORIBA has an important role to play in the environmental understanding, control and reduction of the risks caused by MP's and NP's now and in the future.

Acknowledgments

Many thanks to Dr. Bridget O'Donnell, Dr. Eunah Lee and Dr. Jeff Bodycomb of HII, NJ for their valuable input and discussion during the preparation of this review paper.

* Editorial note: This content is based on HORIBA's investigation at the year of issue unless otherwise stated.

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