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Perspective

Communicating Confidence in the Reliability of Micro- and Nanoplastic Identification in Human Health Studies

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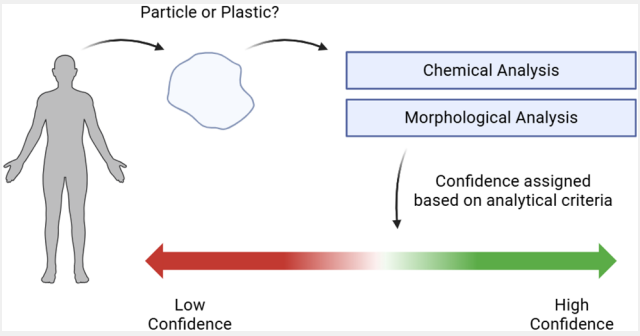
ABSTRACT: Accurately quantifying and characterizing human internal exposure to micro- and nanoplastics are critical for assessing potential health risks. However, the detection of these particles in human tissues, fluids, cell systems, and relevant models remains a major analytical challenge. There is an urgent need for robust, selective, sensitive, and high-throughput methods capable of generating reliable quantitative data. Equally essential is the transparent reporting of methodological limitations and uncertainties, supported by rigorous data collection and standardized practices. These challenges are compounded by the ubiquity of plastic particles, and therefore the risk of sample contamination and their diverse properties (e.g., size, shape, composition), all adding to the complexity of identifying and quantifying them in biological matrices. To address these issues, we propose a framework that integrates orthogonal analytical techniques to enhance the data reliability. Commonly used analytical techniques for the analysis of micro- and nanoplastics are assigned a category based on their specificity when identifying plastic particles. The framework proposes minimum data requirements from orthogonal techniques for the identification of plastic particles at various confidence levels. Clear communication of analytical confidence is vital, and we present a structured approach to support this. We emphasize the importance of scientific integrity, rigorous study design, and transparent reporting in health research. Finally, we call for the universal adoption of harmonized confidence criteria for reporting the presence of plastics in humans, an essential step toward informed decision-making.



KEYWORDS: *microplastics, nanoplastics, human exposure, analytical methods, biological matrices, quantification, detection*

Humans are increasingly exposed to micro- and nanoplastic particles, both through everyday product use and via environmental contamination,[1] with plastic pollution now recognized as a planetary scale problem.[2] Plastic particles are known to occur in every biome and in many of the species that occupy them, including humans.[3] Concerns about the impact of plastic pollution on wildlife and human health have gained traction through scientific research, media attention, and environmental advocacy. This has led to growing calls for regulatory measures, exemplified by the negotiations for a global plastics treaty.[4] The scientific community is instrumental in studying and communicating the extent and impact of plastic pollution to inform the development and implementation of robust policies based on coherent knowledge. High-quality data, accompanied by transparent reporting of limitations and uncertainties, are essential to this effort.

There are increased concerns that micro- and nanoplastic particles may enter the human body. These particles can form through fragmentation (e.g., physical abrasion, ultraviolet (UV) radiation, and chemical degradation) of plastic items present in the environment or be released from products such as food-contact materials, textiles, cosmetics, and tires during their use phase. A central focus of current plastic pollution research is to determine whether micro- and nanoplastics can enter human tissues and understand how they are absorbed, distributed, potentially metabolized, and excreted and which particle characteristics govern these processes. Particular attention should be given to the particle sizes most likely able to cross

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biological barriers, thus contributing to internal exposure. In addition, micro- and nanoplastics can be vectors for plastic-associated chemicals which could leach and be absorbed into the human body, thereby contributing to increased risk.[1,5,6]

When estimating exposure to micro- and nanoplastics, data on particle size, number or mass, and chemical composition (i.e., toxicologically relevant metrics) should be available as well as estimated total amounts within an organ, tissue, or biological fluid. Only then should an initial assessment of biological risk be carried out. Despite studies reporting the presence of plastic particles in human tissues and fluids,[3,7,8] uncertainties persist regarding the mechanisms of uptake and the physicochemical properties that facilitate this process.[1] Detecting trace levels of ubiquitous and diverse types of plastic particles presents challenges, including the risk of sample contamination, and the need for efficient, yet selective matrix removal, which are both essential prerequisites for subsequent analysis.[9−13] A further challenge is the difficulty of unequivocally identifying and reliably quantifying these particles in complex biological media, which is the focus of this work. To establish whether absorption occurs, analytical data must enable definitive identification and sufficiently exclude all other chemical entities from consideration. This underscores the importance of rigorous data collection of all of the connected procedural information and reporting for informed decision-making.

Every analytical workflow has inherent limitations that are influenced by every step, including sample selection, collection, storage, preparation, analysis technique, operator experience, and instrument choice and settings used. It is therefore necessary to calculate measurement uncertainties to ensure confidence in the results obtained. Unfortunately, being a young field of research, the various analytical techniques employed to identify micro- and nanoplastics in human biospecimens are subject to sources of uncertainty that have not yet been fully investigated, nor calculated. Methodologies to describe uncertainties of the measurements, as they are used in well-established trace analyses of well-known organic pollutants, such as polychlorinated biphenyls (PCBs) or perfluoroalkyl and polyfluoroalkyl substances (PFAS), are not yet available for the trace analysis of many micro- and nanoplastics in complex matrices. Key approaches include the use of certified reference materials, commercially available standards harmonized for numerous polymers in suitable size ranges and low concentrations, isotopically labeled standards of the same polymers, and participation in ring tests. The absence of these measures limits our ability to understand and quantify the uncertainties that accumulate throughout the entire sample handling process. The consequence is that without a full understanding of the uncertainties associated with all steps of the analytical process, including the specific technique used, or indeed specific combinations of techniques, it is impossible to implement the necessary quality control and quality assurance procedures to ensure that the data being generated are accurate, precise, and unbiased. It is critical that this is addressed to enable effective research into the potential toxicity of plastic particles in the body and their contribution to adverse health effects.[14]

The issue of uncertainty can be exemplified by the analytical techniques applied. Vibrational microspectroscopy, including both infrared and Raman techniques, has been the most reported technique for assessing internal human exposure to microplastics, recently reviewed by Wright et al.[15] Despite its advantages, vibrational spectroscopy poses several challenges in microplastic analysis. A primary difficulty is distinguishing plastics within complex mixtures, where overlapping spectral features obscure differentiation among polymers, chemicals, organic matrices, and contaminants. Detection of small plastics (<10 μm), including submicron and nanoscale particles, is achievable with advanced techniques (e.g., surface-enhanced Raman spectroscopy (SERS),[16,17] optical photothermal infrared spectroscopy (O-PTIR),[18,19] nano-FTIR[20]), although it can require considerable analytical effort. While capable of detecting smaller particles <1 μm, limitations with Raman micro-spectroscopy include autofluorescence from certain sample components, which can mask Raman signals. Additionally, smaller particles yield weaker signals, and measuring mode can introduce spectral artifacts, complicating accurate identification.[21] Sample heterogeneity may also result in inconsistent spectral readings within the same sample or even for a single particle. Furthermore, environmental degradation can alter spectral signatures, further hindering identification.[21,22] Finally, high spectral similarity between different plastics and nonplastic materials, for example lipids, adds another layer of complexity to the analysis.[15,23]

There are technical advancements, such as automated analysis platforms for microplastics (e.g., laser direct infrared (LDIR)-based particle analysis, Raman-based particle analysis, and focal plane array-Fourier transform infrared (FPA-FTIR) imaging), but these are still subject to the above limitations, highlighting the key challenges in vibrational microspectroscopy.[24] All of these challenges affect the reliability of spectral matching algorithms and the associated hit quality index (HQI) for classifying spectra against a reference library, generally considered good when >70%.[15] Such algorithms are based on correlations, which can be driven by the presence of one prominent peak, leading to a false match that does not integrate the remaining bands in a spectrum. Reliability is further compromised when polymer-only databases are used for spectral matching, as the HQI then reflects only the relative likelihood of one polymer versus another and does not address the critical question of whether a particle is a polymer at all or a matrix signal from endogenous tissue components.[25] Thus, further scrutiny of spectral matches and classification is required as well as careful communication of confidence in both detection and identification.

Pyrolysis-Gas Chromatography/Mass Spectrometry (GC/MS) is another technique capable of identifying signatures of plastics irrespective of the particle size[26] and which has been widely applied to quantify micro- and nanoplastics in human tissues.[3,7] Pyrolysis-GC/MS is, however, an indirect technique and interference from the pyrolysis products of matrix components (e.g., lipids) with pyrolysis products from specific polymers can hinder the quantification of certain plastics, such as polyethylene or polyvinyl chloride.[13,27−29] For example, certain polymers produce pyrolysis products that are identical, or very similar to those produced from certain biological matrix components, potentially leading to overestimation of polymer concentrations and false-positive results.[13,27−30] Development of procedures to remove interfering matrix components prior to analysis has resulted in pyrolysis-GC/MS methods capable of specifically detecting certain plastics in complex media.[27,30] Furthermore, the possibility of applying internal standards (isotopic dilution methods), the possibility for fast sample throughput, and recent advances in the harmonization of the analytical workflow are important steps toward improved human biomonitoring standardization. However, as a destructive technique, pyrolysis-GC/MS does not, on its own, provide the

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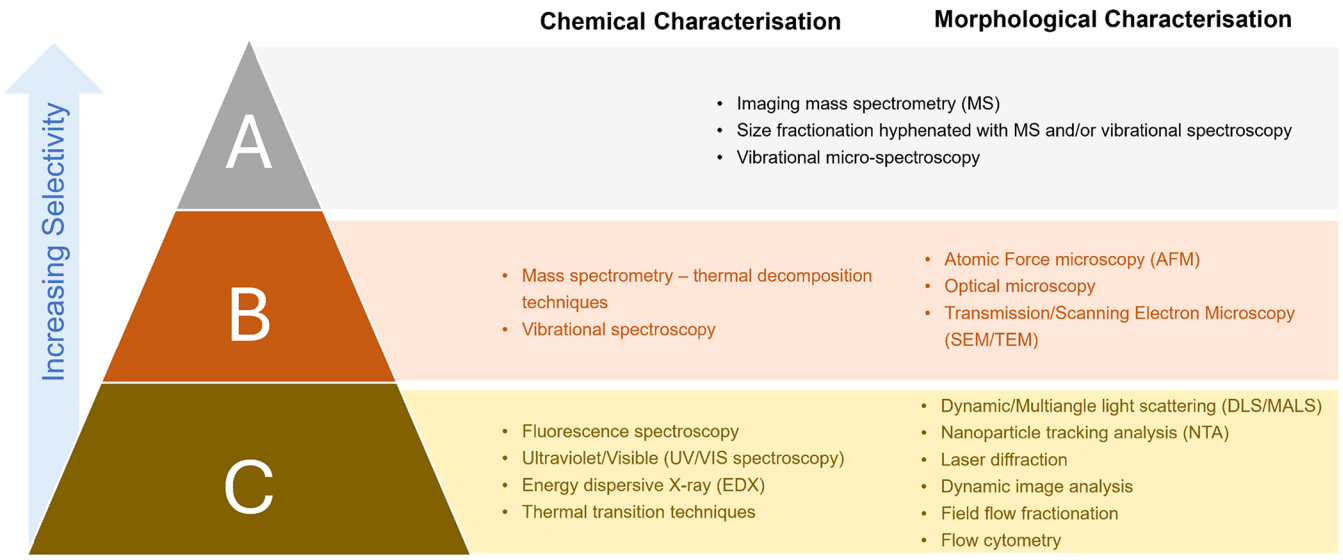

Figure 1. Left: proposed categorization of general analytical techniques for nanoplastic and microplastic analysis. Right: example techniques in each category. Combinations of independent techniques that employ different chemical or physical principles to deliver a measurement of the same property (i.e., orthogonality) should be the approach taken to provide higher identification and quantification assurance. Each technique is either mass and/or size limited and should be used within its accepted limitations, and the uncertainties associated with its use should be fully characterized and reported. If the limitations or uncertainties of a technique are not known, then it should be considered as less reliable and placed in a lower category.

complementary morphological information required for unequivocal plastic particle identification and localization within the sample. Furthermore, particle-size information is not provided directly; it is instead inferred from the sample preparation steps applied (e.g., sequential filtration). Therefore, the capabilities of other emergent techniques need to be thoroughly assessed to determine whether they are suitable to provide this information.

Understanding the uncertainties associated with current techniques for trace plastics analysis in complex media is critical for interpreting findings in both individual studies and enabling cross-study comparisons. To determine if internal human exposure to micro- and nanoplastics is occurring, analytical data must enable identification and sufficiently exclude all nonplastic chemical entities.

Given the existing limitations with commonly used techniques, combined with uncertainties of other analytical steps, clearly communicating the level of confidence linked to the reported data from such analyses is vital. For detection, characterization, and quantification techniques, each with different strengths and vulnerabilities, one approach to increase confidence is to use a framework based on a combination of suitable and, importantly, uncorrelated techniques as is, for example, the accepted gold standard in the forensic sciences. Orthogonal techniques are techniques that apply fundamentally different measurement principles to determine one or more properties of a sample.[31,32] The analysis of a sample with a second orthogonal technique provides additional assurance of the specificity of the primary method and thus limits method-specific biases.

In fact, such an approach is recommended in different fields of analytical chemistry. For example, the European Network of Forensic Science Institutes (ENFSI) published a very detailed best-practice manual for the forensic analysis of isolated natural and synthetic fibers[33] and explicitly recommends using combinations of orthogonal methods to establish particle morphology, color, and chemical composition/characteristics. Outside of forensics, the use of orthogonal techniques has been recommended to increase confidence in analytical findings within pharmaceutical analysis,[34] the analysis of inhalable particles,[35] plant constituents,[36,37] and nanomaterials.[31] Furthermore, the combination of orthogonal measurement techniques has also previously been suggested to tackle the challenge of the analysis of submicron plastic particles,[38] as well as for the analysis of plastics in drinking water.[39]

The prioritization and sequence of technique application are critical for robust identification and for gathering the best quality evidence from a single particle/fiber or sample. ENFSI has clearly shown that of 23 evaluated different techniques for the identification of isolated fibers,[33] even those offering chemical structural information may have critical limitations highly relevant within the plastics field. Similarly, Ivleva (2021) identified comparable limitations in the techniques currently used for the analysis of micro- and nanoplastics in complex environmental samples.[40] These include partial or complete destruction of the sample, applicability to single particle/fiber, discrimination power, requirements for sample preparation, speed, and their general scale of documented usage (and operator's technical experience) in practice. Importantly, sources of uncertainty from sampling, storage, contamination, and analysis through to laboratory and practitioner proficiency evaluation must be carefully characterized, explicitly documented, and clearly communicated.

Currently applied techniques for the analysis of microplastics in environmental and human samples provide different types and amounts of information and varying levels of selectivity, but no technique is free of drawbacks. Considering such differences, analytical tools can be categorized for micro- and nanoplastic analysis and, according to their selectivity, help with interpretation and communicating confidence. Based on the authors' initial discussions, a ranking system to categorize the techniques, and the detail of information each provides, is proposed (Figure 1, Supporting Table 1). Three categories of techniques are proposed, with a provisional assignment of current techniques used for micro- and nanoplastic identification. Category A includes hyphenated (combines two or

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Table 1. Proposed Criteria for the Identification of Plastic Particles at Various Confidence Levels

confidence Level	minimum Data Requirements
unequivocal identification of plastic particles	• positive results from a combination of multiple and justified orthogonal techniques are mandatory • evidence provided for morphology, chemical composition AND physicochemical characteristics • at least TWO orthogonal category A techniques must be used together with at least ONE additional orthogonal category A-C technique on the same particle • the chemical analysis using a spectroscopic technique is conducted at the single-particle level • the raw data for all detected particles AND blanks AND controls are supplied
indicative plastic particle(s)	• positive results from a combination of multiple orthogonal techniques are mandatory • evidence provided for morphology, chemical composition AND physicochemical characteristics • at least ONE category A technique must be used together with at least TWO additional orthogonal category A-C techniques and on groups of particles • the raw data for all detected particles AND blanks AND controls are supplied
presumptive plastic-containing particle(s)	• positive results from a combination of multiple orthogonal techniques are mandatory • evidence provided for chemical composition AND morphology • TWO orthogonal category B techniques used together with at least ONE additional orthogonal category B−C technique on groups of particles and subsamples thereof • the raw data for all detected particles AND blanks AND controls are supplied
a particle	• positive results from a combination of multiple orthogonal techniques are mandatory • evidence provided for particle morphology, with no chemical composition information • at least ONE orthogonal category B techniques with ONE additional orthogonal category B−C techniques can be used • the raw data for all detected particles AND blanks AND controls are supplied

more) techniques which currently provide the highest selectivity for plastic particles, primarily due to their ability to provide the most information about the chemical composition of the material, and detailed particle morphology, or other important physicochemical properties. Category B techniques offer a moderate degree of selectivity for these characteristics. Lastly, Category C tools provide general information on, for example, whether a material is likely to be a particle, and supporting evidence of whether it is plastic-related or not.

It is suggested that the highest assurance in reporting can be achieved through appropriate and justified combinations of orthogonal analytical techniques. To this end, a proposal for a hierarchical confidence matrix was devised (Table 1). For the most stringent 'unequivocal' confirmation of plastic materials present in human samples, it is paramount that multiple techniques are applied to provide comprehensive evidence. For example, the application of fit-for-purpose and validated methods using at least two suitable orthogonal Category A techniques combined with an orthogonal technique from any category could arguably be sufficient. However, this may not always be the case and will depend on how the techniques are used and the quality of the information provided.

In cases in which a Category A technique is not available, results from less selective Category B and C techniques, especially when used in combination, can still contribute meaningfully to the weight of evidence. Where higher-confidence techniques are available, external validation against them can help determine the appropriate weight to assign to these less selective methods. Nevertheless, as limited structural information is provided by the Category B and C techniques, these could arguably only be considered 'presumptive' for a plastic material, especially in complex matrices. Adherence to appropriate quality practices (such as validation of applied procedures, contamination control, analysis of blanks, use of reference materials if available, participation in proficiency testing, etc.), including justification of the techniques employed and reporting applicable limitations, is imperative. Furthermore, making all raw data available for independent assessment must be a fundamental practice to ensure transparency and reliability.

The recommended approach to combine orthogonal techniques refers to investigation of the same sample with at least two techniques. The application of additional orthogonal techniques may require further sample processing, and thus, the sequence of the techniques' application is to be carefully chosen, with destructive techniques applied last. The results obtained are then combined to strengthen the evidence provided by each of them individually on the same sample (or particle).

As discussed above, accurately detecting nanosized plastics (<1 μm) and even particles <10 μm — is a major analytical challenge both in terms of the sensitivity of detecting the particles themselves and the specificity of accurately identifying plastic particles over other particles or matter present in biological samples. Current techniques may not yet be suitable to meet an 'unequivocal' confidence rating for some particles - e.g., nanosized plastics - but ongoing advances in analytical instrumentation may enable specific identification and accurate quantification in the future.

To address human exposure to micro- and nanoplastics, we propose the establishment of an international interdisciplinary working group to define best practices and critically assess the categorization of techniques (Figure 1) and how they can be best used in combination to increase the confidence (Table 1). We advocate that when reporting the findings of such analyses, there is a need to clearly communicate the level of confidence linked to the analytical data that has been collected. To facilitate this, we propose a provisional structured framework for communicating confidence in analytical data when analyzing human tissues and fluids for plastic particles. It is also emphasized that this framework considers only identification using current analytical instrumentation and does not consider uncertainties due to user error through, e.g., lack of experience/understanding of the technique being employed, or the appropriateness of the sample collection and preparation employed prior to analysis. What is critical is that interpretations of the evidential value of each technique, as well as the order in which they are applied are clearly justified, including the communication of confidence levels for each technique applied to justify a positive result. We also propose developing these confidence levels into minimum standards for data use and

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Environ. Health 2026, 4, 804−811

publication. It is acknowledged that minimum standards may vary by context and according to the purpose for which the data is used. However, for specific scenarios, such as where exposure data are associated with clinically or toxicologically relevant end points, or where the data are to be integrated into epidemiological studies or risk assessment, such guidance on minimum standards is of significant value.

To achieve such an improvement in the confidence of the reported studies, wider community involvement is required. It will be crucial for researchers, editors, peer reviewers, and publishers to uphold rigorous standards of scientific integrity to ensure the accuracy and reliability of health research. This includes a robust study design, careful data analysis, and transparent reporting of methods and results. Universal adoption of a defined set of confidence criteria in reporting the presence of plastics in humans is necessary to allow journal editors, reviewers, scientists, funding agencies, policymakers, journalists, industrial stakeholders, and the public to make informed decisions as to the reliability of the data being reported. Existence of reliable data is a prerequisite for a valid assessment of risk, and it is important to note that presence does not necessarily equate to hazard. Publishing human health research studies without contextualizing the quality of the underlying data raises significant risks of overinterpretation—both in terms of both false-positive and false-negative findings, and undermines scientific integrity, with associated ethical implications. Of particular concern are the risks associated with interpreting such data in research fields that may be unfamiliar with the limitations of the techniques used, especially when findings are communicated to the public through popular media. These risks underscore the need for cross-disciplinary dialogue and scientific collaboration to ensure the accurate interpretation and responsible communication of results. A clear framework to report the confidence level of analyses of micro- and nanoplastics in humans is the first key step in improving the rigor of data reporting in this emergent field, and its communication beyond to other associated, as well as nonspecialist audiences.

KEY MESSAGES

- Human exposure to micro- and nanoplastics is a growing concern. Plastic particles are increasingly reported in human tissues and fluids, raising concerns about health risks and the need for accurate exposure assessment.
- Current analytical techniques face significant limitations. Existing methods struggle with sensitivity, specificity, and contamination risks, especially in complex biological matrices. Uncertainties in detection and quantification must be addressed.
- Combining orthogonal techniques enhances confidence. Using multiple, fundamentally different analytical methods improves reliability and reduces bias. A proposed confidence framework is presented that categorizes techniques and guides their combined use.
- Transparent reporting and standardization are essential. Clear communication of methodological limitations, raw data availability, and adherence to quality control practices are critical for scientific integrity and informed decision-making.
- A global framework and community collaboration are needed. An interdisciplinary working group should define best practices and minimum standards for reporting plastic particles in humans, ensuring responsible communication and policy relevance.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.5c00671.

Overview and classification of analytical techniques used for micro- and nanoplastic characterization (PDF)

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Author Contributions

All authors drafted and reviewed the article.

Notes

The authors declare no competing financial interest.

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Leon P. Barron is a Professor of Analytical & Environmental Sciences at Imperial College London where he leads the Emerging Chemical Contaminants group within the School of Public Health. He is an analytical chemist and his research aims to identify, understand, and mitigate chemical threats in the environmental, forensic, and security fields. He is the Chair of the Royal Society of Chemistry (RSC) Separation Science Group and a Fellow of both the RSC and the Chartered Society of Forensic Sciences.

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