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Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics

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Abstract

The ubiquitous pollution of the environment with microplastics, a diverse suite of contaminants, is of growing concern for science and currently receives considerable public, political, and academic attention. The potential impact of microplastics in the environment has prompted a great deal of research in recent years. Many diverse methods have been developed to answer different questions about microplastic pollution, from sources, transport, and fate in the environment, and about effects on humans and wildlife. These methods are often insufficiently described, making studies neither comparable nor reproducible. The proliferation of new microplastic investigations and cross-study syntheses to answer larger scale questions are hampered. This diverse group of 23 researchers think these issues can begin to be overcome through the adoption of a set of reporting guidelines. This collaboration was created using an open science framework that we detail for future use. Here, we suggest harmonized reporting guidelines for microplastic studies in environmental and laboratory settings through all steps of a typical study, including best practices for reporting materials, quality assurance/quality control, data, field sampling, sample preparation, microplastic identification, microplastic categorization, microplastic quantification, and considerations for toxicology studies. We developed three easy to use documents, a detailed document, a checklist, and a mind map, that can be used to reference the reporting guidelines quickly. We intend that these reporting guidelines support the annotation, dissemination, interpretation, reviewing, and synthesis of microplastic research. Through open access licensing (CC BY 4.0), these documents aim to increase the validity, reproducibility, and comparability of studies in this field for the benefit of the global community.

Keywords: Harmonization, standardization, plastic, microplastic, metadata, reproducibility, open science, methods, reporting guidelines, comparability

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Introduction

The state of method reporting for investigations on microplastic pollution is currently at a turning point.[1] As this new research field evolves, it is striving to establish a harmonized community approach to developing, applying, and reporting methodologies. Two of the main purposes for reporting scientific methods are to allow for their replication and enable data to be directly comparable among studies. For example, in the environmental sciences, data from studies might be compared during risk assessments, synthesized for meta-analyses, or used to inform policy creation and monitoring guidelines. Issues with reproducibility and comparability of both data and methods are common across all scientific fields,[2–4] including microplastic research.[1,5,6] Here, this diverse group of 23 microplastic researchers from around the world, present a proposed step towards addressing this issue for microplastics, first by capturing what is already in published literature, and then by prioritizing which types of information should be included in research to reach this goal. Our four aims are to (i) review key reproducibility and comparability problems and solutions for microplastic research; (ii) discuss the open science framework used to identify and prioritize key methodological parameters suggested here; (iii) develop reporting guidelines for researchers to use when reporting, comparing, and developing methods; and (iv) present our vision for future microplastic research.

The Reproducibility and Comparability Turning Point in Microplastics Research

It is well-known that microplastics have a ubiquitous presence in the environment,[7–10] and the potential harm microplastics can cause to species across trophic levels has been recently reviewed.[11,12] While there is mixed evidence for effects, a range of suborganismal, organismal, and population-level responses have been reported.[6,11,13] These results have spurred substantial research activity, as evidenced by the continued exponential growth in the published literature on the topic of microplastics (Figure 1).

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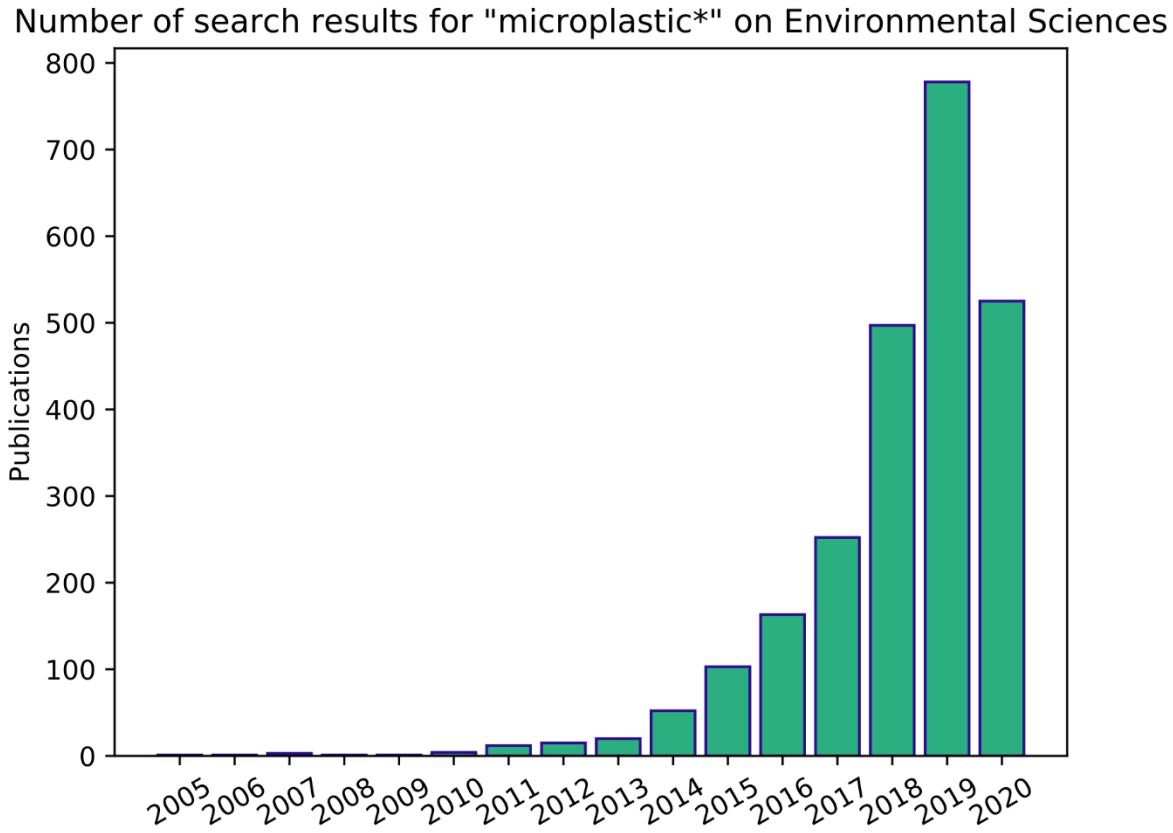

Figure 1. Data acquired from Scopus on 8 April 2020 using the search term "microplastic*" and querying the field of environmental sciences. Publications are annual sums. The figure was created using Python 3.6.9. The rapid expansion of research activities and the resulting data generated in the field of microplastics has resulted in a diverse suite of methods and non-standardized approaches to reporting sample collection, extraction, and analysis.[1,14–21] Each method has its strengths and weaknesses, and there are continued efforts to optimize existing methods and develop new ones that may improve throughput, detection limit, and reproducibility. The development of new methods continues because currently there is no 'catch-all' combination of methods for sampling, extracting, analyzing, and reporting microplastics that is capable of accurately characterizing and quantifying all microplastics present in a sample.[22,23] This is because microplastics are a diverse suite of contaminants that vary greatly in morphology, chemical properties, texture, color, density, and size.[24] Moreover, environments and research goals are diverse and a universal solution is unable to capture this diversity, especially as research matures in this rapidly expanding field. With this in mind, methods should be chosen

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based on the scientific question and reported with enough detail to be comparable and reproducible.

Comparability between studies facilitates meta-analysis,[25,26] which has been difficult for microplastics due to the diversity of methods employed and study details reported.[17–21]

Incomparability is caused by studies published without documenting the elements essential for translating units and metrics to others that are commonly used in the field. For example, studies that employ Raman spectroscopy might not be comparable to those that employ Fourier-transformed infrared (FT-IR) spectroscopy if neither describes their analysis and data transformation steps.[18,27] Additionally, aquatic studies that use water volume grab sampling are not comparable to studies that use net sampling if the studies do not describe the mesh size used, depth of sample collection, or the sample volume.[28] In another example, ingestion studies on the same species of animal are not comparable if they fail to mention which part of the gastrointestinal tract was analyzed (e.g., just the gizzard or the gizzard and proventriculus of birds).[15,17] Moreover, a study using different chemical digestion methods to measure ingestion may be incomparable because some digestion procedures destroy certain plastics.[29] Regardless of diverse methods and wherever possible, reporting raw - or less processed - data would allow reverse engineering and harmonization of some techniques. Still, raw data are seldom reported.[16,30]

Factors that cause incomparability can also hinder the reproducibility of research. Irreproducible research occurs, in part, when the elements that are critical for reproducing similar results are not elucidated. Reproducibility allows responsible decision-making and expansion of protocols. For example, software names should be reported when used because software often has proprietary algorithms and may not be reproducible unless the same software is used. In another example, if a study that employs organic matter digestion does not describe the chemical solution used, its manufacturer, and concentration used to digest the sample, the study cannot be reproduced.

Reporting guidelines provide a structured framework where method information critical to comparing and reproducing research can be referenced. There is a critical need for reporting guidelines in microplastic research as already initiated with the Minimum Information for Microplastic Studies (MIMS) concept for the study of microplastics in seafood,[1] the minimum information for publication of infrared-related data,[27] and other works assessing data quality in

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microplastic studies.[31–33] The reporting guidelines we developed attempt to build on previous work and expand the scope to more methodological components in microplastic research. This study leverages the expertise of a diverse group of researchers from around the world to cover the breadth of the field. To be as transparent as possible, we elaborate on the reasons why each reporting guideline is necessary and provide examples for each. Other fields, like molecular biology,[34] proteomics,[35] and transcriptomics,[36] already have highly successful examples of reporting guidelines that have been widely adopted by their field, and we hope this work serves a similar purpose in our field.

Methods

As a scientific community, we recognize that the need for reporting guidelines for microplastic methods is best addressed through a collaborative open science framework. With this goal in mind, the lead author sent out the following request on Twitter, and tagged several scientists in the microplastic community with a link to a collaborative document:

> *Frustrated with the reproducibility crisis in #microplastics research from poor method descriptions? Now is your chance to change that. I will publish this collaborative document OA* [*Open Access*]. *Add method considerations to this document and cite yourself in the Ack* [*Acknowledgements*].—Win Cowger, @Win_OpenData, 13 June 2019

The collaborative document was hosted open access on Google Drive and researchers were invited to provide input on the reporting guidelines for microplastic research methods. Over the subsequent week, 15 contributors edited the shared document directly. After one week, all initial contributors were invited to be coauthors, and additional coauthors were invited by word of mouth throughout the process using an open-door policy. Overall, there were 23 authors on this project and 26 other people acknowledged for their assistance. In a meeting of coauthors, the threshold for co-authorship was set at one full day of effort (self-defined and self-reported), while the threshold for acknowledgement was to review the document at least once. Authors contributed to this publication and the reporting guideline documents. The first author, Win

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Cowger, led the collaboration and the author order after the first author was randomized by agreement of all coauthors.

The reporting guidelines were identified by referencing standard operating procedures used by various authors and other peer-reviewed publications. All authors agreed not to use language that would imply an intent to standardize methodology or recommend specific methods over others; this was beyond the scope of the work. The task of the authors in developing the reporting guidelines was to outline what should be reported about a method when the method was used to make the method reproducible and comparable. To determine which guidelines were essential to add to the documents, each author was asked to fill out a Google Form survey where they designated each reporting category as required or not. The final reporting guidelines were formed by keeping only the guidelines that 51% or more of the authors agreed upon. During the review process, we received requests by reviewers to add additional reporting requirements. Where they were not already accounted for, we added them to the reporting guidelines and indicated those additions using an asterisk throughout the produced documents. The final reporting guidelines were packaged into three documents which have the same information summarized with specific user groups in mind: (i) thorough, a Detailed Document, (ii) quick and simple, a Checklist (Table I), and (iii) interactive, an online Mind Map (Figure 2).

The reporting guidelines were sent out to other colleagues in the field for an endorsement and critique designated as signatories in the acknowledgments. After the first week, we received 19 endorsements. The manuscript and supporting information were also subject to internal review at the National Institute of Standards and Technology (NIST) and single blind peer review from Applied Spectroscopy. In these ways, we attempted to receive as much feedback as we could to develop reporting guidelines that reflect the diverse group of experts and the broad scope of methods in microplastic research. This framework represents an example of a way that scientists in any field can develop robust collaborations by sharing ideas and learning from one another while developing useful reference documents, even if they have not met before.

Reporting Guideline Document Descriptions

The three documents we created of the reporting guidelines include a (i) Detailed Document, (ii) Checklist (Table I), and (iii) online Mind Map (Figure 2). Each document has the same information summarized with different users in mind. These documents are expected to be useful

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for scientists researching microplastics, peer reviewers asked to evaluate research, and users of the data. These documents outline what needs to be reported for common methods in microplastic research to be reproducible and comparable. The documents can also be used when developing methods internally to quickly identify the essential components of a method to calibrate and control in a lab. The Detailed Document can be used when every detail listed in the reporting guidelines are important to know. The Checklist can be used to quickly reference the reporting guidelines and check off the guidelines relevant to a specific study. The Mind Map is useful for those who prefer interactive information workflows and want to be able to quickly summarize and expand the reporting guidelines at any level of detail.

Any of these documents can be used to reference the report guidelines. All of the documents contain the same information reformatted and summarized. In the documents, the general method groups we define are: Materials, Quality Assurance/Quality Control (QA/QC), Data Reporting, Field Sampling, Sample Preparation, Identification, Categorization, and Toxicology Considerations. Subgroups describe specific method techniques within each group. Some of the groups may be used more than once in a study while some may not be used at all. It is important to note that these documents are templates and one need only consider the guidelines from the groups of methods relevant to a given study. When using the documents, first, assess which groups of methods apply to the study. Subgroups of methods are tab separated to indicate more detailed levels of grouping. Next, assess which of the subgroups apply. These can be highlighted or opened for easy reference. Where the most detailed subgroups apply, all italicized reporting guidelines must be defined, described, or discussed for that method to be reproducible and comparable. All reporting guidelines always apply to groups that do not have subgroups. Importantly, these reporting guidelines are not meant to completely define what should be reported but are a proposal for the minimum guidelines. Below we detail each document individually and outline a path forward for the documents to be updated.

Detailed Document

The Detailed Document (Supplemental Material 1; OSF) is the plain-text thorough version of the reporting guidelines containing the identical information, groups, and order to the Checklist and Mind Map described below. While this document is the primary result of this project, its length precludes including it in the main manuscript. The Detailed Document is meant for those who

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are new to the methods or want a detailed description and reference examples of the reporting guideline. This document may also be useful to those who find the Mind Map format to be challenging to navigate. The Detailed Document is easily printed for reference, which can be especially useful during the design stage of a study. The format of this document follows that the highest level of method grouping is in the largest text font and bolded. Subgroups of methods are in bold and identical font size but further indented if they are a subgroup of a subgroup. The essential elements to report are italicized and all the same font size. The explanation, reason, and examples for each essential element immediately follow the element and are light gray in color.

The Checklist

The Checklist (Supplemental Material 2; OSF; Table I) is meant for those already familiar with the methods and reasons for reporting outlined in the other documents. The format follows the Detailed Document but the explanation, reason, and examples for each reporting guideline are removed for quick reference and reading so that the elements can be checked off when reviewing or writing documents. Citations used in the Detailed document are added at the end of each guideline. The reporting guidelines are italicized and all the same font size as in the Detailed Document.

Table I: This is the Checklist of the reporting guidelines. Asterisks (*) indicate that the guideline was added as part of peer review; all other guidelines were voted on by a majority of the coauthors. The guidelines are grouped using bolded and indented labels. The guidelines are italicized and are the furthest indented for each group. Citations correspond to additional information related to the guideline and good examples of reporting.

Reporting Guidelines Checklist
Components to Report in All Procedures
Materials
All manufacturers of materials and instruments and their calibration[37]
All software used and their calibration[38]

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Quality assurance/quality control
Error propagation
How instrumental, methodological, and/or statistical error was propagated[39–41]
Replicates
Number of replicates[42]
How replicates were nested within samples[43]
Limit of detection
Quantitative detection threshold[44]
Plastic morphology, size, color, and polymer limitations of method[1,29,53,45–52]
Method of accounting for nondetects[19,54]
Blank controls
Number of controls[1,31]
Characteristics of plastics found in blanks with the same rigor as samples[45]
Potential sources of contamination[55]
Point of entry and exit to method[55]
Positive controls
Morphology, size, color, and polymer type of positive controls[1,31,56]
Positive control correction procedure[31,56]
Point of entry and exit to method[56]
Contamination mitigation
Clothing policies[1,57]
Purification technique for reagents[50,58]

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Glassware cleaning techniques[59]
Containment used (e.g. laminar flow cabinet/hoods, glove bags)[1,50,60–62]
Data reporting
Share raw data and analysis code as often as possible[18,22,38,63,64]
Field Sampling
Where (e.g., region) and when (e.g., date, time) the sample was collected[19,65–70]
Size (e.g., m^3, kg) and composition (e.g., sediment, water, biota) of the sample[1,71]
Location at the site that sample was collected (e.g., 3 cm depth of surface sediment)[72]
Sample device dimensions and deployment procedures[14,31,73–75]
Environmental or infrastructure factors that may affect the interpretation of results[75–81]
How samples are stored and transported[1,82,83]
Sample Preparation
Homogenization
Homogenization technique[84]
Splitting/subsetting
Sample splitting/subsetting technique[75]
Drying
Sample drying temperature and time[85]
Synthesized plastic
Synthesized plastic polymer, molecular characteristics, size, color, texture, and shape[86,87]
Synthesized plastic synthesis technique[86,88]
Fluorescent dye

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Dye type, concentration, and solvent used[89–91]
Dye application technique[89]
Sieving strategy
Sieve mesh size[84]
If the sample was wet or dry sieved[84]
Density separation
Concentration, density, and composition (e.g. $CaCl_2$, ZnCl) of solution[82,92,93]
Time of separation[94]
Device used[61,94–98]
Digestion
Duration and temperature of digestion[21,99,100]
Digestion solution composition[21,56,100]
Ratio of digestion fluid to sample[21,56,100,101]
Filtration
Filter composition, porosity, diameter[50,102,103]
Microplastic Identification
Visual identification
Imaging settings
Image settings (e.g., contrast, gain, saturation, light intensity)[18]
Magnification (e.g., scale bar, 50X objective)[104]
Light microscopy
Magnification used during identification[90]

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Shapes, colors, textures, and reflectance, used to differentiate plastic[104–106]
Fluorescence microscopy
Magnification used during identification[90]
Fluorescence light wavelength, intensity, and exposure time to light source[90,91,107]
Threshold intensity used to identify plastic[107]
Scanning electron microscopy (SEM)
The coating used (e.g., metal type, water vapor)[108]
Magnification used during identification[108]
Textures used to differentiate plastic[108]
Chemical identification
Pyrolysis gas chromatography mass spectrometry (py-GC/MS)
Pyrolysis reacting gases, temperature, duration[49,109]
GC oven program, temperature, carrier gas, and column characteristics[49,109]
MS ionization voltage, mass range, scanning frequency, temperature[18,49]
py-GC/MS matching criteria (i.e., match threshold, linear retention indices (LRI), and Kovats index)[49,110]
py-GC/MS quantification techniques[109]
Raman spectroscopy
Acquisition parameters (i.e., laser wavelength, hole diameter, spectral resolution, laser intensity, number of accumulations, time of spectral acquisition)[37,63,111–115]
Pre-processing parameters (i.e., spike filter, smoothing, baseline correction, data transformation)[56,112,115,116]
Spectral matching parameters (i.e., spectral library source, range of spectral wavelengths used to match, match threshold, matching procedure)[37,50,63,70,111–115,117]
Fourier-transform infrared spectroscopy (FT-IR)
Acquisition parameters (i.e., mode of spectra collection, accessories, crystal type, background recording, spectral range, spectral resolution, number of scans)[63,64,103]

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Pre-processing parameters (i.e. Fourier-transformation (FT) parameters, smoothing, baseline correction, data transformation)[18]
Matching parameters (i.e., FT-IR spectral library source, match threshold, matching procedure, range of spectra used to match)[38,50,64,112]
Differential scanning calorimetry (DSC)
Acquisition parameters (i.e., temperature, time, number of cycles)[20]
Matching parameters (i.e., parameters assessed, reference library source, comparison technique)[20]
Microplastic Categorization
Shape, size, texture, color, and polymer category definitions[24,118,119]
Microplastic Quantification
Units (e.g., kg, count, mm)[1,120]
Size dimensions (e.g., Feret minimum or maximum)[18]
Quantification techniques[18]
Toxicology Considerations
Dosed plastic age, polymer, size, color, and shapes[121–130]
Animal husbandry[131,132]
Exposure concentration, media, and time[132–138]
*Effects evaluation metrics (e.g., what markers were evaluated?)**
*Biota metrics (e.g., which tissues were analyzed?)**

Mind Map

The Mind Map (Supplemental Material 3; LINK; OSF; Figure 2) was developed because we recognized a need to have many intermediate levels of detail between the detail provided by the Detailed Document and the Checklist. Interactive mind map documents allow the user to query to the level of detail they need quickly. This is meant for users who prefer spatially structured interactive information queries. The Mind Map was formatted using www.mindmeister.com, a

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free collaborative mind map creator that can reformat mind maps into tiered documents. The Mind Map is structured the same as the Detailed Document, where general method groups flow from the primary term "Microplastics Reporting Guidelines". These general groups are further refined by subgroups of method types and instrument groups, where the terminal node of every branch leads to essential methodology elements (italicized) that should be reported. Each reporting guideline is described by an explanation, reasons to report, and/or examples from published microplastic literature.

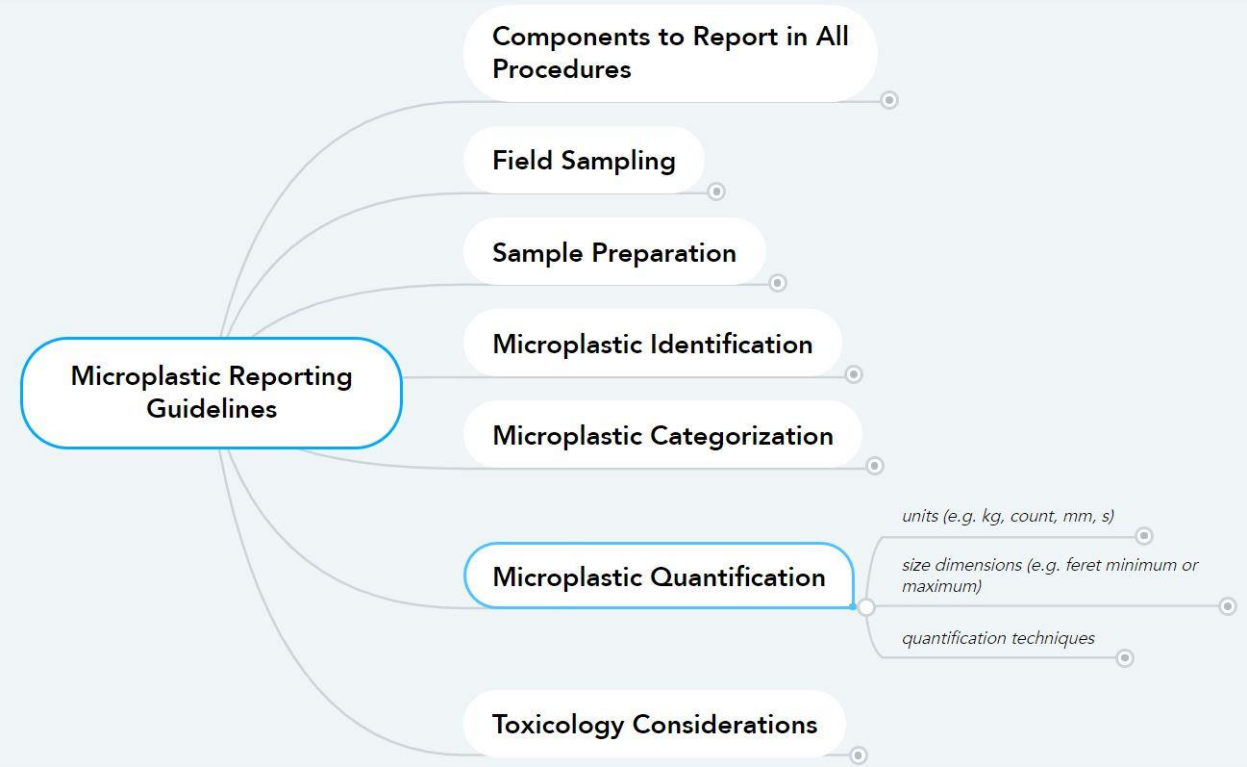

Figure 2. A screenshot of the Mind Map (LINK) showing the components and flow of reporting guidelines for microplastic studies. The first nodes branching off of "Microplastic Reporting Guidelines" are the general groups of the guidelines, subgroups follow in bold until the second to last nodes are the reporting guidelines (in italic) and the terminal node is the description of the guideline

Strategy for Updating the Reporting Guidelines

The field of microplastic research is rapidly evolving, and we expect that our documents, like most things in science, will need to be adapted, expanded, and revised. We recognize that as the

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field of microplastic pollution develops and grows, there will be new techniques and methods developed that will have reporting guidelines. We also acknowledge other methods are already useful to report that are not yet covered here. These documents are expected to be updated over time as new techniques are developed. That is why all documents are completely free and hold open access licenses (CC BY 4.0). The license allows for redistribution and adaptation with attribution to the original document. Additionally, we created an Open Science Framework project (OSF) for each document where researchers can reach out with suggestions and comments to update future editions of these documents. The authors will monitor the comments on the project and respond, as necessary. Future versions will be updated periodically on the OSF project site using version control. Additionally, we submitted this reporting guideline and others reported in the literature[1,27] to the reporting guideline portal at https://fairsharing.org/. We hope that these documents and online forums are widely used for the benefit of the global community.

Our Vision of the Future of Research on Microplastics

We envision a future where research on microplastics is comparable, reproducible, and transparent. We aim for researchers in the field to be able to read a paper and use the methods for their work and/or use the data in a synthesis paper or meta-analysis. We aim for policymakers and managers to be able to review the literature and have the ability to compare data across sources, pathways, and geographies to inform the decision-making process. We envision a field where communication is clear amongst different stakeholders in the world of microplastics and where collaboration and research translation are made simpler. With our collaborative and open access framework, we aim to improve future work on microplastics and provide a framework for other emerging contaminants.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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Supplemental Material

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Reporting guidelines for microplastic research: Checklist

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How to use:

The Checklist is meant for those already familiar with the methods and reasons for reporting outlined in the other documents. This document outlines the essential elements to report about microplastic research methods to make them reproducible and comparable. This attempts to cover most of the common methods in the field but some methods have not been covered and researchers will need to develop their own guidelines for those. Groups of methods are in bold. To use these guidelines, first assess which groups of methods apply to your study. Next, assess which of the subgroups (tab separated to indicate more detailed levels of grouping) apply to your study. These can be highlighted for easy reference. At the level of the most detailed subgroups that apply to your study, all italicized criteria must be defined, described, or discussed for the method to be reproducible and comparable. All criteria always apply to groups that do not have subgroups. When units are given, they are examples not prescriptive. Whenever “i.e.” is used we think that all the stated components are important to report. Guidelines indicated with an asterisk (*) were recommended during the review process and added. All other guidelines were voted on by at least a majority (51%) of coauthors of this document. Finally, we recognize that these

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guidelines should not be the sole criteria for determining what is suitable for publication, but should be a tool used to help reporting methods in publications improve in general.

Reporting Guidelines Checklist
Components to Report in All Procedures
materials
all manufacturers of materials and instruments and their calibration[1]
all software used and their calibration[2]
quality assurance/quality control
error propagation
how instrumental, methodological, and/or statistical error was propagated[3–5]
replicates
number of replicates[6]
how replicates were nested within samples[7]
limit of detection
quantitative detection threshold[8]
plastic morphology, size, color, and polymer limitations of method[9–19]
method of accounting for nondetects[20,21]
blank controls
number of controls[9,22]
characteristics of plastics found in blanks with same rigor as samples[12]
potential sources of contamination[23]
point of entry and exit to method[23]
positive controls
morphology, size, color, and polymer type of positive controls[9,22,24]

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positive control correction procedure[22,24]
point of entry and exit to method[24]
contamination mitigation
clothing policies[9,25]
purification technique for reagents[17,26]
glassware cleaning techniques[27]
containment used (e.g. laminar flow cabinet/hoods, glove bags)[9,17,28–30]
data reporting
share raw data and analysis code as often as possible[2,31–34]
Field Sampling
where (e.g. region) and when (e.g. date, time) the sample was collected[20,35–40]
size (e.g. m3, kg) and composition (e.g. sediment, water, biota) of the sample[9,41]
location at the site that sample was collected (e.g. 3 cm depth of surface sediment)[42]
sample device dimensions and deployment procedures[22,43–46]
environmental or infrastructure factors that may affect interpretation of results[46–52]
how samples are stored and transported[9,53,54]
Sample Preparation
homogenization
homogenization technique[55]
splitting/subsetting
sample splitting/subsetting technique[46]
drying
sample drying temperature and time[56]
synthesized plastic
synthesized plastic polymer, molecular characteristics, size, color, texture, and shape[57,58]
synthesized plastic synthesis technique[57,59]
fluorescent dye
dye type, concentration, and solvent used[60–62]
dye application technique[60]

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sieving strategy
sieve mesh size[55]
if sample was wet or dry sieved[55]
density separation
concentration, density, and composition (e.g. CaCl2, ZnCl) of solution[53,63,64]
time of separation[65]
device used[29,65–69]
Digestion
duration and temperature of digestion[70–72]
digestion solution composition[24,70,72]
ratio of digestion fluid to sample[24,70,72,73]
Filtration
filter composition, porosity, diameter[17,74,75]
Microplastic Identification
visual identification
imaging settings
image settings (e.g. contrast, gain, saturation, light intensity)[31]
magnification (e.g. scale bar, 50X objective)[76]
light microscopy
magnification used during identification[61]
shapes, colors, textures, and reflectance, used to differentiate plastic[76–78]
fluorescence microscopy
magnification used during identification[61]
fluorescence light wavelength, intensity, and exposure time to light source[61,62,79]
threshold intensity used to identify plastic[79]
scanning electron microscopy (SEM)
coating used (e.g. metal type, water vapour)[80]
magnification used during identification[80]
textures used to differentiate plastic[80]

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chemical identification
pyrolysis gas chromatography mass spectrometry (py-GC/MS)
pyrolysis reacting gases, temperature, duration[16,81]
GC oven program, temperature, carrier gas, and column characteristics[16,81]
MS ionization voltage, mass range, scanning frequency, temperature[16,31]
py-GC/MS matching criteria (i.e. match threshold, linear retention indices (LRI), and kovats index)[16,82]
py-GC/MS quantification techniques[81]
raman spectroscopy
acquisition parameters (i.e. laser wavelength, hole diameter, spectral resolution, laser intensity, number of accumulations, time of spectral acquisition)[1,33,83–87]
pre-processing parameters (i.e. spike filter, smoothing, baseline correction, data transformation)[24,84,87,88]
spectral matching parameters (i.e. spectral library source, range of spectral wavelengths used to match, match threshold, matching procedure)[1,17,33,40,83–87,89]
Fourier-transform infrared spectroscopy (FTIR)
acquisition parameters (i.e. mode of spectra collection, accessories, crystal type, background recording, spectral range, spectral resolution, number of scans)[33,34,75]
pre-processing parameters (i.e. fourier-transformation (ft) parameters, smoothing, baseline correction, data transformation)[31]
matching parameters (i.e. FTIR spectral library source, match threshold, matching procedure, range of spectra used to match)[2,17,34,84]
differential scanning calorimetry (DSC)
acquisition parameters (i.e. temperature, time, number of cycles)[90]
matching parameters (i.e. parameters assessed, reference library source, comparison technique)[90]
Microplastic Categorization
shape, size, texture, color, and polymer category definitions[91–93]
Microplastic Quantification
units (e.g. kg, count, mm)[9,94]
size dimensions (e.g. feret minimum or maximum)[31]
quantification techniques[31]
Toxicology Considerations

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dosed plastic age, polymer, size, color, and shapes[95–104]
animal husbandry[105,106]
exposure concentration, media, and time[106–112]
*effects evaluation metrics (e.g. what markers were evaluated?)**
*biota metrics (e.g. which tissues were analyzed?)**

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Reporting guidelines for microplastic research: Detailed Document

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How to use:

This document outlines the essential elements to report about microplastic research methods to make them reproducible and comparable. This attempts to cover most of the common methods in the field but some methods have not been covered and researchers will need to develop their own guidelines for those. Groups of methods are in bold. To use these guidelines, first assess which groups of methods apply to your study. Next, assess which of the subgroups (tab separated to indicate more detailed levels of grouping) apply to your study. These can be highlighted for easy reference. At the level of the most detailed subgroups that apply to your study, all italicized criteria must be defined, described, or discussed for the method to be reproducible and comparable. Descriptions follow each reporting requirement in gray. All criteria always apply to groups that do not have subgroups. When units are given, they are examples not prescriptive. Whenever "i.e." is used we think that all of the stated components are important to report. Guidelines indicated with an asterisk (*) were recommended during the review process and added. All other guidelines were voted on by at least a majority (51%) of coauthors of this document. Finally, we recognize that these guidelines should not be the sole criteria for determining what is suitable for publication, but should be a tool used to help reporting methods in publications improve in general.

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Components to Report in All Procedures

materials

all manufacturers of materials and instruments and their calibration

The suppliers of instruments, labware, consumables, and chemicals (e.g. dyes, digestion materials, density separation salts) used should be clearly reported. For instruments, the model and manufacturer should be stated. For general labware, relevant material properties (glass, plastic, other etc) and sizes/volumes should be clearly defined, and the supplier stated. Where relevant, quantities of consumables used should be specified. Many types of calibration are mandatory for normal functioning of the instrument. For example, laser alignment can change over time in an Fourier-transformed infrared (FTIR) or Raman spectroscopy instrument. The lasers must be aligned so that the peaks are not shifted (e.g. a peak for silicon should appear at 520.7 cm^{-1} on the Raman shift) and the intensity of the peak should be within an acceptable range to produce quality spectra with an acceptable signal to noise ratio. Calibration is necessary to ensure that spectra can be compared to other spectra obtained on a different instrument in order to be independent of instrument-specific artefacts. Examples: (Oßmann et al. 2018)

all software used and their calibration

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Software can have different analysis steps and assumptions built into their functioning and when the software is proprietary, these steps may be unknown. Software is used with most analytical sampling instruments and statistical analyses and should be stated as the name of the software. Any tuning parameters specific to the software should also be described. Examples: (S. Primpke et al. 2017)

quality assurance/quality control

error propagation

how instrumental, methodological, and/or statistical error was propagated

Many types of error and uncertainty can be calculated and propagating this error typically requires detailed steps which should be described. Examples: (Hurley, Woodward, and Rothwell 2018; Kedzierski et al. 2019; Haave et al. 2019)

replicates

number of replicates

Replication is how variability is calculated and accounted for. The number of replicates determines the interpretation of the accuracy of the measurements. Therefore the number of replicates should always be reported. Examples: (Cable et al. 2017)

how replicates were nested within samples

The scale of replication can be nested to allow for calculating the variability at multiple levels. For example a field sample could be replicated at a certain time or in a specific location to measure the variability or compare variables. Examples: (Frias, Sobral, and Ferreira 2010)

limit of detection

quantitative detection threshold

The percent, count, mass, or volume of plastics that a given method is able to detect is a central metric for assessing the error of a method. This can be calculated using blank and positive controls described below or based on the instrument specifications. The quantitative detection threshold of plastics should be reported in its least aggregated form by describing the quantity for shape, size, polymer, and/or color categories. Examples: (Martin et al. 2018)

plastic morphology, size, color, and polymer limitations of method

All analytical methods have limitations based on morphology, size, color, and/or polymers. Plastics, particularly at the microscale, can be mistaken for non-plastics and vice versa. There can be substantial contamination from certain shapes (e.g. fibers, (McNeish et al. 2018)**).** (M. Liboiron et al. 2016) **and** (Song et al. 2015) **found that visual identification with a microscope became unreliable for plastics under 1 mm in size. Based on transparency some plastics may not be visible using visual methods. Based on the color of some contamination sources, a study may exclude a whole group of colored particles. Some polymers like Polytetrafluoroethylene (PTFE) (2.2 g ml^{-1}) may be more dense than density separation procedures and other polymers may be vulnerable to some digestion procedures** (Enders et al. 2017)**. These considerations warrant specific discussion of method limitations based on shape, size, color, and polymer. Examples:** (Nel et al. 2019; Hermabessiere et al. 2018; Dehaut, Hermabessiere, and Duflos 2019; Lorenz et al. 2019; Devriese et al. 2015; Vandermeersch et al. 2015; Hendrickson, Minor, and Schreiner 2018)

method of accounting for nondetects

Nondetects are measurements which are below the limit of detection. Non-detects can be handled with appropriate statistical testing (Helsel 2006)**. There are many different methods, so the method used should be described. Examples:** (Brander, submitted)

blank controls

number of controls

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It is important to know that the number of controls determines the measurement of uncertainty of the control results. Examples: (Hermsen et al. 2018; Dehaut, Hermabessiere, and Duflos 2019)

characteristics of plastics found in blanks with same rigor as samples

Controls are often used to correct the full results. Characterizing controls with the same rigor as the samples allows for a complete comparison between control and sample results. Examples: (McNeish et al. 2018)

potential sources of contamination

It is important to discuss what may have contributed to any contamination in the field or lab for interpretation of results. This includes but is not limited to the types of clothing/safety vests worn by field and lab personnel, the lack of air filtration systems, or the proximity to chairs, carpet, curtains or other sources. Examples: (Miller et al. 2017)

blank correction procedure

In blank correction, the value observed in blanks is removed from the value counted in samples taking into account multiple variables for a thorough correction (Dehaut et al. 2019). Some common procedures are subtraction, mean value subtraction, and comparisons of distributions in blanks and samples. These procedures are not standardized and should be described. Examples: (Miller et al. 2017)

point of entry and exit to method

If a blank sample is a field blank, it enters the method workflow in the field. If it is a lab blank, it enters the method in the lab. Exit point is the time and location that the sample was quantified (e.g. "blanks were quantified at the same time and location as the sample was.") Examples: (Miller et al. 2017)

positive controls

morphology, size, color, and polymer type of positive controls

The recovery efficiency of a positive control determines the bias of the method. Factors that can affect recovery and interpretation of results are morphology, size, color, and polymer type. Examples: (Dehaut, Hermabessiere, and Duflos 2019; Hermsen et al. 2018; Dehaut et al. 2016)

positive control correction procedure

Take the results of positive controls into account to discuss the performance of the methods. In positive control correction, the loss or gain value observed in positive controls is removed from the value counted in samples taking into account multiple variables for a thorough correction. Some common procedures are subtraction, mean value subtraction, and comparisons of distributions in controls and samples. These procedures are not standardized and should be described. Examples: (Hermsen et al. 2018; Dehaut et al. 2016)

point of entry and exit to method

If a positive sample is a field control, it enters the method workflow in the field. If it is a lab control, it enters the method in the lab. Exit point is the time and location that the sample was quantified, (e.g. "controls were quantified at the same time and location as the sample was.") Examples: (Dehaut et al. 2016)

contamination mitigation

clothing policies

Contamination in samples can come from clothing and gloves (Dehaut, Hermabessiere, and Duflos 2019). The material of clothing in the field and lab should be described. Examples: (Van Cauwenberghe and Janssen 2014).

purification technique for reagents

There is no microplastic-free standard solution available on the market (Dehaut, Hermabessiere, and Duflos 2019). Reagent producers are not familiar with microplastic contamination and atmosphere in production facilities plants can contain microplastics. It is important to filter all used reagents. The degree of filtration determines the degree of potential contamination and the method for filtering reagents should be reported. Examples: (De Witte et al. 2014; Lorenz et al. 2019)

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glassware cleaning techniques

Glassware can adsorb microplastics particles which contaminate samples. Muffling, thermal destruction of microplastics, can be used to treat glassware (500°C) but may be limited to small glassware. Rinsing - using filtered reverse osmosis or Milli-Q water can also be employed to remove residual microplastic particles before use. Whatever method is used it should be thoroughly described. Examples: (Tanaka and Takada 2016)

containment used (e.g. laminar flow cabinet/hoods, glove bags)

Containment devices like laminar flow hoods, glove bags and other enclosed devices decrease contamination. Without containment, small fragments and fibers can contaminate the samples (Wesch et al. 2017; Lorenz et al. 2019)**. Not all containment devices have the same capabilities** (Wesch et al. 2017)**, it is thus very important to use and describe containment devices used to physically protect samples from particles. Examples:** (Claessens et al. 2013; Torre et al. 2016; Dehaut, Hermabessiere, and Duflos 2019; Lorenz et al. 2019)

data reporting

share raw data and analysis code as often as possible

Raw data is data in its least aggregated form. The level of aggregation typically depends on the methods used and as a general rule, the data recorded onto a field or lab sheet or files created from an instrument will be the rawest form of data possible. Raw data can allow for the reverse engineering of methods and improve the ease of comparability. Analysis code records all analysis steps and makes method reporting simpler. Raw data and analysis code should be shared with all publications. Examples: (S. Primpke, Dias, and Gerdts 2019; Cabernard et al. 2018; Sebastian Primpke et al. 2018; S. Primpke et al. 2017)

Field Sampling

where (e.g. region) and when (e.g. date, time) the sample was collected

Location determines the ecology, geology, climate, geopolitical standing, and socio-economic setting of the sample. The location where field samples are collected can be expressed using latitude and longitude or other coordinates with coordinate system reference datum and a map (Bagaev et al. 2017)**. Research has shown that different seasons** (Spear, Ainley, and Ribic 1995; Cheung, Cheung, and Fok 2016)**, weather such as storms** (Lattin et al. 2004)**, wind** (Browne, Galloway, and Thompson 2010)**, time between repetition of sampling** (Smith and Markic 2013)**, and other time- and date-dependent factors affect plastic distribution and types. This is as true for shoreline, benthic, and water sampling where particle movements are influenced by the weather. In biotic sampling seasons, time of day and other factors affect feeding behaviour. Noting these trends is important for understanding environmental relationships. The date and time of sample collection should be recorded, including information on seasons, during data collection. When time is reported, a standardised format should be used, such as UTC (Coordinated Universal Time) or include the time zone and information about how the date is recorded. This data can be correlated with season, weather events, tides, and other time-based factors. For example,** (Cheung, Cheung, and Fok 2016) **found high seasonal variance between wet and dry seasons in microplastics concentrations in the estuary of a subtropical macro-scale drainage basin in South China, to the degree that "if the mean abundance of microplastics was determined solely by winter sampling, an underestimation of 73% would occur"** (Cheung, Cheung, and Fok 2016)**. A study by Smith and Markic on shorelines "in eastern Australia indicates that estimated daily accumulation rates [of plastics] decrease rapidly with increasing intervals between surveys, and the quantity of**

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available debris is underestimated by 50% after only 3 days and by an order of magnitude after 1 month." (Smith and Markic 2013). Examples: (Brander, submitted)

size (e.g. m3, kg) and composition (e.g. sediment, water, biota) of the sample

With increased sample size there is an increase in statistical power and certainty in interpretation. Volume and mass of many samples are typically dominated by water content. These sample size units should be described as wet or dry. Differences in sample composition could reflect the heterogeneity in plastics distribution, and the composition itself may affect the results (e.g. wet weight of silty versus sandy sediment, the fraction of suspended particulate matter, or the influence of algae blooms on the size of the plankton sample).The composition of the sample taken must be stated (e.g. sediment, water, plankton and biota (including tissue vs organ) samples). Examples: (Dehaut, Hermabessiere, and Duflos 2019; Leslie et al. 2017)

location at the site that sample was collected (e.g. 3 cm depth of surface sediment)

Plastic can be stratified by depth and width at intervals that are not standard across media (Willis et al. 2017). The location at the sample site where the sample was taken from, describing this typically involves describing the depth and width or area of the sample where the sample was taken within the site (e.g. the stream flow sample was taken from the stream bed to 0.5 m above the stream bed).

sample device dimensions and deployment procedures

Device dimensions and deployment procedures affect the sample (e.g. environmental layer, life stage) and results of microplastic detection in marine environmental samples. The mesh size of a net or sieve will determine the lower size limit of particle retention and the aperture will determine the upper limit. The width, length, height, aperture size, and mesh size of the device should be stated. How the device is deployed (e.g. from the side of the vessel vs behind the vessel, at what speed) will also affect the sample concentration and should be stated. Examples: (Hermsen et al. 2018); Mesh Size: ("Interim Report of the Working Group on Marine Litter (WGML)," 2018; Hidalgo-Ruz et al. 2012; Covernton et al. 2019) Device deployment: (Amy L. Lusher et al. 2014)

environmental or infrastructure factors that may affect interpretation of results

Environmental factors can greatly affect the concentrations of microplastics in field samples. Report environmental factors (e.g. winds/ocean currents) (Reisser et al. 2015) or extreme weather events (storms) associated with time samples were collected. Mention any sources of anthropogenic influence (e.g. storm drains, run-off, etc), pH, Salinity, Dissolved (in)organic content. Timing and influence of prior cleanup events. Information on the sample device positioning during sampling should be reported relative to these fluxes. Examples: (Amy L. Lusher et al. 2014; Enders et al. 2015; Hardesty et al. 2017; Iwasaki et al. 2017; Kukulka, Law, and Proskurowski 2016; Frère et al. 2017)

how samples are stored and transported

Transporting samples in plastic materials could contaminate the sample or create a static charge on the sample. Additionally the solution the sample is transported and stored in could be important because formaldehyde or ethanol could lead to volume change in particles or affect the use of enzyme downstream. If samples are stored without biocide, the particles could become fouled and affect other measurements later on (Crichton et al. 2017). Rough handling of samples can lead to fragmentation of plastic particles. Examples: (Courtene-Jones et al. 2017; Dehaut, Hermabessiere, and Duflos 2019)

Sample Preparation

homogenization

homogenization technique

Some homogenization techniques can break up plastic particles. Therefore it is important to report what techniques samples homogenized by (e.g. sonication with probes, agitation). Examples: (Wagner et al. 2017)

splitting/subsetting

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sample splitting/subsetting technique

Some sample splitting and subsetting techniques introduce more error than others, and if the subsample is not representative of the whole sample, this may lead to over- or under-estimation of particle count, and/or improper particle diversity characterization. If the whole sample was not analyzed and instead was split in some way (e.g. for application in different methods), what were the parameters or techniques for splitting? Parameters include how the sample was mixed before and during splitting, what volume or proportion of the total sample was taken, whether the sample was split before filtration/isolation, or afterwards. If before, were they split by size fractionation, or some other technique? If afterwards, what area of the filter was subsampled and where. Examples: (A. L. Lusher et al. 2017)

drying

sample drying temperature and time

Some plastics are destroyed by high temperatures and the time of drying sets the degree of drying. Therefore, drying temperature and time are important to report. Examples: (Dekiff et al. 2014)

synthesized plastic

synthesized plastic polymer, molecular characteristics, size, color, texture, and shape

Microplastic reference materials (MRMs) are either purchased, synthesized or ground up from post-consumer material. MRMs may be used in method development/validation/recovery tests, as positive blanks or controls, in toxicology studies, or in studies investigating processes such as weathering, transport, settling rates and resuspension. To improve reproducibility, it is important to describe the origin of MRMs, including how they were prepared and what their physicochemical properties are. Essential parameters to report are polymer composition, particle size distribution, particle shape/morphology (spherical, irregular, fibrous, foam, film etc) and color of the particles. Where possible, the presence of any other known chemicals or materials associated with the MRM should be specified (e.g. surfactants, impurities, additive chemicals), molecular weight distribution (MWD), melt-flow-index, melting point, glass transition temperature, crystallinity, additive chemical profile (from non-target screening analysis), metal content. Reporting Examples: (Vicentini et al. 2019) **characterized the plastic particles on its morphology, diameter and suspension stability as well as performed toxicity assays with the synthesized particles.** (Wang et al. 2017) **quantified the concentrations of depolymerized building block compounds such as bisphenol A (BPA) and para-phthalic acid (PTA). The polycarbonate (PC) and PET MPs concentrations in the environmental samples from an activated sludge reactor were estimated by comparing the samples with BPA and PTA chromatographs.**

synthesized plastic synthesis technique

If the polymers were synthesized in-house, what techniques, glassware/reactors, chemicals, reactions, and additives were used? A brief description of the techniques used, chemicals used and their function on the reaction, proceedings and any other details that may be related to the synthesis process. If the material is synthesised or produced by milling (or similar), method details should be clearly described. The synthesis technique may describe potential reactions and aid in listing excipients as possible alterations for the toxicity. Reporting Examples: (Lu, Qu, and Forcada 2009) **reported the whole detailed technique for synthesis and** (Vicentini et al. 2019) **reported the methodological adaptations for the same synthesis elaborated by** (Lu, Qu, and Forcada 2009)**.**

fluorescent dye

dye type, concentration, and solvent used

Fluorescent dyes can be used for the selective staining of common polymers, aiding in sample sorting and speeding object identification for spectroscopy. Different dyes have different fluorescent properties. Dye concentration determines the extent of stain and background fluorescence. Solvents have a variety of solubilities for the dye and some solvents can dissolve some plastic. Describe what solvent (e.g. acetone, hexane, n-propanol) was used to make the nile red stock solution. Examples: (Karakolis et al. 2019; Wiggin and Holland 2019; Maes, Van der Meulen, et al. 2017)

dye application technique

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If dye is applied before some steps (like digestion) there could be reduced fluorescence. This will affect the fluorescence intensity and the chance of false positives and false negatives. Therefore, explain how and when was the microplastics exposed to the stain (e.g. dilution, directly). Describe the incubation conditions (e.g. concentration, temperature, duration) of the plastics and dye solution. Examples: (Karakolis et al. 2019)

sieving strategy

sieve mesh size

Sieve mesh size sets the smallest diameter of the particle extracted. Examples: (Wagner et al. 2017)

if sample was wet or dry sieved

Wet sieving and dry sieving typically have different separation efficiencies because particles may be left behind in the sieve. Therefore, whether the sieving was done wet or dry is important to report. Reporting Examples: (Wagner et al. 2017)

density separation

concentration, density, and composition (e.g. CaCl2, ZnCl) of solution

Depending on the density used for separation some polymer types of plastics might not be completely separated. The concentration of the salt in mass per volume of the solution used in density separation should be stated. The target, estimated, or measured density of the solution should also be stated. Concentration and density, while possibly sharing the same units (g/mL) are different measurements and both should be reported. For example, a 30% solution of NaCl is 30 g of NaCl per 100 mL of liquid and the density is the total mass of the combined solution divided by its volume. Salts can react with the matrix in many ways and salts adhered to the surface of the plastic can influence spectral interpretation. Therefore, report the type of salt used to create the high-density fluid and methods used to remove the salts from the isolated microplastics. In (Ivleva, Wiesheu, and Niessner 2017), an example graph shows the different limitations of separation fluids. Examples: (Crichton et al. 2017; Mani et al. 2019)

time of separation

Separation efficiency is a function of time with increases in time generally increasing separation efficiency. The duration of separation or settling should be specified. Examples: (Imhof et al. 2012)

device used

Devices have different recovery rates and particle size limitations. Describe the device used for density separation (e.g. flotation & supernatant filtration) (Thompson et al. 2004), elutriation (Claessens et al. 2013), sediment fluidization (Wessel et al. 2016), froth flotation (Imhof et al. 2012), separation funnel (Masura et al. 2015). Examples: "Sediment-Microplastic Isolation unit" (Coppock et al. 2017). "Munich Plastic Sediment Separator (MPSS)" (Imhof et al. 2012).

digestion

duration and temperature of digestion

Duration and temperature combination can have different deleterious effects on plastics (Munno et al. 2018; Thiele, Hudson, and Russell 2019). Duration (using a clear format (e.g. hours)) and temperature of incubation (°C) should be stated. Examples: (Thiele, Hudson, and Russell 2019; A. Lusher, submitted)

digestion solution composition

Some chemicals can alter plastic by changing their color or destroy them (Dehaut et al. 2016). Composition of the digestion solution along with its concentration must be provided when used. Examples: (Thiele, Hudson, and Russell 2019; A. Lusher, submitted)

ratio of digestion fluid to sample

Digestion efficiency can present opposite results even if used with the same concentration, incubation temperature and duration but with a different ratio of chemical per mass of sample (Comparison between (Dehaut et al. 2016) and (von Friesen et al. 2019). When higher ratios of digestion fluid to sample are used, there is a higher ability for that sample to be degraded by the solution. This ratio can be provided as a volume of solution per mass of sample (ml g^{-1} for example)

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or volume of solution per volume of sample. Either way, these units should be described. Examples: (Thiele, Hudson, and Russell 2019; A. Lusher, submitted)

filtration

filter composition, porosity, diameter

Filter composition can have an impact on background signal if Raman or FTIR are used to identify microplastic (Oßmann et al. 2017; Löder and Gerdts 2015)**. Filter integrity can be compromised by incompatibility with chemicals in the sample. Filter porosity will influence the smallest size of microplastic recovered and filter diameter influences maximum surface area for particles to concentrate. Filter composition, porosity, and diameter should be provided. Example:** (Lorenz et al. 2019)

Microplastic Identification

visual identification

imaging settings

image settings (e.g. contrast, gain, saturation, light intensity)

The settings for the light influence the interpretation of the colors and shininess of objects should be specified when reproducing an image is important for reproducing results of a study. Examples: (Cowger, submitted)

magnification (e.g. scale bar, 50X objective)

Interpretation of plastic size depends on the magnification of the image. Include scale bars in all images generated and describe magnification used during assessment. Examples: (Fries et al. 2013)

light microscopy

magnification used during identification

The limit of detection based on size depends on the magnification used to identify particles. Examples: (Wiggin and Holland 2019)**.**

shapes, colors, textures, and reflectance, used to differentiate plastic

These factors determine the cutoffs used to distinguish a positive identification of plastics. Examples: (Murray and Cowie 2011; Rowshyra A. Castañeda, Suncica Avlijas, M. Anouk Simard, Anthony Ricciardia 2014; Fries et al. 2013)**.**

fluorescence microscopy

magnification used during identification

The limit of detection based on size depends on the magnification used to identify particles. Examples: (Wiggin and Holland 2019)**.**

fluorescence light wavelength, intensity, and exposure time to light source

The wavelength of excitation light and the intensity determines the fluorescence emission intensity and wavelength (Wiggin and Holland 2019; Erni-Cassola et al. 2017; Maes, Jessop, et al. 2017)**. Additionally, some fluorescence pigments undergo photobleaching which will decrease their intensity over time when exposed to a light source** (Karakolis et al. 2019)**.**

threshold intensity used to identify plastic

The threshold intensity is an arbitrary unit that defines the cutoff range of intensities that result in a positive plastic identification. This range should be described as well as the maximum and minimum intensity values in the analyzed samples. Examples: (Erni-Cassola et al. 2017)

scanning electron microscopy (SEM)

coating used (e.g. metal type, water vapour)

The coating changes the quality of the SEM image, the conductivity of the sample, and the Energy Dispersive X-Ray Spectroscopy (EDS) measurement if an EDS measurement is taken.

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Additionally, the gas pressure/vapor content needs to be reported for similar reasons. Examples: (Zbyszewski, Corcoran, and Hockin 2014)

magnification used during identification

The limit of detection based on size depends on the magnification used to identify particles. Examples: (Zbyszewski, Corcoran, and Hockin 2014)

textures used to differentiate plastic

SEM is commonly used to describe microplastic textures, these textures should be described with images provided for reference where possible. Examples: (Zbyszewski, Corcoran, and Hockin 2014)

chemical identification

pyrolysis gas chromatography mass spectrometry (py-GC/MS)

pyrolysis reacting gases, temperature, duration

Pyrolysis temperature (in °C) and duration (in s or min) for identification using this technique should be clearly reported as those two parameters can influence the degradation products formation and then influence polymer identification (Hermabessiere et al. 2018)**. In addition, if thermochemolysis is realized, the volume and concentration of the chemical should be reported. Example:** (Fischer and Scholz-Böttcher 2017)

GC oven program, temperature, carrier gas, and column characteristics

All parameters related to the GC separation parameters including GC oven temperature program (temperature ramp rates and hold durations), interface temperature, injection port temperature, split ratio, column characteristic (length, diameter, thickness, phase, manufacturer), gas carrier and its velocity must be reported as those parameters are mandatory to reproduce GC analyses (Hermabessiere et al. 2018)**. Information too extensive for the material & methods section can be reported in the form of a table in the supplementary material. Examples:** (Fischer and Scholz-Böttcher 2017)

MS ionization voltage, mass range, scanning frequency, temperature

All parameters related to the MS acquisition parameters including interface temperature, ion source type, ion source temperature, ionization voltage, mass range, and scanning frequency must be reported to allow reproducible MS data between studies (Hermabessiere et al. 2018)**. Information too extensive for the material & methods section can be reported in the form of a table in the supplementary material** (Cowger, submitted)

py-GC/MS matching criteria (i.e. match threshold, linear retention indices (LRI), and kovats index)

Plastic identification after py-GC/MS can be done using two techniques. First, the average mass spectrum is sent to a software where comparison is made between the spectrum and a library. For this purpose, the software name and supplier in addition with the library name and origin (supplier or homebase) should be reported. The minimum matching threshold (e.g. 80%) should be also given (Hermabessiere et al. 2018)**. Secondly, identification can be made by comparing characteristic peaks of a pyrogram with the available literature. LRI and kovats index should be used instead of the retention time as this metric can be used to compare across laboratories** (van Den Dool and Dec. Kratz 1963)**.**

py-GC/MS quantification techniques

If quantification of plastic is performed with py-GC/MS, several other pieces of information should be provided. Particular attention should be given to quality control and assurance, including lab blanks and matrix-matched positive controls. Which quantification approach (external standard, internal standard, or standard addition) used should be described. The source of the calibration materials, concentrations, and how and when they were handled and tested need to be included. Indicate which characteristic peak of a polymer was used to perform the quantification. This characteristic peak must be specific to the polymer and present in relative abundance. Calibration curve and equation must be provided in the results section in order to evaluate any interference issues with the samples (Fischer and Scholz-Böttcher 2017)**.**

raman spectroscopy

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acquisition parameters (i.e. laser wavelength, hole diameter, spectral resolution, laser intensity, number of accumulations, time of spectral acquisition)

Laser wavelength determines the likelihood of interference from fluorescence and should be reported in nm. The hole diameter and slit width (µm) can also affect the spectral resolution of the spectrum. Spectral resolution can change the peak shape and intensities observed and should be reported as the step size of the resolution. Spectral resolution is calculated using laser wavelength (nm), grating (grooves/mm), and charge-coupled device (CCD) size (pixels). Spectral resolution may be used to determine appropriate settings to use with instruments from various manufacturers in order to achieve comparable spectra among differing instrumentation. The intensity of the laser can be adapted to higher or lower values to improve the quality of the spectrum (Cabernard et al. 2018)**. The maximum laser power of the instrument should be reported (in mW) to provide context to the laser intensity used to collect spectra. The laser intensity may be reported as a filter (%) of maximum laser power. Multiple spectra are averaged by a predetermined number of accumulations, and increasing the number of averaged spectra may improve the quality of the final spectrum. Increasing spectral acquisition time may improve the quality of the spectrum by increasing the signal or aid in mitigating fluorescence; however, increased time may also lead to photodegradation of the sample. A delay time may also be added to mitigate fluorescence** (Munno et al. 2020)**. Additional spectral acquisition times can destroy samples or improve the signal. As a good practice, a record of the parameters used should be stored (e.g. spectrum metadata) in case the spectrum must be produced again from the same particle and instrument. Examples:** (Lenz et al. 2015; Karami et al. 2018; Cabernard et al. 2018; Oßmann et al. 2018; Käppler et al. 2016; Collard et al. 2015; Munno et al. 2020)

pre-processing parameters (i.e. spike filter, smoothing, baseline correction, data transformation)

A spike filter can remove small window peaks. Smoothing parameters can smooth out peak signals. Baseline correction and data transformations can warp the peak signals in many ways. Data transformations can change the peak shape. Therefore, these factors need to be rigorously described. Depending on the software used, the type and degree of baseline correction, smoothing and data transformation may be automatic, and may vary from spectrum to spectrum. A range of possible pre-processing parameters may be reported if the parameters vary. For spectra processed in batches, or for automated and semi-automated methods, the specific pre-processing parameters should be reported. Examples: (Lenz et al. 2015) **(baseline correction, smoothing, normalization),** (Ghosal et al. 2017; Collard et al. 2015; Dehaut et al. 2016)

spectral matching parameters (i.e. spectral library source, range of spectral wavelengths used to match, match threshold, matching procedure)

Spectral reference databases can affect the accuracy of spectral matching and results. Discuss whether the libraries are made in house or purchased, and if the libraries are specific to only polymers or include other chemicals. Matches can only be made to available entries in the library. State whether the libraries include spectra for environmentally aged and degraded polymers. Examples: (Käppler et al. 2016; Karami et al. 2016; Cabernard et al. 2018; Munno et al. 2020)**. When using Raman spectroscopy for polymer identification, many characteristic polymer peaks occur within the 100 – 3500 cm-1 range** (Smith and Markic 2013)**. It is important to report the range of wavenumbers included for spectral matching purposes. Some portions of the range are more susceptible to masking by other substances (e.g. pigments masking polymer peaks in the fingerprint region)** (Lenz et al. 2015)**. The range affects which peaks will be described. For example,** (Oßmann et al. 2018) **found that for Raman spectroscopy analysis, values larger 2000 cm-1 can be excluded for a successful analysis. Examples:** (Oßmann et al. 2018; Collard et al. 2015; Käppler et al. 2016; Cabernard et al. 2018) **The minimum threshold for a 'successful match' may impact the likelihood of assigning an incorrect match or matching only one component of a multi-component polymer (e.g. a copolymer or a polymer and pigment/additive combination).** (Munno et al. 2020) **observed a relatively high proportion of spectra identifying only pigments that were considered successful matches prior to the creation of a microplastics specific reference database.**

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Minimum matching threshold creates the cut-off point of what is accepted as a 'good match'. (Lorenz et al. 2019) did a large reanalysis of the determined matches for a minimum threshold for different polymer types. (Cabernard et al. 2018) investigated the full matching behavior of their method and determined a minimum matching threshold. Example: (Karami et al. 2018). Different matching procedures (e.g. correlation, Euclidean distance) have different accuracies and should be reported.

Fourier-transform infrared spectroscopy (FTIR)

acquisition parameters (i.e. mode of spectra collection, accessories, crystal type, background recording, spectral range, spectral resolution, number of scans)

These factors affect the raw spectra that are collected by the FTIR. While attenuated total reflection (ATR) only measures on the surface of the sample, transmission is measured throughout the whole thickness of the particle/material. The surface penetration depth can also depend on the ATR crystal type, incidence angle, and wavelength. Reflectance mode is a third mode option, which uses the reflection of light from the surface of the particle to extract the particle's spectrum. Reflectance is commonly used in automated chemical image mapping where the particles are resting on a surface and the light beam does not transmit throughout the particle. Reflectance spectroscopy can only identify the surface polymer of the particle. Transmission mode may cause total absorbance to the spectrum at which no measurable light reaches the detector at distinct wavelengths. ATR may need a correction to obtain similar peaks if compared to transmission FTIR. Example: (Sebastian Primpke et al. 2018). Describe any accessories used (e.g. microcompression cell, gold-plated microscope slide). Example: (Cabernard et al. 2018) used gold coated polycarbonate filters and gold coated mirrors for method comparison. The material of the crystal used should be stated. How often the background atmospheric signal was recorded should be described. (Sebastian Primpke et al. 2018) found that different spectral ranges had an influence on the number of detected polymers. Spectral range resolution and number of scans are common optimization parameters (Löder and Gerdts 2015).

pre-processing parameters (i.e. fourier-transformation (ft) parameters, smoothing, baseline correction, data transformation)

These parameters affect the spectral shape and or intensity and subsequent match. Examples: (Cowger, submitted)

matching parameters (i.e. FTIR spectral library source, match threshold, matching procedure, range of spectra used to match)

Matches can only be made to available entries in the library. The threshold sets the probability of false positives and negatives. Different matching procedures have different accuracies. The spectral match that is the most likely may not be the match with the highest hit quality index (HQI). Overlapping peaks can create additive effects or masking effects that may lead to false confidence in matching. Sometimes, manual interpretation of a spectrum leads to matches that differ from the highest HQI result. The method for determining matches, if not based on HQI, should be discussed. This may include visual interpretation and disregarding of matches that are unlikely (e.g. a large pellet matching with a pharmaceutical drug). The spectral range used to match affects which peaks are available for spectral comparison. Peaks for pigments and other substances may appear in the same region as polymer peaks (Lenz et al. 2015). Reporting Examples: (Lorenz et al. 2019) did a large reanalysis of the determined matches for a minimum threshold for different polymer types. (S. Primpke et al. 2017) investigated the matching behavior of seven methods and checked the minimum matching threshold. (Sebastian Primpke et al. 2018) screened a spectral region for its ability to separate different polymer types.

differential scanning calorimetry (DSC)

acquisition parameters (i.e. temperature, time, number of cycles)

The measured signals are affected by the heating rate, temperature, and number of cycles. Reversible thermal transitions will occur within every cycle of a measurement while overlapping effects like the evaporation of solvent or residual monomers occur only in the first run. To

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determine characteristic DSC information it is mandatory to report data from the first (irreversible transitions) and second cycle (reversible transitions). Examples: (Sebastian Primpke, submitted)

matching parameters (i.e. parameters assessed, reference library source, comparison technique)

Parameters assessed, reference library or data source and comparison technique are not standardized. These factors affect what the material is matched to and how the match is characterized. Examples: (Sebastian Primpke, submitted)

Microplastic Categorization

shape, size, texture, color, and polymer category definitions

The definition of sizes, shapes, color, and polymer classes are not standard. For example, some studies use the particle type term "thread" or "line" to refer to both fragmented fishing gear and line, as well as microfibers, while others break them out as separate types with different assumed sources. As such, what counts as a "thread" or "line" or other shape category needs to be clearly defined in a study. Unique circumstances (such as microfibers that also have threads entwined) should be described. Common size classes include (micro, meso, macro, nano) and these need to be defined by measurable units. The method used to determine and classify color should be discussed. The way that polymers are grouped should be discussed (e.g. grouping low density polyethylene (LDPE) and high density polyethylene (HDPE) together into one category). For a study that defines texture patterns, see (Corcoran, Biesinger, and Grifi 2009)**. For a study that describes the unique circumstance of a microfiber bundle, see** (Rochman et al. 2019)**. For a source that describes different plastic shapes, see** (F. Liboiron et al. 2018)**.**

Microplastic Quantification

units (e.g. kg, count, mm)

Mass, count, and volume are not the same units. The mass of a single particle could be equal to the mass of numerous smaller particles. Therefore, report the units (e.g. count, mass, volume) documented and how they were acquired (e.g. extrapolated or measured). Report multiple measurements in as many units as can be measured. Examples: (Simon, van Alst, and Vollertsen 2018; Dehaut, Hermabessiere, and Duflos 2019)

size dimensions (e.g. feret minimum or maximum)

Nomenclature for microplastic size is not yet standardized for size and different units reported hamper reproducibility. If a length is described discuss what it represents (e.g. feret minimum, feret maximum, square root of area). Also explain any physical manipulation of the dimensions (e.g. stretched out). Examples: (Cowger, submitted)

quantification techniques

There are automated and manual quantification techniques, these use different assumptions and have different errors. Examples: (Cowger, submitted)

Toxicology Considerations

dosed plastic age, polymer, size, color, and shapes

These factors can influence the toxicity of plastics. Virgin (fresh plastic) will likely have unpolymerized monomers or additives (e.g. stabilizers, dyes) within it or sorbed to it. These can leach out and affect a

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toxicology experiment. Aged plastic will likely already have lost any remaining monomers and so will be less likely to cause this problem, but other factors can make aged plastic more likely to contain sorbed pollutants. Plastic can also be solvent-rinsed (e.g. using methanol) and dried thoroughly to clean it prior to use in exposures. (Walpitagama et al. 2019) showed migration of unpolymerized photoinitiator, 1-hydroxycyclohexyl phenyl ketone (1−HCHPK) from a 3D printed plastic to the surrounding water occurred within 24 hours. See also (Liu et al. 2019) for differences in sorption of pollutants between virgin and aged plastics. The toxicity of the particles themselves might be linked to the polymer type. A non-target screening for the presence of additive chemicals associated with the plastic is also useful to describe. Ideally, toxicity studies should describe both particles and a chemical leachate derived from the particles in order to identify the source of any observed toxicological response. If using commercially available test materials for toxicity studies, the presence of surfactants must also be documented and accounted for. Related to the polymer, nearly all absorption takes place in amorphous (non-crystalline) regions of the polymer; therefore crystallinity will significantly affect sorption, especially for large (bulky) organic pollutants (Hüffer and Hofmann 2016; Hartmann et al. 2017). For example, larger polypropylene (PP) microplastics of similar surface area to smaller HDPE microplastics had greater sorption because PP has lower crystallinity (Sanchez 2019). Crystallinity may be either measured (e.g. with DSC) or cited from literature (well documented for most polymers). Particle size and shape can affect the uptake and toxicity of microplastics. Factors such as the size range of prey items for particular study species should be considered when selecting microplastics for use in experiments to increase environmental relevance. Particle size-dependent toxicity has been observed in rotifers (Jeong et al. 2016) and grass shrimp (Gray and Weinstein 2017). Nano-polystyrene particles had 3 orders of magnitude higher surface area than micro-sized polyethylene; this caused polystyrene to sorb significantly more polychlorinated biphenyls (PCBs) even though it is a glassy polymer (Velzeboer, Kwadijk, and Koelmans 2014). Polypropylene was observed to have >3x more surface area than the same particle size of high-density polyethylene, and sorbed significantly more triclocarban (Sanchez 2019). Additionally, free space or volume within the polymer matrix varies with chemical structure (larger space = easier for organic pollutants to absorb) (Pascall et al. 2005; Hartmann et al. 2017; Rochman et al. 2013). Finally, some organisms prefer plastics that look similar in color to their food (Schuyler et al. 2014).

animal husbandry

The length of acclimation and starvation, as well as controlling many environmental factors, can affect organism responses to microplastic exposure. If animals were sampled from the wild, how long were they depurated prior to exposures beginning. In what conditions and how long were animals acclimated for, temperature, tank size, number of animals per tank, were they starved prior to exposures (for how long), feeding mode (e.g. filter feeder, deposit feeder), age (days post fertilization or days post hatch if early life stage is used), average size (length, weight), male/female if applicable, water changes, feces removal, open or closed water circulating system, artificial or natural water, Licensing and ethical aspects (e.g. if vertebrates, refer to Institutional Animal Care and Use Committee (IACUC) protocol). Are holding and exposure tanks/rooms and diets free of background microplastic or associated pollutant contamination? If not, report potential sources. Examples: (Devriese et al. 2017; Athey et al. 2020).

exposure concentration, media, and time

Dose determines toxicity. The dose metric used to describe the exposure concentration should be presented (e.g. number of particles per L of water or per gram of sediment). Describe what media (e.g. solvents, sediment, organic matter, water, or air). In feeding experiments, concentrations in exposure media should be checked (via cell counter, etc.) before organisms are added and after they are removed. Additionally, certain life stages may be exposed differently if they are in the water column vs sediments. These factors also must be tracked closely to ensure that dosing of organisms is accurate. Reporting Examples: sediment - (Wright et al. 2013), water (Athey et al. 2020), food - (Watts et al. 2015). The amount of time the animal was exposed to the sample. Was the exposure constant or pulsed? This is typically measured in days but can be in other units as well. Units should be indicated. Different

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toxic substances will have different equilibrium times with different polymers; this is necessary to accurately determine differences in sorption. Biologically, smaller organisms generally have much shorter elimination times (e.g., time from consuming to defecation) than larger organisms. Depending on the objective, shorter or longer exposure times will be necessary to observe effects due to the substance being studied, therefore, toxicology experiments make decisions on exposure time based on animal physiology and life history. Examples: For equilibrium time of organic pollutants: (C. Wu et al. 2016; P. Wu et al. 2019; Rochman et al. 2013)**. For biological considerations:** (Key et al. 1998) **(organic pollutants);** (Rochman et al. 2014) **(microplastics).**

effects evaluation metrics*

Which parameters (markers) were evaluated?

biota metrics*

Which tissues were analyzed (e.g. stomach only or whole digestive tract)? Tissue weight (wet and dry) for tissues assessed and for the whole organism can aid in interpreting exposure. Typical reporting metrics for species (e.g. shell width and height for bivalves) should be reported to increase comparability with other studies.

Disclaimer:

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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