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Quality of nanoplastics and microplastics ecotoxicity studies: Refining quality criteria for nanomaterial studies

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A B S T R A C T
It is becoming increasingly important to develop assessment criteria for the quality of nanoplastics studies. This study is an attempt to establish such criteria based on those developed for engineered nanomaterials, the GUIDEnano and DaNa criteria being two representatives. These criteria were applied to studies on polystyrene nanoparticles (PS NPs), which currently represent the majority of studies on nanoplastics. We compiled a list of existing nanomaterial-related criteria that are not fully relevant to PS NPs and propose additional nanoplastic-specific criteria targeting polymer chemical composition, source, production and field collection, impurities/chemical additives, density, hydrophobicity, colour, and chemical leaching. For each criterion, scientific justification is provided. We conclude that the existing study quality assessments originally developed for nanomaterial toxicity studies can, through refinements, be applied to those dealing with nanoplastics studies, with a further outlook on microplastics. The final quality criteria catalogue presented here is intended as a starting point for further elaborations considering different purposes of an assessment.

1. Introduction

Plastic contamination has become a central issue in environmental research. Over recent years, in addition to numerous reports on microplastics pollution (Free et al., 2014; Rezania et al., 2018; Wang et al., 2017; Zhang et al., 2020) nanoplastics have also been identified as emerging contaminants of concern (Koelmans, 2019; Lambert and Wagner, 2016). They are one of the least studied types of plastic waste but considered potentially one of the most hazardous (Koelmans, 2019). According to the definitions of Gigault et al. (Gigault et al., 2018) and Hartmann et al. (Hartmann et al., 2019), nanoplastics are solid particles of synthetic or heavily modified natural polymers with a sizes between 1 and 1000 nm, whereas microplastic particles have a size between 1 and 1000 µm. Nanoplastics can be either produced intentionally, referred to as primary, or unintentionally leading to secondary nanoplastics. Primary nanoplastics can be bottom-up synthesized or top-down milled; in any case they contain pristine non-surface modified material. Milling is a common technique in industrial settings, and is also applied to polymer particles to reduce the size. Primary nanoplastics for testing may be purchased or extracted from products, for which they are intentionally produced and added, such as coatings, biomedical products, cosmetics, drug delivery, medical diagnostics, electronics, magnets and optoelectronics (Koelmans et al., 2015; Mitrano et al., 2019). In cases where nanoplastics are made by milling or grinding of larger plastic items, for example for research purposes, these could also be referred to as primary nanoplastics as they are intentionally produced, usually in a certain nano-size range (Hartmann et al., 2019). These particles however can be used as laboratory models to simulate mechanical weathering and formation of secondary nanoplastics. Unintentional formation occurs during wear and degradation of larger plastic objects and resulting particles exhibit colloidal behaviour (Gigault et al., 2018; Jahnke et al., 2017). Also, nanoplastics can form unintentionally from microplastics inside the products, like in personal care products (Hernandez et al., 2017) or from food and beverage packaging (Paul et al., 2020). A vocabulary defining the most important terms relevant for this study can be found in the Supplementary information.

Recently, data has emerged on the presence of nanoplastics in environmental samples, e.g. in North Atlantic subtropical gyre seawater (Ter...
Engineered nanomaterials by definition are primary particles as they are designed intentionally. To enable proper interpretation and regulatory decisions, the nature of their toxicity strongly influences their regulatory relevance (González-Fernández et al., 2018; Hartmann et al., 2017). Assessment of study quality is always context- and purpose-dependent with different approaches serving different backgrounds. For example, NanoCRED (Hartmann et al., 2017) is a relatively recent framework, which provides a transparent and flexible framework to assess both the reliability and regulatory relevance of engineered nanomaterial ecotoxicity studies, especially for regulatory purposes. It provides a structured, transparent approach to assist expert judgment (Hartmann et al., 2017). The DaNa criteria checklist was created to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018). Both approaches include criteria for evaluation of study quality and design in addition to criteria describing physical-chemical properties and particle behaviour. The DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Kruger et al., 2018).
To facilitate the transfer of lessons learned from nanoeotoxicological research into common practice in nanoplastics ecotoxicity studies, in this paper, we evaluated whether existing criteria, developed for engineered nanomaterials safety studies, mainly composed of metals, metal oxides or inorganic carbon, apply to nanoplastics and whether additional, nanoplastics-specific criteria, are needed to cover certain peculiarities occurring for this material group. For this purpose, we have reviewed the literature on nanoplastics ecotoxicity published until the end of 2019. As the majority of studies were published on polystyrene nanoplastics (PS NPs) these served as a case study to apply the two quality assessment approaches for engineered nanomaterials as described above, the GUIDEnano and the DaNa criteria.

Our approach followed a two-step procedure: In step 1 we performed a general assessment of the suitability of GUIDEnano and DaNa criteria, respectively, by applying them to (eco)toxicological studies employing primary PS NPs as test material. Based on this assessment we made a refined selection of applicable criteria and developed additional criteria to capture considerations specific to PS NP testing. In Step 2, we evaluated the more general applicability of these criteria to primary nanoplastics of other polymers as well as secondary nanoplastics with environmental relevance (various polymer types and mixtures thereof). Finally, we discuss the relevance of the nanoplastics criteria for the ecotoxicity studies of the larger microplastic particles (Fig. 1).

2. Methods

2.1. Case study with the most commonly tested nanoplastics: the polystyrene nanoparticles

The literature on nanoplastics ecotoxicity was collected using established search engines (WoS and Science Direct, last search on polystyrene nanoparticles done in October of 2019; last search on other nanoplastics done August 2020) and selected papers were subsequently evaluated using GUIDEnano and DaNa criteria. This literature search resulted in 47 studies testing polystyrene nanoplastics (PS NPs) and 5 studies testing nanoplastics other than polystyrene (Baudrimont et al., 2020; Booth et al., 2016; Greven et al., 2016; Ji et al., 2020; Venâncio et al., 2019). To maximise comparability, we applied the GUIDEnano and the DaNa criteria to the 47 studies on PS NPs, whereas the 5 studies on other polymer types served as further “case studies” to expand the criteria to other polymer types and acquire information on additional criteria relevant for quality assessment of nanoplastics studies in general. Before applying the quality assessments, we extracted basic information for each of the studies to clarify which test organisms were used (Supplementary information Fig. S2) and in particular the characteristics of the tested PS NPs in terms of size, functionalisation, fluorescent labelling and manufacturer. This allowed us to compare the studies that may have used exactly the same PS NPs supplied by the same manufacturer. Furthermore, some papers were excluded because the size of the particles exceeded the limit for nanoplastics (1000 nm). However, in the majority of the studies, nanoparticles with a size of less than 100 nm were used.

2.2. GUIDEnano quality assessment approach

We applied the GUIDEnano approach closely following the questions and categorization rules published by Fernández-Cruz et al. (Fernández-Cruz et al., 2018). Two scores were evaluated: the K-score, which defines the reliability of the study, and the S-score for substance characterisation. Both give a common final value for quality (Q-score). The K-score is composed of 3 groups of questions: 1. the characterisation of the organism, 2. the description of the study design and 3. the documentation of the study results. Some of the crucial questions (“red questions”) must be answered positively in order to pass the evaluation. On the basis of the final evaluation, the work is rated as follows: K1 - study reliable without restrictions; K2 - study reliable with restrictions and K3 - study not reliable (Supplementary information Table S1). The S-score consists of two groups of questions: 1. substance characterisation and 2. other specific engineered nanomaterial characterisation, including the characterisation of pristine nanoparticles and nanoparticles in the exposure medium. For the substance characterisation, the work is scored as follows: S1 - very good acceptability of the substance characterisation; S2 - characterisation of the substance acceptable and S3 - characterisation of the substance not acceptable (Supplementary information Table S2).

Based on the S- and K-scores, the quality categorisation (Q-score) of the ecotoxicity study is determined out according to the following rule: $Q = 1$/very high quality (K1-S1), $Q = 0.8$/high quality (K1-S2 or K2-S1), $Q = 0.5$/medium quality (K2-S2) and $Q = 0$/unacceptable quality (K1-S3, K2-S3, K3-S1, K3-S2, K3-S3).

Fig. 1. Representation of the two-step approach to derive criteria to assess the quality of nano- and microplastics ecotoxicity studies based on existing criteria for engineered nanomaterial studies. In Step 1, GUIDEnano and DaNa criteria are applied to studies of polystyrene nanoparticles as a case study. Criteria are then adapted and in Step 2 expanded and discussed in relation to other primary and secondary nanoplastics as well as microplastics.
2.3. DaNa criteria checklist

The evaluation of the DaNa criteria checklist was performed according to the document published on the website of the DaNa4.0 project (DaNa). These criteria apply in general to toxicity studies and were not compiled for ecotoxicity studies specifically. The questions relate to: 1. physico-chemical engineered nanomaterial properties, 2. sample preparation, 3. test parameters and 4. general aspects (Supplementary information Table S3). Again, some of the criteria are mandatory to pass the assessment while others are optional. The final outcome is a simple ‘acceptable’ versus ‘non-acceptable’ categorisation. The evaluation according to DaNa criteria checklist is not based on a point system but the checklist provides an overview of whether the mandatory criteria are met, and which of the additional criteria were fulfilled. The mandatory criteria need to be fulfilled for all studies to be accepted, while the need for fulfillment of the additional criteria will depend on the purpose the study results will be used for in the context of the public knowledgebase. It also has a descriptive part to justify the decision and make it also comprehensible and transparent later on. While it thus allows some inclusion of expert judgment into the final decision, this primarily has the function of ensuring that the assessment is context-relevant, i.e. whether the study data is useful will depend on the type of information that is needed for a specific part of DaNa website. For the purpose of this study, the context for the evaluation of the papers was defined as “providing reliable information on ecotoxic effects of PS NPs”.

2.4. Data analysis

The results of our study analysis are presented as the proportions of the studies that agree or disagree with each of the questions on the GUIDEnano and DaNa checklists. The overall quality assessment of ecotoxicity studies with PS NPs is presented as the proportion of studies with very high (Q1), high (Q2), medium (Q3) and unacceptable quality (Q4) according to GUIDEnano and acceptable/not acceptable by the assessment with DaNa criteria.

3. Results and discussion

3.1. Overall quality of polystyrene nanoparticles ecotoxicity studies

According to the GUIDEnano approach, which predominately concerns regulatory purpose, none of the 47 studies was classified as very high quality, 36% of the studies were of high quality, 23% were of medium quality and 41% were unacceptable. Most of the studies were unacceptable due to low score of physico-chemical characterisation of particles. According to the DaNa2.0 criteria, 82% of all studies were found to be acceptable, and thus only 18% of the studies on PS NPs were found unacceptable. All of the latter were also evaluated as unacceptable according to the standard GUIDEnano criteria. Also, within DaNa, the majority of these studies were rejected due to poor characterization of test material. The detailed results of this evaluation can be found in the Supplementary information (Fig. S3). In the following, based on this initial evaluation, each of the criteria for engineered nanomaterials, from GUIDEnano and DaNa, respectively, was checked with regard to appropriateness and sufficiency for nanoplastics.

3.2. Implementation of GUIDEnano and DaNa approaches for polystyrene nanoparticles studies to identify relevance of existing criteria for PS NPs

Most of the evaluated PS NP studies complied to GUIDEnano “red questions”, which address the most important aspects of the development/reporting of the study and are considered as mandatory requirements. Also, almost all studies met the criteria on the test organisms and design (K1-K7) (for specific details on criteria see Table S1). Only 50% of the studies were conducted according to internationally standardised protocols (K10). However, the use of standard protocols is not guaranteeing study quality when it comes to nanomaterials or nanoplastics, if they were not especially adapted for these materials. This is due to the fact that most standardised methods are optimised for soluble chemicals. Using adapted methods may hence deliver more reliable results. Only 17% of the studies explicitly mention the use of a reference chemical (or toxicant as used in GUIDEnano). The majority of the PS NPs studies did not present the results as effect values (LC50, EC50, NOEC, LOEC, 25% effect, BCF, BAF,…) (K9), but the biological effect was quantified as a significant difference in response compared to the control (Fig. 2A). The lack of reporting of the effect values may be because the effects were generally limited or not exceeding 50% at the highest test concentration of PS NPs tested in the respective study. Further, the prime aim of the majority studies was not to provide data for regulatory use, but rather to provide first indications of potentially hazardous effects of PS NPs (Heinlaan et al., 2020) or mechanistic insights (Chae and An, 2020).

Much lower scores were obtained for substance characterisation (S-scores, S1-S19; please refer to whole description in Table S2) (Fig. 2A). Most studies provided information on the identity and supplier of the PS NPs, but the concentration of stocks was rarely reported (S3). According to the GUIDEnano guidelines, this criterion is considered fulfilled as long as the information on the concentration of stocks is available on the supplier’s website. In most cases, therefore, we found this information, provided by the suppliers/manufacturer, online. However, in some cases it is difficult to trace which nanoplastics were actually used and this information may not be permanently available online. We therefore suggest that stock concentrations should be consistently reported in the study. Almost 30% of the studies did not provide information on dispersal protocols (S7). This is important information as the mode of dispersion largely influences the exposure conditions of the test organism (Hartmann et al., 2015; Kennedy et al., 2009). In most studies, the physico-chemical properties of the test exposure medium, for example pH, temperature and dissolved oxygen, alkalinity, were not measured (S11, S12), but it was assumed that this criterion is fulfilled if a medium was reported to be prepared following guidelines protocols (i.e. OECD, ISO, etc.). This information is very important to report as it can affect the fitness and survival of organisms and the final outcome of the study.

In terms of reporting properties that are specific to engineered nanomaterials, most of the studies provided the nominal size and shape of pristine PS NPs, but only 60% of the studies described the surface charge (S15). As the surface functionalisation plays a key role in the toxicity of PS NPs (Gonzalez-Fernandez et al., 2018; Manfra et al., 2017), this should be reported for PS as well (Tallec et al., 2019). Impurities were rarely reported (S5), although this is a very relevant property for nanoplastics (see chapter 3.3 on Nanoplastics specific criteria). The concentration of PS NPs in the exposure medium is almost never measured, whereas nominal concentrations were reported. Hence, we suggest to measure the final polymer concentration in the exposure medium as the dispersion of nanoplastics can be a complex process involving several steps (Hartmann et al., 2015) and therefore may result in losses. However, we are aware that this is still challenging for unlabelled particles. Ideally, both mass-based and number-based concentrations should be reported as the latter facilitates the comparison with environmental monitoring data. The stability of PS NPs concentration during the exposure period (S8) was not reported for any of the 47 evaluated studies. In GUIDEnano the stability refers to the dissolution or ion release from engineered nanomaterials, as well as does not apply to solid and insoluble nanoplastics, such as PS NPs. For nanoplastics, however, dissolution can be considered analogous to leaching of polymer-associated chemicals or residual monomers from the plastics (see chapter 3.3 on Nanoplastics specific criteria). In addition to the GUIDEnano interpretation of stability, there are other factors influencing the consistency of PS NP exposure during the test. More specifically, and as discussed for engineered nanomaterials in the nanoCRED framework (Hartmann et al., 2017), nanoparticle exposure should also
be monitored over the duration of the test, quantitatively as well as qualitatively, in terms of exposure concentrations (actual suspended PS NPs), agglomeration state, particle size distribution and surface charge/zeta potential. Agglomeration state is covered by Q18 (see text below), however we recommend that the remaining characterisation measurements are also reported. None of the 47 evaluated studies reported the specific surface area of PS NPs (S14). Specific surface area is usually measured on dry powders with BET technique, most of the PS NPs, however, were supplied in suspension and hence this parameter was not assessed. BET is considered as an important physico-chemical property of engineered nanomaterials that governs their surface reactivity (Van Hoecke et al., 2008). Hence, it may be assessed for plastic particles that exist as powders prior to biological experiments, e.g. if generated from milling of larger plastic items. If the specific surface area of nanoplastics can be used as predictor of their effects remains to be elucidated. However, this information is important when organisms are exposed simultaneously to nanoplastics and another chemical pollutants as the adsorption of chemicals depends on the surface area of polymeric particles (Xu et al., 2018). Further, differentiation between physical adsorption (adhesion of molecules onto a material surface) and chemical adsorption (diffusion into the material) is important, in order to quantify the total amount of chemical pollutants associated to the plastic particle. It will depend both on the polymer chemical composition and the chemical which of the processes will prevail (Sørensen et al., 2020). Almost 80% of the studies reported the hydraulic diameter of PS NPs (S18), but most of them only at the beginning of the experiment. Since the aggregation/agglomeration behaviour of the particles defines the exposure conditions of test organisms to nanoparticles, hydraulic diameter should preferably be reported also at the end of exposure (Hartmann et al., 2017; Tallec et al., 2019). Some of the engineered Nanomaterial-specific properties (S17), such as magnetic properties, acidity/basicity, redox potential, catalysis, were rarely reported for PS NPs. Currently, reports on the intrinsic reactivity of polymer materials as well as their potential to induce oxidative stress in organisms are inconclusive (Liu et al., 2020; Magni et al., 2020). Most probably, pristine / primary nanoplastic particles are not prone to ROS generation under common test conditions and time-frames. The relevance for PS NPs and other primary nanoplastics is discussed in Table 1.

The majority (average 88% for related questions) of the studies met the mandatory DaNa criteria (Table S3) and those regarding the test parameters, which are associated with a good overall study reliability (Fig. 2B). However, only half of the studies clearly stated whether the test concentrations were “overloaded” (Q16). This criterion initially referred to in vitro studies using cells lines and inhalation studies, where “overload” refers to a situation where natural defence or clearance mechanisms are overwhelmed and other effects such as inflammation overlay the actual toxicity of a material. This is due to the fact that the evaluation is not tailored for ecotoxicity studies, but refers to toxicity studies in general. For this reason, we did not consider this criterion (Q16) to be necessary for the reviewed studies to pass the assessment. Very few studies reported the use of a reference material. A reference material, in the sense of a reference particle, is commonly used in human inhalation studies or in vitro studies using cells lines. In PS NP ecotoxicity studies, however, this could refer to a particle control such as a natural particle of similar dimensions, to elucidate the attribution of any effect to PS NP versus a general particle effect. In addition, the use of a positive control, which may be either a chemical or a particle with a known ecotoxicological effect, is also recommended. It is applied to check the sensitivity of the test species, test protocol and the respective endpoint. We thus suggest that, for PS NP studies, reporting of results with particle references as well as with a chemical or particle positive control are included as criteria. None of the studies provided data on specific surface area (Q4), crystalline phase (Q7), radical formation (Q9), porosity and magnetic properties (Q10).

Based on these two assessments, we compiled a list of GUIDEnano and DaNa criteria that in its present formulation we found less relevant for polystyrene nanoplastics in suspension (Table 1). We also discuss how these criteria are relevant for other primary nanoplastics that have different characteristics as PS NPs and exist also as powders.

3.3. Expanding polystyrene nanoplastics criteria to general primary nanoplastics-specific criteria

In the section above, we have provided recommendations on the need for additional quality criteria, based on the analysis of PS NP studies. This includes recommendations on (1) consistent reporting of stock concentrations, (2) monitoring exposure, including dissolution and hydrodynamic diameter, throughout the duration of the test, (3) reporting of measured PS NP concentration in the exposure medium as both mass and number, and (4) inclusion of particle and chemical reference.

As presented in Table 1 most of the criteria that are not directly relevant for PS NPs are also not relevant for other primary nanoplastics. However, some physico-chemical properties that cannot be determined for PS NPs are relevant for other types of primary nanoplastics that exist in powder form prior to their dispersion in test media. For example, specific surface area and porosity can be directly determined for powders and the relevance of this parameter has already been discussed in Chapter 3.2.

In addition to the existing criteria, initially developed in DaNa and GUIDEnano for engineered nanomaterials and applicable for nanoplastics in their original or modified form, we have identified further relevant criteria. The source and the production protocol of the nanoplastics should be thoroughly reported as they can strongly influence their physico-chemical properties and toxicity potential. In particular, it is important to report on the extraction protocol from consumer and industrial sources where nanoplastics is intentionally added. This echoes concerns for engineered nanomaterials in terms of synthesis by-products/catalysts and trustworthy sources in the nanoCRED framework (Hartmann et al., 2017). In case of extraction of nanoplastics from a product, the protocol should report on the probability of remaining chemicals from the products in the final nanoplastics sample. The type of mechanical processing applied to obtain nanoplastics affects the surface area and topography (Potthoff et al., 2017), which can affect the interaction of organisms with the nanoplastics as has been previously shown for microplastics (Kalcikova et al., 2017). Also, surface modification could alter the adsorption potential of nanoplastics for other pollutants (Hüffer et al., 2018), which is an important factor in mixture toxicity studies. If nanoplastics are fluorescently labelled, the leaching of probes should be evaluated, as this can cause erroneous results on the fate of nanoplastics (Schür et al., 2019). As plastics can contain a number of chemical additives, as well as monomers, these should be analysed and their leaching evaluated under experimental conditions (Groh et al., 2019). Namely, it has been shown that for some types of microplastics plastic chemicals can be the main driver of their toxicity (Zimmermann et al., 2020). Also some monomers, like styrene, can induce toxicity (Gibbs and Mulligan, 1997). In this respect, nanoplastics made from recycled plastics pose a particular challenge, as they contain a cocktail of chemical mixtures originally derived from plastics of different origins. Although producers of nano(micro) plastics could potentially not disclose the additive content in their products, further chemical analysis is possible in research laboratories (Jemec Kokalj et al., 2021). An important property is also the density of nanoplastics, which influences the behaviour of the particles in the test medium and thus their bioavailability to organisms (Potthoff et al., 2017). As density of many common polymers, such as polyethylene and polypropylene, is often below the density of water, floating of particles on the surface of...
exposure media may dominate, and hence exposure of organisms to plastic particles is not given. On the contrary, for inorganic, often metal-based engineered nanomaterials, sedimentation processes are predominant. Similar to engineered nanomaterials, the dispersibility of nanoplastic in exposure media is influenced by hydrophobicity, which may hamper proper dispersal. Hence, the use of dispersant aids is often reported, meaning either some kind of pre-wetting treatment (e.g. ethanol) or the addition of substances such as detergents to reduce the surface tension of the particles (Potthoff et al., 2017). In any case, the procedures involved as well as substance added to the media need to be reported, as interferences with organisms may occur, which also needs consideration for selection of proper controls for the tests with organisms. As described for engineered nanomaterials in the nanoCREd framework, when a dispersant/stabiliser/solvent is used then it must be considered if this is within an appropriate concentration range in the test media and data on tests with a dispersant/stabiliser/solvent control should be included (Hartmann et al., 2017) (Table 2).

For soil toxicity testing, it is of importance to analyse the presence and concentration of plastic-associated chemicals in soil pore water. Namely, some organisms are predominately exposed to soil pore water and not via ingestion of soil particles. Plastic particles may affect the soil biophysical environment, such as for example water holding capacity and the number of water stable aggregates (de Souza Machado et al., 2018). In practise this means that the moist content should be adjusted to desired value after the plastic particles had been mixed in soil. For engineered nanomaterials this was not as relevant. This protocol should hence be reported in the study.

We compiled a complete list of criteria for nanoplastics ecotoxicity studies representing a combination of existing GuideNANO and DaNa criteria we considered relevant for nanoplastics as well as the new

<table>
<thead>
<tr>
<th>Original question (No. and framework)</th>
<th>Relevance for PS NPs suspension</th>
<th>Relevance for other primary nanoplastics suspension/powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardised tests methods used? (GUIDEnano K8; DaNa Q21)</td>
<td>The use of standard protocols (if not especially adapted for nanomaterials) is not a guarantee for quality when it comes to nanomaterials or nanoplastics. PS NPs are insoluble.</td>
<td>The same as for PS NPs.</td>
</tr>
<tr>
<td>Was the stability of the substance concentration measured during the exposure period? Stability refers specifically to ion release and dissolution (GUIDEnano S8), Water solubility (discriminate between soluble, metastable and persistent particles; metastable: soluble within days or weeks) (DaNa Q13)</td>
<td>Cannot be directly measured for nanoplastics in suspension, which is the common form of commercially available / lab synthesized PS NPs. As PS NPs are most often well-defined and spherical particles in solution, surface areas can be approximated based on particle size.</td>
<td>Surface area can be assessed for plastic particles that exist as powders prior to biological experiments, e.g. if generated from milling of larger plastic items. This property affects the potential of nanoplastics to interact with test organism as well as to adsorb and/or absorb chemicals. How surface area affects the ecotoxicity of nanoplastics is currently unknown.</td>
</tr>
<tr>
<td>Pristine nanoparticle surface area (GUIDEnano S14) Specific surface area of powders (e.g. BET surface) (DaNa Q4)</td>
<td>Not all information included in this criterion is relevant for PS NPs. The following properties are relevant: 1. Hydrophobicity: relevant as it influences the adsorption of chemicals on plastics as well as adsorption of nanoplastics on the surface of organisms (Thormann et al., 2008) (→ justification of criterion on hydrophobicity in Table 2) 2. Crystal structure: relevant information to distinguish amorphous and crystalline polymers. The crystal structure affects the absorption of chemicals (Giu et al., 2018), 3. Radical production capacity: Polymers per se do not release radicals in such extent as for example photocatalytic TiO₂ or CeO₂ nanoparticle nanomaterials. (→ justification of criterion on radical production in Table 2) 4. Magnetic properties: not relevant for nanoplastic particles, however when magnetic particles are used as additives, these have to be characterised. Magnetic particles are not common additives for PS NPs, hence this criterion is not relevant.</td>
<td>The same as for PS NPs. Magnetic properties may be relevant when magnetic particles are used as additives. This is true for magnetic particles which are embedded in polymers for medical applications (Feldman, 2016). This may be relevant for secondary nanoplastics if derived from these materials. We could not find evidence for primary magnetic composite nanoplastics in the current literature.</td>
</tr>
<tr>
<td>Porosity (DaNa Q10)</td>
<td>Cannot be assessed for nanoplastics in suspension.</td>
<td>Porosity of polymers affects the adsorption of chemicals (Kyriakopoulos et al., 2005). Hence this is relevant information.</td>
</tr>
<tr>
<td>Dosage used classified clearly to be ‘non-overload’ or ‘overload conditions’ (DaNa Q16)</td>
<td>Originally this DaNa criterion was aimed for toxicology studies, including in vitro toxicity as well as inhalation studies. In ecotoxicity studies for PS NPs it cannot be used in its present formulation, but is relevant with regard to attachment and subsequent physical effects in organisms. Analogously studies could report the justification for test concentrations used in relation to environmental relevance.</td>
<td>The same as for PS NPs</td>
</tr>
</tbody>
</table>

Table 1
Existing GUIDEnano and DaNa criteria for engineered nanomaterials that in its present form do not entirely apply for polystyrene nanoplastics suspension (PS NPs). Their relevance for PS NPs, including the need for modifications, is described as well as if/how this applies to other primary nanoplastics, either powder or suspension is discussed. These criteria were selected from the list presented in Supplementary information S1–S3.
Table 2
Suggestions for additional criteria to evaluate the quality of ecotoxicity studies for primary and secondary nanoplastics in general.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Why this criterion is important</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin of nanoplastics (commercial supply, laboratory production, field collection)</strong></td>
<td>The origin of nanoplastics can provide indications of the potential presence of plastic-associated chemicals such as additives.</td>
</tr>
<tr>
<td><strong>Protocol of nanoplastics production and collection</strong></td>
<td>For nanoplastics produced through fragmentation of larger plastic materials, the type of mechanical processing may affect the surface and structural properties of nanoplastics and could be a source of impurities in the test material. In case of nanoplastics produced through synthesis, information on synthesis protocols is important to evaluate the potential for synthesis by-products/catalysts.</td>
</tr>
<tr>
<td><strong>Nanostructures characteristics</strong></td>
<td>Secondary nanoplastics are mixtures of plastics of different origin.</td>
</tr>
<tr>
<td><strong>Polymer chemical composition (CAS number)</strong></td>
<td>Secondary nanoplastics are mixtures of plastics of different origin.</td>
</tr>
<tr>
<td><strong>Impurities</strong></td>
<td>Impurities for secondary nanoplastics are chemicals that are used to harvest nanoplastics from the environment and in subsequent process to concentrate the particles. Such are for example different enzymes or chemicals that change the density of particles (e.g. salt solutions).</td>
</tr>
<tr>
<td><strong>Chemical additives</strong></td>
<td>Secondary nanoplastics can contain mixtures of additives.</td>
</tr>
<tr>
<td><strong>Other chemical pollutants</strong></td>
<td>Chemicals can become associated to the plastic during its life cycle and may originate from air, water or soil. Identification of associated pollutants is important to interpret the source of toxicity.</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td>Also relevant. Density alters due to weathering and biofouling.</td>
</tr>
<tr>
<td><strong>Hydrophobicity</strong></td>
<td>Also relevant. Changes due to weathering processes.</td>
</tr>
<tr>
<td><strong>Formation of radicals, (photo-)catalytic activity</strong></td>
<td>Particles subjected to weathering or harsh environmental conditions may release ROS formed by chemical degradation of the polymers. The production of reactive radicals increases the reactivity of nanoplastics posing a risk for oxidative damage in organisms. Both biocorona and biofouling are relevant. Biofouling may provide a nutrition source in tests as well modulate the adsorption of pollutants. It affects the fate and bioavailability of nanoplastics.</td>
</tr>
<tr>
<td><strong>Biocorona/biofouling</strong></td>
<td>Secondary nanoplastics are mixtures of plastics of different origin.</td>
</tr>
<tr>
<td><strong>Toxicity testing protocol description</strong></td>
<td>Also relevant.</td>
</tr>
<tr>
<td><strong>Verification of background nanoplastics/microplastics contamination</strong></td>
<td>Also relevant.</td>
</tr>
<tr>
<td><strong>Concentration of leached chemicals from nanoplastics under experimental conditions</strong></td>
<td>Also relevant.</td>
</tr>
<tr>
<td><strong>Protocol for application of water in soil described in detail</strong></td>
<td>Also relevant.</td>
</tr>
</tbody>
</table>

Criteria (Supplementary information, Table S4). We applied the criteria regarding nanoplastics primary characteristics and their properties in exposure medium to five studies that have tested nanoplastics other than PS NPs (Baudrimont et al., 2020; Booth et al., 2016; Greven et al., 2016; Ji et al., 2020; Venancio et al., 2019). Almost all tested nanoplastics were primary nanoplastics, produced in the laboratory though either top-down or bottom-up methods. Baudrimont et al. (2020), however, produced secondary nanoplastics from field-collected plastics. Some of the criteria were found to be fulfilled by all studies, such as the polymer chemical composition, origin and protocol for nanoplastics production. Other properties were less commonly reported, for surface charge and chemistry, density, impurities and other chemical pollutants. None of the studies provided information on the specific surface area, porosity, crystal structure, chemical additives and radical formation by nanoplastics. Also, none evaluated the leaching of chemicals and biocorona/biofouling under exposure conditions (Fig. 3). This survey implies the need for incorporation of nanoplastics specific criteria, which can be used to design relevant ecotoxicity studies, improving the quality and critical interpretation of test results.

3.4. Secondary nanoplastics specific considerations?

Secondary nanoplastics are complex mixtures with regard to their composition in comparison to primary nanoplastics. Being subjected to
environmental weathering considerably alters their physico-chemical properties and interaction with the environment. Further, secondary nanoplastic particles are a mixture of plastics of different origin (e.g. polymer composition, additive content). For this reason, particular attention should be paid to their characterisation. In line with this, polymer-associated chemicals should be thoroughly analysed. The particle size, shape, surface topography and area of secondary nanoplastics may be very diverse due to chemical, physical and biological degradation in the environment (Jahnke et al., 2017). Perhaps the most evident transformations in the environment are weathering, changing the physical appearance and structure of the particles, as well as the formation of organic matrices (i.e. biofouling or biocoronas) on the nanoplastics (McGivney et al., 2020; Rummel et al., 2017). Biofouling on nanoplastics may provide a nutrition source in tests as well increase the adsorption of pollutants compared to plastics without the biofilm (Kalčíková et al., 2020). Biocoronas have been hypothesised to influence biological interactions and thus potential for toxic effects (Marques-Santos et al., 2018). Hence, if sampling secondary nanoplastics from the environment for ecotoxicity testing purposes, harsh protocols should be avoided to preserve original properties of the secondary nanoplastics as they are present in the environment (Table 2).

3.5. Can nanoplastics study quality criteria be applied to studies of microplastics?

Although nano- and microplastics differ in size (nano: 1–1000 nm; micro: 1000 nm–1000 µm (Hartmann et al., 2019), they occur on a size continuum and may even have the same origin. Both the DaNu and GUIDEnano criteria are specifically designed for studies of engineered nanoparticle (eco)toxicity and consist of two main particle specific parts: the evaluation of the reliability of the test protocol and of the particle characterization. The evaluation criteria for the test protocols define the need to report on how the test dispersion was prepared and how the particles change during exposure, and could therefore be also applied for microplastics. For microplastic studies it is likewise important to report on the basic physicochemical properties in terms of particle size, morphology, functionalization, density, hydrophobicity, radical formation, surface topography and charge. In addition, also other primary and secondary nanoplastics specific criteria (Table 2), such as the origin of the plastics, extraction protocols, mechanical processing protocol, nano (micro)plastics background contamination, composition of chemical additives and leaching under exposure conditions apply to microplastics. For microplastics also some new criteria become relevant, such as for example the colour which can be important for organisms with selective feeding strategies (Chen et al., 2020). A summary of all particle characteristics relevant for quality of studies corresponding specifically to engineered nanomaterials (ENMs), primary or secondary nano(micro) plastics and their overlaps is presented in Fig. 4. Recently, study quality evaluation criteria for microplastics were proposed (de Ruijter et al., 2020) with 20 quality criteria in four main categories (particle characterization, experimental design, applicability in risk assessment, and ecological relevance). Their approach takes a different starting point and develops criteria based on microplastic studies that examined a wide variety of plastic materials. Hence, considering our suggestions (Table S4) and those by de Ruijter et al. (2020) would aid much in the quality of future microplastics studies.

4. Conclusions and outlook

This is the first study to propose specific quality criteria for primary and secondary nanoplastics(eco)toxicity studies. We built on two selected study quality evaluation frameworks, GUIDEnano and DaNa, that were developed for engineered metal-, inorganic and carbon-based nanomaterials and introduce new nanoplastics-specific criteria. The GUIDEnano and the DaNa criteria were tested for suitability on scientific papers on the ecotoxicity of the most intensively studied nanoplastics, polystyrene nanoparticles, which served as a case study. We conclude that by adding some refinements of terminology and new nanoplastics...
specific considerations, existing study quality evaluation approaches, originally developed for nano(eco)toxicology studies, can be adopted to the evaluation of studies dealing with nanoplastics. The main part of the necessary adaptations relates to polymer-specific properties and their behaviour in test media. Also, some soil specific issues need to be addressed as plastic particles may affect soil properties. So far, we consider the modified study quality evaluation approach to be valid for primary and secondary nanoplastics. It could likely be implemented as well for primary and secondary microplastics. As the assessment of study quality is always purpose dependent, the list provided here is intended as a starting point for further elaboration, taking different purposes for an evaluation into account (e.g. planning a study, evaluating regulatory reliability). Our suggested criteria can thus serve as inspiration in the further development or adaptation of frameworks to suit the evaluation of nanoplastics studies, including adaptations to DaNa, GUIDEnano, nanoCRED and others. This list could also be a starting point to develop reporting information standard for nano- and microplastics analogously to those developed for nanomaterials which ensures interoperability of data in the FAIRification process. In general, further development will be necessary for secondary micro(nano)plastics sampled from the environment, as the situation for these mixtures of various particles is much more complex. With the advancement of knowledge in this field, additional study quality criteria for secondary micro(nano)plastics will likely be suggested in the future.

CRediT authorship contribution statement

Anita Jemec Kokalj: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Funding acquisition,
Nanna B. Hartmann: Data curation, Writing - review & editing,
Damjana Drobné: Writing - review & editing, Funding acquisition,
Annegret Potthoff: Writing - review & editing, Kühnel Dana Kühnel: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Funding acquisition All authors have read and agreed to the published version of the manuscript.”

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125751.
References


DaNa criteria, 2016.


