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

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ABSTRACT

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 (nano)ecotoxicity 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...
Halle et al., 2017) and more recently in sand water extracts after serial filtration and analysis by Pyrolysis GC-MS (Davranche et al., 2020). The current scarcity in environmental exposure data is mainly due to the difficulty of sampling, separating, characterising and quantifying nanoplastics in environmental media as well as complex biological matrices (Gigault et al., 2017; Nguyen et al., 2019). It is expected that nanoplastics could be more hazardous than microplastics due to their increased potential for interacting with biological systems including internalisation due to endocytosis or phagocytosis, increased surface reactivity due to higher surface area as well as different kinetics for release of potentially toxic chemical additives (Mattsson et al., 2018). Size-dependent effects have already been demonstrated for polystyrene nano- and microplastics (Mattsson et al., 2018; Sjöllema et al., 2016).

The number of environmental science studies on nanoplastics has been increasing steadily since 2017, and increased by 60% from 2019 till 2020. The majority of studies on nanoplastics concern polystyrene nanoparticles (PS NPs) (Supplementary information Fig. S1), as summarized in most recent reviews (de Ruijter et al., 2020; Ferreira et al., 2019; Koelmans, 2019; Lehner et al., 2019) and confirmed through our own analysis of existing scientific literature. Polystyrene nanoparticles are commercially available, inexpensive and also easy to synthesize and process into nanoparticles, which is the main reason for their broad use in (eco)toxicity studies as test material (Lehner et al., 2019). Additionally, they are commercially available as fluorescently labelled particles, which facilitates detection and tracing by e.g. flow cytometry, fluorescence microscopy and confocal microscopy, thereby overcoming the general analytical difficulties associated with nanoplastics. The use of these particles as models for nanoplastics research has been criticized, as they do not cover the multitude of plastic particles occurring in the environment (Gigault et al., 2018; Koelmans, 2019; Lehner et al., 2019; Pessoni et al., 2019). Commercially produced PS NPs are monodisperse, spherical and may contain traces of chemical additives from synthesis (Pessoni et al., 2019; Pikuda et al., 2018). Often PS NPs are chemically functionalized with a carboxylic acid or amino groups, resulting in a negative or positive surface charge, respectively, which strongly influences their toxicity (González-Fernández et al., 2018; Manfra et al., 2017). How this functionalization relates to environmentally relevant nanoplastics is unknown.

For these reasons, there is a general motivation and drive to develop new model nanoplastic particles for toxicological research (Magri et al., 2018; Pessoni et al., 2019) as alternatives to those commercially available. Examples include: Top-down approaches based on laser ablation of polymers to form polyethylene terephthalate nanoplastics (Magri et al., 2018), ultra-sonication of field-collected microplastics (Baudrimont et al., 2020), dissolution of low-density-polyethylene-pellets (LDPE) in toluene to obtain polyethylene nanoplastics (Balakrishnan et al., 2019), microemulsion polymerization of methyl methacrylate (Venancio et al., 2019), mechanical breakdown using dispersants and differential centrifugation (Ji et al., 2020) and soap- and metal-free polystyrene latex particles (Pessoni et al., 2019). To our knowledge, only few eco-toxicity studies have been performed with nanoplastics other than commercially available PS NPs, like methyl methacrylate-based (Booth et al., 2016; Venancio et al., 2019), polycarbonate nanoplastics (Greven et al., 2016), polyethylene terephthalate nanoplastics (Ji et al., 2020) and nanoplastics produced from field-collected microplastics (Baudrimont et al., 2020).

A rapid expansion of ecotoxicity studies, similar to what we are currently observing with micro- and nanoplastics, has previously been observed for engineered nanomaterials (Krug, 2014). The definition of engineered nanomaterials differs from the one for nanoplastic given above in the relevant size range 1–100 nm in at least one dimension (ISO, 2010), as well as in the composition, as they consist of a broader variety of raw materials (e.g. metals and metal oxides, inorganic carbon, particles derived from natural polymers, e.g. nanocellulose). Engineered nanomaterials by definition are primary particles as they are designed intentionally. To enable proper interpretation and regulatory use of data from (eco)toxicological studies on engineered nanomaterials, thorough tests material characterisation has been considered crucial (Hartmann et al., 2017). Several criteria to assess the quality of nanomaterial studies for regulators, as well as for knowledge communication have been developed. This is both to ensure the quality of studies to be used for regulatory purposes (Fernández-Cruz et al., 2018; Hartmann et al., 2017), as well as to provide grounds for unbiased, quality-assured reporting in a public knowledge base specialized in engineered nanomaterials (Krug et al., 2018; Marquardt et al., 2013). Several approaches have been developed compiling criteria for metadata reporting (Mills et al., 2014; Stefaniak et al., 2013) or judgement of study quality (Fernández-Cruz et al., 2018; Hartmann et al., 2017). Assessment of study quality is always context- and purpose-dependent with different approaches to serve 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 judgement (Hartmann et al., 2017).

The aim of the current paper is thus to provide quality criteria tailored to (nano)plastics safety research based on two other existing approaches: GUIDENano (Fernández-Cruz et al., 2018) and DaNa criteria checklist (DaNa, 2016). These are both simple and objective scoring systems, with minimal reliance on expert judgement. This allows for a more ‘automated’ evaluation of study quality, which we found to be well suited for our present work. We will however draw on other, more complex assessment frameworks, including nanoCRED, in subsequent discussions of suggested quality criteria for nanoplastics studies. GUIDENano and DaNa address different purposes of study quality assessment. GUIDENano has a regulatory purpose (Fernández-Cruz et al., 2018) whereas the DaNa criteria checklist is developed to assess general study quality to ensure knowledge transfer from reliable, scientific sources to the general public (Krug 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 GUIDENano approach to quality assessment uses a scoring system based on considerations of test design and reported parameters, including K-Score for the assessment of study reliability and the introduction of a new score for the physicochemical properties of engineered nanomaterials (S-Score) (Fernández-Cruz et al., 2018). The main motivation of this approach was to reduce the need for expert judgement. On the other hand, the DaNa checklist criteria are less stringent as they were originally intended as a form of screening tool for the public knowledge base on the DaNa website (www.nanoobjects.info), hence without implications for regulatory decision making (Krug et al., 2018; Kühnel et al., 2017; Marquardt et al., 2013). More specifically, the DaNa criteria checklist was created to evaluate the quality of nanosafety studies in order to provide relevant information that is subsequently communicated to the general public. The public knowledge base includes information on human and environmental health related to different types of engineered nanomaterials. The criteria concerning study quality and design are thus kept rather general and does not include criteria exclusively referring to studies in the field of ecotoxicology. Also, the overall number of criteria was kept as low as possible reducing the effort for study evaluation, as for certain types of engineered nanomaterials, e.g. silver, titanium dioxide, a huge amount of studies had to be evaluated (Nau et al., 2016). The DaNa criteria checklist comprises a number of general (e.g. statistics, standardization), biological (e.g. appropriate controls, dosing procedures and physical chemistry (e.g. particle size and surface area, impurities) checkpoints. These criteria may also be used to design relevant nano(eco)toxicity studies and to assess the potential reasons for no-effect results. The DaNa approach allows for a high degree of flexibility as the use of information in a public knowledge base is highly dependent on the context in which the information is used. This was considered appropriate, as in addition the study results are used in an aggregated way (i.e. in the form of brief texts on the website) and not for making any regulatory decisions.
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 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).

![Existing quality criteria for engineered nanomaterial studies](image)

**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 nanoplastics 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 this allows some inclusion of expert judgement 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 was 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, and hence 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), nanomaterial 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 S18 (see text
below), however we recommend that the remaining characterisation
measurements are also reported. None of the 47 evaluated studies re-
ported 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 reac-
tivity (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
absorption (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 hydrodynamic 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 nanoplastics, hydrodynamic
diameter should preferably be reported also at the end of exposure
(Hartmann et al., 2017; Tallec et al., 2019). Some of the engineered
(Sanomaterial-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 param-
eters, which are associated with a good overall study reliability (Fig. 2B).
However, only half of the studies clearly stated whether the test concen-
trations 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 overlap 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 rec-
ommended. 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 pow-
ders 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 nano-
plastics in their original or modified form, we have identified further
relevant criteria. The source and the production protocol of the nano-
plastics 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 frame-
work (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 inter-
action 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
Table 1

| Original question (No. and framework) | Relevance for PS NPs suspension | Relevance for other primary nanoplastics suspension/powder |
|--------------------------------------|---------------------------------|-----------------------------------------------------------|
| Standardised test methods used? (GUIDEnano R8; DaNa Q21) | The use of standard protocols (if not especially adapted for nanoplastics) is not a guarantee for quality when it comes to nanomaterials or nanoplastics. PS NPs are insoluble. | The same as for PS NPs. |
| 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) | 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. | 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 organisms as well as to adsorb and/or absorb chemicals. How surface area affects the ecotoxicity of nanoplastics is currently unknown. |
| Pristine nanoparticle surface area (GUIDEnano S14) Specific surface area of powders (e.g. BET surface) (DaNa Q4) | 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 nanoplastics. (→ 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. | 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. |
| Porosity (DaNa Q10) | Cannot be assessed for nanoplastics in suspension. | Porosity of polymers affects the adsorption of chemicals (Kyricalopoulos et al., 2003). Hence this is relevant information. |
| Dosage used classified clearly to be 'non-overload' or 'overload conditions' (DaNa Q16) | Originally this DaNa criterion was aimed for toxicity 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. | The same as for PS NPs. |

The use of standard protocols (if not especially adapted for nanoplastics) is not a guarantee for quality when it comes to nanomaterials or nanoplastics. PS NPs are insoluble. The majority of nanoplastics are insoluble polymers. Solubility of nanoplastics might instead be interpreted as the leaching of plastics-associated chemicals and residual monomers (→ new criterion on additives, monomers and leaching in Table 2). Stability should consider 'stable exposure conditions' as a whole and thus, in addition to S18 on agglomeration, also include monitoring of dispersed concentrations, surface charge/zeta potential and particle size distribution during the test period. 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 organisms as well as to adsorb and/or absorb chemicals. How surface area affects the ecotoxicity of nanoplastics is currently unknown. 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.
### Table 2

Suggestions for additional criteria to evaluate the quality of ecotoxicity studies for primary and secondary nanoplastics in general.

| Criterion | Primary nanoplastics “intentionally produced in certain nano size by defined procedures in the lab (either bottom-up synthesis or top-down milling of a defined plastic powder)” | Secondary nanoplastics “formed by weathering and/or fragmentation in environment or during use” |
|-----------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Origin of nanoplastics (commercial supply, laboratory production, field collection) | The origin of nanoplastics can provide indications of the potential presence of plastic-associated chemicals such as additives. | The same applies as for primary nanoplastics. In addition, the presence of other pollutants is probable through sorption of chemicals to nanoplastics from the surrounding environment. Type of field collection should be described as it may affect physico-chemical properties of nanoplastics. |
| Protocol of nanoplastics production and collection | For nanoplastics produced though 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. |  |
| Nanoplastics characteristics | Secondary nanoplastics are mixtures of plastics of different origin. |  |
| Polymer chemical composition (CAS number) | The information on polymer composition provides clues on potential hydrophobicity, and density of particles. The polymer composition is particularly important when primary nanoplastics is obtained from recycled plastics where mixture of polymers may be present. | 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). |
| Impurities | Impurities can be remains from extraction of primary nanoplastics from products (e.g. personal care products) or synthesis of primary nanoplastics and may affect the toxicity. For example, PS monomers are toxicological relevant (Lambert et al., 2017). |  |
| Chemical additives | Additives are substances that give polymers certain properties, e.g. colour, strength, elasticity. Identity and amount of additives is important to interpret the source of toxicity (Groh et al., 2019). The complexity of additives may increase if nanoplastics arise from recycled plastic items. | Secondary nanoplastics can contain mixtures of additives. |
| Other chemical pollutants | Not relevant for primary nanoplastics synthesised in the laboratory. Relevant from nanoplastics generated from larger plastic items that may have had absorbed and/or adsorbed pollutants. Relevant for nanoplastics isolated from personal care products. | 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. Also relevant. Density alters due to weathering and biofouling. |
| Density | Density affects the agglomeration/aggregation behaviour and floating/settling properties in test medium. Defines the bioavailability of nanoplastics to organism. | Also relevant. Changes due to weathering processes |
| Hydrophobicity | Hydrophobicity governs the potential of nanoplastics to interact with test organism as well as to adsorb chemicals. It greatly affects the dispersal in aquatic media, but this is less relevant for soil toxicity tests. |  |
| Formation of radicals, (photo-) catalytic activity | Not relevant for polymers, but additives may produce radicals. | 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. |
| Biocorona/biofouling | Biocorona formed in exposure medium alters biological interactions of nanoplastics with organisms. Biofouling not relevant. |  |
| Toxicity testing protocol description | Protocols to avoid and verify background contamination with micro- or nanoplastics should be reported to enable the interpretation of data pending for particular test nanoplastics. Important for the interpretation of nanoplastics sources of toxicity. For soil testing the concentration of leachates in soil pore water should be reported, especially for organisms exposed to pore water. | Also relevant. Also relevant. |
| Verification of background nanoplastics/microplastics contamination |  |  |
| Concentration of leached chemicals from nanoplastics under experimental conditions |  |  |
| Protocol for application of water in soil described in detail | Nanoplastics may affect water holding capacity of soil resulting in different soil moisture and evapotranspiration, a finding shown for nanoplastics isolated from personal care products. |  |

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 (Marcques-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 DaNa 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 Drobne: Writing - review & editing, Funding acquisition.
Annegret Potthoff: Writing - review & editing.
Kuhnel 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.
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