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GUIDANCE DOCUMENT ON TESTING NANOMATERIALS USING OECD TG No. 312 “LEACHING IN SOIL COLUMNS”

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NO. 342

Guidance Document on testing Nanomaterials using OECD TG No. 312 "Leaching in soil columns"

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Paris 2021

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or contact:

**OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

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FOREWORD

This document is the *Guidance Document (GD) on Testing Nanomaterials Using* ***OECD TG No. 312*** *"Leaching in Soil Columns"*. The GD was developed by Canada and Germany with the support from the Joint WNT-WPMN Expert Groups on Ecotoxicity and Fate. An inter-laboratory comparison test (ILC) took place to evaluate the benefit of the proposed modifications for nanomaterials. The report of the ILC is made available as a separate document, No. 341 in the OECD Series on Testing and Assessment. The GD and the ILC report were approved by the WNT in April 2021.

This Guidance Document is published under the responsibility of the Chemicals and Biotechnology Committee.

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Background

The need for a Guidance Document (GD) for testing the behaviour of engineered nanomaterials (NMs) in soils using the OECD Test guideline (TG) No. 312 was identified as a priority goal by the OECD's Working Party on Manufactured Nanomaterials (WPMN). Under the authority of the WPMN, a first expert meeting on ecotoxicology and environmental fate of NMs took place in January 2013 in Berlin. During the meeting, it was concluded that the OECD TG No. 312 is generally applicable for the testing of NMs. However, an additional GD needed to be developed with specifications for the testing of NMs (OECD 2014). As a follow-up to this conclusion, a Standard Project Submission Form (SPSF) was submitted to OECD WNT in November 2016 and the project was approved by the WNT-29 in April 2017.

In 2017, a preliminary draft guidance was developed by Canadian and German experts, which was further elaborated based on feedback provided by the OECD Joint WNT-WPMN Expert Group on Ecotoxicity and Fate of Nanomaterials (hereafter JEG) via teleconferences. The first draft was sent to the JEG in June 2018 for commenting. A 2nd draft version was presented for discussion at the meeting of the JEG held in December 2018 in Arona, Italy and opened another round of written comments.

Based on the discussion at the JEG meeting and subsequent comments, it was decided to conduct an inter-laboratory comparison test (ILC)[1] to evaluate the benefit of the proposed recommendations for NMs as well as to verify the reproducibility of the results. The ILC took place from June 2019 to December 2019 (with some extension for some partner laboratories to perform analytics). Results were obtained from seven laboratories for two different NMs (silver, cerium oxide) in two soils featuring different characteristics to enable differentiated leaching patterns. Results of the ILC and conclusions derived from them for consideration for the draft GD were summarized in a report. Based on the results and experiences from the ILC, further adaptions of the draft GD were made and discussed by the leads, Canada and Germany (with support by additional international experts), resulting in a draft guidance presented for a first round of comments by the WNT in December 2020 and January 2021. Based on the comments received, the draft GD was revised and submitted for approval to the WNT.

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[1] The report is published as No. 341 in the OECD Series on the Testing and Assessment

1. Introduction

It is accepted that the existing OECD Test Guidelines (TGs) are generally applicable to test engineered nanomaterials (NMs), but there are needs for adaptation of certain TGs or additional Guidance Documents (GDs) (for further abbreviations please see Chapter 5.2). The OECD TGs are designed primarily for organic, soluble or readily soluble chemicals. Their utilization for testing NMs is often limited by the particulate character of the NMs. Therefore, specific guidance is needed to account for this material characteristic. Accordingly, the OECD Council published a recommendation on the safety testing and assessment of manufactured NMs stating that for the investigation of NMs, OECD TGs should be applied and adapted as appropriate to take into account the specific properties of NMs (OECD 2013).

The need for an OECD GD for testing the fate of NMs in soils using the OECD TG No. 312 (OECD 2004) was identified during the "Joint WNT-WPMN Meeting on Environmental Fate and Ecotoxicology of Nanomaterials (JEG)" which took place in January 2013 in Berlin. At this meeting, it was concluded that "the OECD TG No. 312 is generally applicable to the testing of NMs". However, "a preamble or an additional guidance with specification for the testing of engineered nanomaterials" is needed in order to reliably report on the mobility and fate of NMs in soils (OECD 2014).

For chemicals, it is assumed that thermodynamic processes mainly determine their distribution in the environment because they tend to reach equilibrium between the different environmental compartments. However, equilibrium partitioning does not apply to solid NMs as they do not form solutions but colloidal dispersions that are thermodynamically unstable. Thus, methods based on equilibrium processes are not applicable for reporting on the fate of NMs in environmental matrices. The fate of NMs in the environment is subject mainly to irreversible kinetic processes such as (hetero-)agglomeration and sedimentation (Praetorius et al. 2014) so that they will never end up in a thermodynamic equilibrium. The OECD TG 106 ("Adsorption-Desorption Using a Batch Equilibrium Method") was developed to test the fraction of a chemical adsorbed to soil (OECD, 2000). However, this TG is not appropriate for NMs in soils, because it assumes a thermodynamic equilibrium between the dissolved and adsorbed substance. Therefore, an adapted version of OECD TG No. 312 becomes of particular importance for the determination of the behaviour of NMs in soils in a robust and reproducible manner, focused on kinetically-driven methodology. Thus, the GD has the potential to generate kinetic descriptors to characterise the fate of NMs in soils.

This document provides specific guidance for test preparation, implementation, performance, analysis and reporting using the OECD TG No. 312 for testing the mobility and retention of NMs in different types of soils. The GD is likely also relevant for colloidal materials of greater size ranges, because transport of these materials occurs via the same kinetically dominated processes that determine the fate of NMs. The document informs on necessary modifications and additions to the standard test protocol including preparation and application of the test materials, analytics and data reporting. The modifications and additions proposed in the GD are included in order to generate relevant, accurate and reproducible data on NM retention and mobility in soils. Estimations of parameters such as K_{oc} (organic carbon normalised adsorption coefficient) and K_{om} (organic matter normalised distribution coefficient) as presented in the parent TG No. 312 are not applicable for NMs.

The GD is structured in a way that recommended modifications and additions to the test method and performance are corresponding to the chapters of the OECD TG No. 312. An overview of the proposed modification and additions in deviation to OECD TG No. 312 is given in tabular format in Chapter 6.1 to this GD.

2. Scientific Background

Recent studies have highlighted the limitations of testing NM retention and mobility in soils and discussed aspects to be considered during testing, including the need for adapted sample preparation (Cornelis et al. 2010; Kuhlbusch et al. 2012; Cornelis et al. 2013; Cornelis et al. 2014; Hoppe et al. 2014; Petosa et al. 2012; Saleh et al. 2008; Praetorius et al. 2014).

In general, the transport of NMs through soil (and other unconsolidated porous media) as well as their fate is governed by (i) the physical and chemical characteristics of the respective NM, including particle size, shape, concentration, surface properties, stabilizing agents used, aging behaviour, aggregation and deposition behaviour, and (ii) the characteristics of the bulk soil and (iii) the soil pore water. Soil characteristics such as grain size distribution, surface roughness and percentage of clay material have an important role on NMs fate. Moisture content and chemical composition of the medium (e.g. presence of organic matter, iron oxides and hydroxides, oxygen, surfactants, and microorganisms) are of relevance as well. A third key factor defining NM transport is the pore water chemistry; namely, its chemical composition, ionic strength, pH, the presence of multivalent ions, concentration of dissolved organic matter and colloidal material as well as hydraulic properties such as flow velocity and direction that determine residence time.

Most of these conclusions have been reached based on column studies with homogeneous materials (e.g., quartz sand), as they provide some degree of control on the composition and morphology of the porous medium. Compared to these studies, transport studies of NMs with natural soils are less frequent. Some of these studies are described below.

In Cornelis et al. (2014) the literature on the fate of NMs in soils and their bioavailability was summarized. Most of the processes determining the fate of colloids in soil also determine the fate of NMs. The main fate-determining processes in solid matrices are leaching of NMs from biosolids (e.g., after contaminated sewage sludge is added to soil), homoagglomeration (agglomeration of NMs with each other – relevant mainly under artificial lab conditions), heteroagglomeration (agglomeration of NMs with other particles such as natural colloids or other types of NMs), deposition (attachment of NMs after they approach a pore wall by Brownian diffusion, direct capture on grain surfaces or sedimentation), straining (physical entrapment of NMs in pore spaces) as well as transport processes in soils. The mobility of NMs in soils is predominantly controlled by salinity, texture, pH, concentration and the nature of mobile organic compounds, degree of saturation, and the presence of macropores. Interactions with solutes, i.e., inorganic (e.g., sulfate, phosphate) and organic molecules such as dissolved organic matter (DOM, e.g., humic and fulvic acids, exudates from soil organisms) which are ubiquitous in soil pore waters significantly alter the physicochemical characteristics and therefore the fate of NMs in the environment. DOM adsorption often renders NM surface potential more negative and increases NM transport because of steric and electrostatic stabilization. However, this may not be considered uniform as DOM adsorption depends on DOM characteristics, NM properties and multivalent cations in soil solution (Degenkolb et al. 2019b; Zehlike et al. 2019). Studies from lab model systems suggest that NMs are released from grain surfaces. In particular, slow diffusion-controlled NM release occurs under constant physicochemical conditions depending on the depth of the NM-grain surface interaction energy minima (Hahn et al. 2004). Only a small fraction of retained NMs may be released under these conditions. In contrast, studies on the release and/or remobilization of NMs from natural soil or saturated porous media are still scarce. Hoppe et al. (2015) found NMs released from farmland soil due to sorption to mobile colloids. Individual NMs were not detected in soil pore water of column experiments. NM transport in an artificial riverbank filtration scenario was not induced by a simulated rain event, which was concomitant with a decrease in ionic strength (Degenkolb et al. 2019a). Similarly, lab batch studies demonstrated a change in hydrochemical conditions alone not to be sufficient to trigger NM mobility. This was only achieved in combination with mechanical forces (Degenkolb et al. 2018). Up to now, there seems to be little knowledge on NM release form heteroaggregates or breakup of heteroaggregates. While there is ample knowledge on the disaggregation of homo-aggregates (for instance Metreveli et al. 2015), these processes play only a minor role under environmental conditions.

Several researchers have investigated the influence of soil and NM properties on NM transport. For example, the transport of silver nanoparticles coated with polyvinylpyrrolidone (PVP-coated AgNP) in 11 natural soils (grains < 2mm) under saturated conditions was studied by means of column tests (Cornelis et al. 2013). Prior to transport experiments with AgNP, tracer tests using bromide were carried out. HNO_3 or NaOH were used to adjust the pH for the respective soil and KNO_3 was used as background solution (artificial pore water). AgNP breakthrough was analyzed by applying a model that made use of two-site kinetic attachment–detachment. Results suggest increased deposition of AgNP and/or increased straining after heteroaggregation of AgNP with mobile soil colloids. PVP-coated AgNP can easily interact with natural colloids in soils, which significantly reduces their mobility.

Fang et al. (2009) studied the transport behaviour of TiO_2 NMs using column experiments packed with 12 different natural soils under saturated conditions. They observed that the pH, ionic strength and dissolved organic carbon content affect NM mobility in soil columns. In soils with higher clay content, no transport of TiO_2 NMs was observed.

Kasel et al. (2013b) examined the mobility of multi-walled carbon nanotubes (MWCNTs) in two natural, undisturbed soils at water contents close to saturation (85-96%). Their experiments showed that the soil acted as a strong sink for the NMs. The same research team also studied the transport of the MWCNTs in water saturated sand having different grain sizes (Kasel et al. 2013a). MWCNTs have a high aspect ratio (length to width) and the researchers proposed that the retained NMs may create a porous network trapping additional particles. Thus, particle shape can strongly influence concentration-dependent particle transport.

Quevedo and Tufenkji (2012) studied the transport of two types of quantum dots (QDs) and a nanoplastic in quartz sand and loamy sand over a range of ionic strengths in KCl or $CaCl_2$ solutions. NM deposition rates were at least an order of magnitude greater in the loamy sand than in the quartz sand. Also, the three types of NMs exhibited different transport potential in the loamy sand, likely due to differences in the binding affinities of the NM surface coatings for specific soil constituents such as clays.

Babakhani et al. (2017) recently performed a meta-analysis on NM transport in porous media by re-analysing 493 breakthrough curves from 50 column transport studies with a wide range of different NMs. Their conclusion was that attachment-detachment was mainly controlled by the concentration of the NM coating, ionic strength of the pore water, porosity of the granular medium and its surface charge.

Adrian et al. (2018) studied the transport of a surfactant- and PVP-stabilized AgNP in natural silicate-dominated sediment under saturated conditions. Their results indicate that increasing ionic strength, divalent cations, presence of silt and clay, and decrease in flow velocity enhances the retention of AgNPs. AgNP breakthrough was modelled using one or two irreversible retention sites that accounted for Langmuirian blocking on one site. AgNP retention was mainly attributed to the increased residence time and cation bridging in the presence of fine grains and calcium which was always more pronounced for PVP-AgNPs compared to surfactant-stabilized AgNPs. Also, low surface charge values contributed to the retention of AgNPs.

There exist several other studies and reviews on NM transport in sands and soils. These studies are generally aimed at understanding the impacts of pore water chemistry, soil chemistry and grain size, as well as NM size and surface chemistry on NM mobility. Soil column studies are useful for understanding the transport and hence contamination risks associated with NMs in natural subsurface environments as well as designing application protocols for nanopesticides in agricultural settings or reactive NMs in environmental remediation.

3. Specific guidance on NMs

3.1. Applicability domain of the test

The guidance given in this document is applicable for all NMs for which accurate and sensitive analytical strategies are available. Different analytical techniques and strategies might be needed depending on the physicochemical properties of the tested NMs and on the background NMs present in the tested soil. Even though the accuracy of advice given in this GD was experimentally checked to only a limited number of NMs (i.e. nanosilver, cerium oxide), they are expected to be valid for the current commonly known NMs fulfilling the criteria mentioned. In cases this GD is intended to be followed for NMs strongly deviating in properties currently known, the advices should be carefully examined for applicability. However, this GD is not applicable to NMs with high solubility in simulated environmental water (further information on solubility testing of NM can be found in the OECD GD No. 318 (OECD 2020)). Furthermore, following the GD will not produce reliable results for NMs which cannot be sufficiently dispersed in order that an adequate amount of test material can be added to the soil column.

3.2. Information on the test substance

Chapter 7.1 list information on the tested NMs which should be available or determined prior to soil leaching testing for well-informed test performance and adequate interpretation of test results. This information will replace the information needed prior to testing as given in the parent TG No. 312.

It is recommended to apply the NMs in concentrations that ensure their detection by appropriate analytical techniques (see below). Detection limits will vary according to the type and size of the NMs, the possibility of NM labelling, as well as the chosen detection method. On the one hand, the concentrations of applied NMs to the soil columns should be high enough such as to allow discrimination from natural background levels (e.g., in the case of metals/metal oxides, and carbon-based NMs). However, on the other hand, the amount of applied NMs should be as low as possible. High concentrations will enhance clogging in the first centimetres of the soil column and may provoke increased agglomeration. This will limit recovery during testing and may alter transport and leaching. To overcome this dilemma for NMs with high natural background, one option might be to label the NMs in order to be able to detect them. If labelled NMs are used, deviations from the physicochemical properties of the original (unlabelled) NMs should be reported. In all cases, the amount of NMs finally applied to the soil needs to be reported.

Guidance on how to determine dispersion stability, solubility and dissolution rate of the NM in the test medium can be found in the OECD TG No. 318 on dispersion stability of NM in simulated environmental media (OECD, 2017) and the OECD GD for the testing of dissolution and dispersion stability of NMs, and the use of the data for further environmental testing and assessment strategies (OECD 2020). These data will support the interpretation of test results and should be considered for testing before beginning soil leaching tests, or in parallel.

3.3. Reference substance

It is recommended to study the transport behaviour of a standard inert tracer substance to characterise the hydrodynamic properties of the packed soil column, because these properties are required to calculate kinetic fate descriptors for NMs such as the attachment efficiency. Examples of inert tracer are potassium bromide, sodium nitrate, brilliant blue, uranin, or tritiated water. The use of a tracer that does not interact with the soil medium allows the user to determine the effective porosity and dispersion coefficient of the packed medium, values that can be used for more accurate determination of NM transport parameters (see chapter 3.7).

The tracer should be injected into the column before introducing NMs to avoid interaction with the NMs. All (100%) of the applied tracer material should be recovered at the end of the tracer test. In soils of pH below 7, bromide might not be a conservative tracer (Goldberg and Kabengi, 2010), and thus, care must be taken to validate the choice of tracer for a given soil. Salts (e.g. sodium chloride) have the advantage that they can easily be detected using a conductivity meter, but as a salt tracer will induce alterations in physico-chemical properties in the soil columns, it could be more advantageous to use dye (e.g. brilliant blue) or fluorescent tracers (e.g. uranin). However, care needs to be taken to ensure that no adsorption onto soil surfaces takes place. In addition, isotopic tracers (deuterium, tritiated water) might be an alternative.

As injection of the tracer before introducing NMs to the columns can lead to long lasting and laborious test performances and delays in analytical assessments, it is possible to add the tracer only to control columns (without introduced NMs). If the tracer behaviour varies significantly across the control columns replicates, it must be assumed that non-uniform packing also occurred in the test columns and repetition of the test is advisable, paying attention to making the soil packing as homogenous as possible. It is also possible to inject a tracer/electrolyte solution after the introduction of NMs to evaluate the remobilization of retained NMs (Tufenkji and Elimelech 2004). In this case any possible effect of the tracer on NM remobilization should be taken into account.

3.4. Definitions and units

See chapter 5.1 to this GD.

3.5. Quality criteria

The parent TG OECD No. 312 uses recovery ranges as well as repeatability and sensitivity of the analytical method as quality criteria for test conduction. In accordance to the TG, a recovery of at least 70% (for non-labelled NMs) is considered acceptable also for NMs. Recovery is understood as the sum of NM found in the soil segments and the leachate at the end of the experiment expressed as percentage of the initial NM amount introduced to the column. It is acknowledged that the success of recovery of NMs in soil columns strongly depends on many different variables (e.g. particle type, the choice of application and applied amount of the test substance, type of soil used) which may challenge the determination of recovery, depending on the NM to be tested and/or the specific test parameters. Therefore, in case the required recovery is not achieved, it is recommended to check different steps and/or elements of test performance for improvement. These include the analysis of sand layers and tubing used to check for sorption, the stability of the stock dispersion to check for sedimentation as well as the reconsideration of chosen digestion methods. For tests using natural soils where NMs are present in high natural background, and for which recovery of 70% will be hard to achieve, the use of labelled NMs is advised. The effect of labelling products on the physicochemical properties of the particles must be minimised as far as it is reasonable possible, since it has to be ensured that labelling will not affect transport behaviour. The use of artificial soil/substrate to exclude natural background should be avoided as the deviation to the recommended soil types will be large and this will lead to unreliable extrapolations of results.

To reliably quantify the tested NM, the analytical measurement should at least be performed in triplicates. It is not recommended to rely on pre-defined detection and quantification limits when evaluating the success of the performed test. Instead, LOD/LOQ should be determined based on the method used for the analytical assessment as values will strongly depend on the used medium, NMs and background levels.

Control experiments should be conducted with soil columns (at least in duplicates) that have not been previously exposed to the tested NM. The purpose of these control experiments is to determine the release of soil colloids that may interfere with the analytical method(s) (i.e. background concentrations of the tested NM).

3.6. Considerations on the test method

3.6.1. Test system and laboratory equipment

Leaching columns should be made of inert material such as glass or stainless steel as the likelihood of NM attachment to these surfaces is low. The utilization of glass columns features the advantage of transparency of the material, which allows the user to visually observe the occurrence of air pockets or non-uniform packing of the porous material. However, if NMs are sensitive to photochemical processes, light-transmissive glass should be avoided or should be wrapped with aluminium foil to protect them from light. If specific materials are used for the column tubes or at the column outlet (e.g. porous mesh), which cannot be chemically analysed, it has to be ensured that no NMs are retained on them. Recovery is dependent on the material at the outlet end itself and on its porosity. Materials with a high enough porosity (but small enough to retain the smallest fraction of soil grains used) are recommended. To avoid attachment of the tested NM to test equipment like tubing and valves, equipment made of an inert material (e.g. Teflon) is recommended. The same applies to containers that are used to store NM suspensions collected and used during the experiment. However, attachment of NMs to materials may vary (as a function of NM type and any NM coatings) and should therefore be considered individually for every NM tested. A blank experiment (no soil, at least two replicates) can be conducted to verify that NMs are not retained on column materials or tubing.

The minimum diameter of the column should be 4 cm. As low mobility is expected for most of the NMs in soils, the length of the column is recommended to be 10-20 cm (final length to be derived from the needed soil height). This is in contrast to the column length recommended by the parent TG No. 312 (30 cm). In case of complete (100%) breakthrough, the test should be repeated with longer columns in order to achieve more detailed data on the mobility.

To prevent soil release from the column into tubing and fittings, it is recommended to place a mesh of an inert material (with pore size smaller than the smallest size fraction of the soil) immediately underneath the soil (prior to introducing soil into the empty column). Additionally, it is recommended to add a thin layer (~ 2 mm) of high-purity clean quartz sand (~400 µm grain size) or small high-purity glass beads (e.g. 5 mm in diameter) between mesh and soil.

To ensure uniform distribution of water over the column cross-sectional area, it is recommended to add a thin layer (~ 2 mm) of quartz sand above the packed soil. The amount of this layer should be as small as possible as sorption of the NM tested cannot be excluded. However, sand layers (or other material) should not be deployed to fill up larger columns in cases of tests which only require a low soil height to avoid effects on NM transport in the sand.

Sand layers but also filters used should be checked for potential effects on NM transport and sorption by pre-testing. Additionally, the extent of recovery should be checked to consider this issue in the mass balance. This can be done by various approaches, such as (i) by a simplified column test with reduced work load (e.g. considering a reduced number of pore volumes of leachate and/or a faster flow rate) or (ii) with filtration experiments (e.g. filtration of a representative NM dispersion through the sand layers / filters).

In order to enhance reliability, leaching columns should be reproduced at least in duplicates, but triplicates are strongly recommended as high variability in NM properties in the different test samples is likely, e.g. due to problematic sample preparation.

In addition to the laboratory equipment and chemicals mentioned in the parent TG OECD No. 312, it should be considered to use a probe sonicator to prepare aqueous NM dispersions before introducing them to the test columns, if the NM is provided as a powder. A magnetic stirrer in the container holding the NM in dispersion may be necessary to ensure a sufficiently homogeneous suspension. However, damage of NMs of specific morphology like fibres and rods might be possible when using probe sonicator and thus, morphology changes should be carefully evaluated.

3.6.2. Test substance

It is recommended to apply the test substance as a dispersion to the surface of the soil column. The application can be performed as a pulse application or as a step injection. To study dynamic deposition interactions (e.g., blocking or ripening), it can be beneficial to use continuous injection in volume coinciding with over four times of the pore volume, but it should be considered that a continuous injection can result in high total NM concentrations in the soil that may therefore lead to misinterpretation of the interaction mechanisms.

The amount of test substance applied to the soil columns should be sufficient to allow discrimination from the natural background during both depth profile and breakthrough curve analysis. It may not be possible to detect the test substance in all segments of the soil column. However, too high concentrations should be avoided as they may alter transport and leaching by increased agglomeration and clogging in the first cm of the soil column. In case high amounts of the test substance are needed to enable the distinction of the test substance from the natural background, the following options may be considered to reduce the required amount of test material introduced to the test system:

1. For geogenically occurring metals or metal oxides, the isotopic ratios of the natural metals / metal oxides can differ from those of manufactured NMs. These differences in the ratio between NM spiked soil and control soil can be measured and compared (using e.g. ICP-MS) (Gondikas et al. 2014; Praetorius et al. 2017; Montano et al. 2014).
2. NMs can contain impurities which can be used as tracers.
3. Labelled materials might be used to distinguish them from naturally occurring counterparts while it has to be ensured that labelling will not affect transport behaviour (see paragraph 28).
4. An alternative soil featuring a lower background of the NM studied can be selected. In contrast, the use of artificial soil / substrate, as a substitute to soil to high natural NM background, should be avoided as the deviation from the recommended soil types will be large and this will lead to unreliable extrapolations of results.

If available, analysis by SEM-EDX or TEM-EDX may also help to distinguish between natural and anthropogenic metal or metal oxide NMs by observing and describing the morphological characteristics of (nano)particles in spiked soil and control soil. In order to compare data and to consider concentration dependency, it is recommended to use a range of test concentrations (e.g., three test concentrations where the lowest and highest differ by one order of magnitude).

3.6.3. Soils

The selection of soils should be of environmental relevance, rather than selected based on the properties of the soils that promote leaching of NMs based on their physicochemical properties. However, as there might be various reasons for performing soil column tests, soils of interest might also deviate from those of environmental relevance. For testing the leaching of NMs, at least 2 soils, different in either pH, organic carbon content, clay content and/or texture, should be considered. Fewer soil textures compared to the OECD 312 are suggested for practical reasons. Soils with high clay content (soil 1, TG 312) tend to block during leaching experiments with NMs and sandy soils with high carbon content (soil 5, TG 312) have limited availability. Main emphasis, for the selection of soil textures, should be on differences in texture. In order to promote comparability and reproducibility of data, it is recommended that the properties of the chosen soils relate to those mentioned in Table 3.1. It has to be noted that the chosen parameters differ from the parameters recommended in the parent TG No. 312 and are aimed explicitly for testing NMs in soils. In case there is a need to investigate the fate of NMs in soils with higher organic carbon content or different texture, such a soil should be tested in addition, instead of in substitution, to other soils. Soils with high clay content should be avoided because the hydraulic conductivity of these soils is very low, which requires high flow pressures and makes saturated column tests difficult. In addition, many NMs tend to attach strongly to clay minerals (Cornelis et al. 2012; Cornelis et al. 2011), resulting in absence of NM breakthrough (Cornelis et al. 2013). Moreover, particle transport in clay-rich soils occurs predominantly in macropores (Ryan and Elimelech 1996), making experiments with saturated, stacked soil cores unrepresentative for NM transport rates in clay soils in the field.

Table 3.1 Guidance for selection of soils for leaching studies using nanomaterials

Soil No.	pH value (0.01 M $CaCl_2$)	Organic carbon %	Clay content %	Texture
1	5.0 ± 0.5	0.8 – 1.5	<10 - 15	loamy sand
2	6.5 ± 0.5	1.2 – 2.0	15 - 30	loam / silt loam
3	5.0 ± 0.5	3.0 – 4.5	<10	loamy sand / sand

Note: The soil pH can be measured using the method reported by EN 15933 (Sludge, treated biowaste and soil - Determination of pH) (EN 2012), or ISO 10390 Soil quality – determination of pH (ISO 2005).

3.6.4. Test conditions

A low flow rate of 2-3 $Lm^{-2}h^{-1}$ (corresponds to 0.2-0.3 $mLcm^{-2}h^{-1}$) can be used as a realistic worst-case scenario. Low flow rate represents realistic conditions of NM mobility in soils for which considerably longer residence time is expected compared to water soluble chemicals, and thus avoids an artificial breakthrough of NMs. However, the low flow rate results in long test durations which may be impractical in many cases. Although a higher flow rate (e.g., 100 $Lm^{-2}h^{-1}$ for sandy soils) can be more practical, it should be considered that this practice would require high pressure that deviates from realistic environmental conditions and can result in unrealistic interpretation of the NM leaching behaviour.

As a leaching solution, which mimics artificial rain, an aqueous solution of 0.005 M KCl or NaCl is suggested. This features a reduction of the recommended salt concentration of the parent TG OECD No. 312 (i.e. 0.01 M $CaCl_2$) and a change to a monovalent salt instead of divalent. The use of a divalent salt such as $CaCl_2$ would not provide a “worst-case scenario” test as it would favor (homo)agglomeration and thus, reduced mobility. For this reason, we recommend the use of a monovalent salt, such as KCl or NaCl. The chosen salt should consider environmental relevance and should not react with the NM by accelerating its dissolution. For NMs reacting with Cl (e.g. Ag) and forming barely water soluble, precipitating salts, suitable anions such as NO^{3-} (KNO_3) have to be used. Moreover, as recommended earlier, the stability of the NM suspension should be measured prior to the column test. Nonetheless, where the soil pore water is dominated by divalent cations a solution of 0.005M $CaCl_2$ can also be used to mimic such conditions (however, cannot be considered as “worst case scenario” conditions).

Conducting soil column tests under unsaturated water conditions is very challenging and requires a high level of technical expertise. To test a worst-case scenario, it is recommended to conduct experiments under conditions where the soil column is fully saturated with the leaching solution prior to introduction of the NMs.

In general, columns can be packed with either dry or wet soil. Dry soil is meant to be air-dried soil (preferably between 20-25 °C) while wet soil is adjusted to a certain water content. The decision of dry versus wet packing of columns is of general relevance for testing the behaviour of chemicals in soils and is the subject of ongoing scientific discussion. In this GD, it is recommended to preferentially pack the columns using dry soil. While wet packed soil might decrease the extent of air entrapment and water repellency, it is impossible to recommend standardised guidance for adequate water content as water adjustments depend on the physico-chemical properties of the selected soil and need to be individually chosen. As it cannot be excluded that different results will be obtained depending on whether soil is packed wet or dry, dry packing is suggested as preferred method to reduce variance in the results.

As mentioned in OECD TG No. 312, equilibration of the soils with the leaching solution should be performed in upflow mode in order to avoid air filled soil pores. In order to mimic the natural conditions of particle transport, leaching of the test substance should be performed in downflow mode. In addition, all solutions and dispersions should be degassed by sonication to avoid introduction of air bubbles into the column. NMs are known to interact strongly with air-water interfaces and air bubbles would thus introduce artefacts in the test. Sonication may alter NM dispersion characteristics, and therefore the conditions of the devices, duration and energy input need to be chosen carefully.

3.6.5. Test performance

At least duplicate leaching columns should be used for testing, but in general, triplicates are strongly recommended (see also paragraph 36).

The columns are preferably dry packed (see paragraph 45) with air-dried (preferably 20-25 °C) and sieved soil (< 2 mm) up to a height of approximately 10-20 cm. To obtain near uniform packing, the soil is added to the column in small portions with a ceramic spoon and pressed with a plunger under simultaneous gentle column vibration until the top of the soil column does not sink in further. Near uniform packing is required for obtaining reproducible results from leaching columns. The medium for equilibration (leaching solution without NMs) can then be added by flushing up-flow with a degassed aqueous solution. In this way a high degree of water saturation can be achieved.

Before adding the test substance, the columns should be leached with at least two pore volumes of leaching solution but also until the turbidity and conductivity of the effluent reach stable values to avoid unstable conditions during test substance transport in the column.

The application of the test substance as dispersion is recommended to achieve a more realistic emission scenario. It is not recommended to add NM powders directly to the soil. . Stock or test dispersions can be prepared using probe sonication (40 W, 10 minutes) using a volume of approximately 50-250 mL, with the exception of fibres and rods which may be damaged as a result of this preparation. Probe sonication allows treating large volumes of dispersion and enables reproducible energy input (compared to e.g. bath sonication). A highly concentrated stock suspension should be avoided as this will promote homoagglomeration and sedimentation in the stock suspension and thus introduce variability in the test dispersion. The sonication step can also be followed by a centrifugation step to remove large, undispersed agglomerates. However, in such a case, the surfactant NM concentration should be measured. If technically possible, the test dispersion should be prepared directly in order to avoid errors due to dilution of a stock suspension. If magnetic stirring is used to hold the NM in dispersion, care must be taken to ensure that stirring does not lead to increased agglomeration of the NMs or modifications of the surface of the NM affecting behaviour in the soil column. A homogeneous distribution of the dispersion over the cross section of the column should be ensured (by adding a thin layer of clean quartz sand over the packed soil). During application of the dispersion, the occurrence of supernatant of the test dispersion on top of the columns should be avoided. This can be achieved by lowering the speed of pulse application or step injection, respectively. The application can be performed as a single pulse application (with maximum volume corresponding to 5 % of the total pore volume of the packed porous matrix) or as a step injection (corresponding to 4 pore volumes). The decision on the most suitable application procedure depends on amount of NMs to be added considering the sensitivity of analytical method and the background concentration. Pulse application is suitable for NMs which can be added in a low amount due to sensitive chemical analyses and low background. For NMs which have to be added in higher amounts, step injection is to be preferred. Due to variables such as chemical identity of the NM to be tested, the chosen soil with its background concentration as well as available analytical devices, more precise recommendations on the most suitable application method cannot be provided. To study dynamic deposition interactions (e.g., blocking or ripening), it can be beneficial to use continuous injection corresponding to a volume of more than four times the pore volume~~s~~, but a continuous injection will inevitably add relatively high total NM concentrations in the soil. This may lead to interaction mechanisms that are not realistic for scenarios of spurious NM emissions to soils leading to low NM concentrations. The exact duration of the injection will depend on the experimental conditions, the NM tested and the type of soil. Stability of the NM dispersion should be verified (e.g., using OECD TG No. 318) prior to NM application to the test column. Dissolution of the NMs should also be characterised in the test solution medium (e.g., under consideration of OECD GD No. 318) to determine the fraction of NMs that have been dissolved. For estimation of pore volumes in the columns please refer to see paragraph 53.

Following introduction of the test substance, the column should be purged with the background leaching solution (particle free).

The leachates should be sampled every 0.5 ± 0.1 pore volume over a collection period equivalent to twice the number of pore volumes injected into the column (at least eight times the pore volume). The optimal material of the recipients in which the leachates are captured depends on the NM studied and should be checked individually prior to the start of the experiment. In the case of metal and metal oxide NMs, polymer materials or even Teflon are preferable instead of glass, because NMs may attach to the latter material. Glass may however, be a preferable material in the case of hydrophobic NMs such as carbon nanotubes or graphene.

Estimation of the pore volume in the columns: The pore volume in the columns is estimated using two separate replicates. The filled and weighed columns are saturated with water in upflow mode (see also paragraph 46). Surplus water is allowed to drain off until the water level equals the soil surface in the column. The column is weighed again. The difference between the two weight measurements (column with air dried soil and column with saturated soil) is used as an indicator of the pore volume. The pore volume can be estimated as follow:

(1) $$PV\ (cm^3) = V_{col} \times \theta$$

with

PV = pore volume (cm^3)

$\theta = 1 - M_{col}/(\rho_R \times V_{col})$

ρ_R = grain density ($g \cdot cm^{-3}$)

$V_{col} = l \times \pi \times r^2$

l = height of soil column (cm)

r = diameter of soil column (cm)

M_{col} = dry matter of soil column

The time required to leach of one pore volume can also be calculated as follow:

(2)

$$t\ (h) = \frac{PV}{Uo(\pi r^2)}$$

with

Uo = superficial fluid velocity ($cm^3 \cdot cm^{-2} \cdot h^{-1}$)

PV = pore volume (cm^3)

r = radius of the column (cm)

A more exact determination of the effective pore volume may be obtained based on tracer breakthrough data (see simulation models in paragraph 61, for mathematical basic see Šimůnek et al. (2006).

3.6.6. Analytics

Mass concentration of the NMs, pH, electrical conductivity, and turbidity should be determined in the leachates. Depending on the specific testing goals, measurement of particles features (e.g. size distributions) might also be considered. All analyses should be done at least with two replicates, although a higher number of replicates is recommended. Acid digestion of the leachates, immediately after they are obtained, is highly recommended for metal and metal oxide NMs. NMs may attach to walls of the recipients and/or agglomerate and sediment leading to underestimation of the leached NM concentration. Analysis of the leachate samples can occur without prior digestion of the leachates in some cases, but recovery of the NM mass concentration in non-digested samples should at least be compared with digested samples. In the case of NMs where digestion destroys the NM material itself (e.g. carbon-based NMs), leachate analysis should occur as soon as possible.

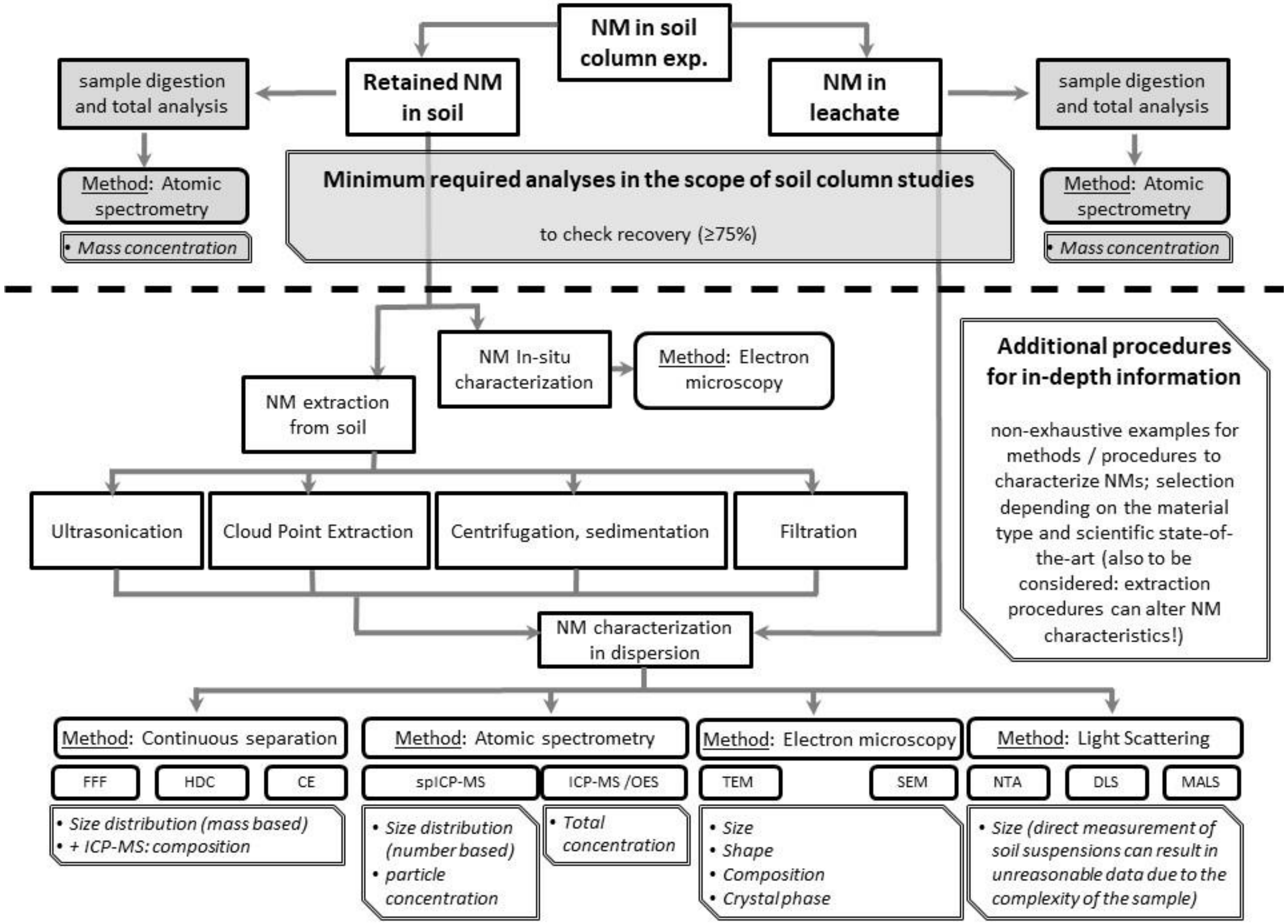
For analysis of the NMs retained in the soil (depth profile), the upper 10 cm of the column should be sectioned at minimum into 2 cm layers, the rest of the soil in 5 cm layers because for most NMs, only little transport of NM is expected. Care must be taken not to excessively disturb the soil such as to cause release of NMs from the soil grain surfaces Tufenkji and Elimelech 2004; Tufenkji and Elimelech 2005). To avoid broad scattering of data, every soil layer has to be homogenized before sample analysis. These layers should be analysed choosing an appropriate number of replicates of samples per layer in accordance with the diameter of the column (for the recommended minimum number of replicates see paragraph 47). In the case of metal and metal oxide NMs, the most common analysis of the retained NM mass involves an acid digestion followed by atomic spectrometry analysis (ICP-MS or ICP-OES). Standard digestion methods applicable to metal analysis in soils such as US EPA method No. 3052 (1996) can be used and involve appropriate blanks and controls for recovery.

The analytical method which should be used for the detection of the NMs in the soil and eluate samples is dependent on the type of information which is needed for assessment of the column experiments, e.g. NM mass in the samples, particle number size distribution, agglomeration state, etc. Based on the needed information, standard and/ or more advanced analytical tools can be used to answer these questions. All of these methods have advantages and disadvantages that have to be taken into account. Currently, no universal method for the analysis of NMs in soils exist. We therefore recommend to carefully review the scientific literature and to select the method that best provides the analytical data of interest. Furthermore, the specific requirements for the chemical analysis which will be used have to be taken into account before the experiments are conducted and if standard methods exist they should be applied (e.g., the ISO standard for spICP-MS (ISO 2017) or the ISO standard for asymmetrical-flow and centrifugal field-flow fractionation (ISO 2018). Scientific literature may also provide useful methodologies (Peters et al., 2015; Hadioui et al., 2014; Hadioui et al., 2015). Furthermore, the requirements and chosen analytic method and procedure should be reported in detail, to guarantee that sufficient data interpretation and comparison with other data is possible.

Advice for selecting appropriate methods for analysis of NMs in soil samples and leachate as well as parameters which will be addressed by applying these methods is provided in Figure 1. In this figure, relevant methods and corresponding endpoints are listed. A distinction is made between methods that are required as a minimum analysis (i.e. for determination of recovery) with regard to soil column studies (grey boxes), and methods that are available for more thorough analysis (white boxes). Methods for additional analysis depend on the tested NM and/or the individual scope of the study. Please consider that the list of methods is non-exhaustive and a selection of methods will depend on the NM under investigation and scientific question to be answered. With respect to the recommended techniques in Figure 1, it is important to note that once NMs are in soil, some of their properties will alter by interaction with the soil matrix. These properties include agglomeration/aggregation state, surface functionalization, zeta potential, IEP, dissolution kinetics.

These parameters are much more difficult to determine in the soil than in the soil pore water, thus, the sample preparation for characterisation and interpretation of results should be considered with caution.

Figure 1. Selecting appropriate methods for analysis of NMs in soil samples and leachate (for abbreviations see chapter 5.2; exp. = experiment)



3.7. Data and reporting

The utilization of the parent TG OECD No. 312, upon which this GD is drawn will provide information on retention and mobility. Parameters such as K_{oc} and K_{om} as presented in the parent TG are not useful or applicable for NMs (Praetorius et al. 2014), as they assume uniform distribution while not considering kinetic processes of NMs as aggregation and sedimentation. Alternatively, calculations of particle attachment efficiency (α) can be carried out as per Yao et al. (1971); Tufenkji and Elimelech (2004). α is a kinetic parameter expressing the probability that NMs will attach when they collide with the soil grain surface. The calculation of this parameter takes into account random effects caused by the way the soil matrix happens to be structured.

A quantitative estimation of α can be obtained from interpretation of column transport tests where a continuous NM input is applied into the column and NM concentration level is monitored over time at the column outlet. The following equation can be used.

$$\alpha = -\frac{2d_c}{3(1-\theta)L\eta_0}\ln\left({}^{C}\!/_{C_0}\right)$$

Where C is the steady-state concentration level measured at the column outlet (this corresponds to the constant concentration level observed at the outlet after particles have been injected into the column for a sufficient amount of time), C_0 is the mass concentration of the NM stock dispersion added to the column. d_c (d_{50}) is the median grain size of the soil, θ is the porosity of the soil. The porosity value obtained during column preparation can be used as described above, although the effective porosity obtained from inert tracer data using modelling software is more accurate (see paragraph 62). L is the length of the column and η_0 is the single-collector contact efficiency calculated according to Yao et al (1971) or Tufenkji and Elimelech (2004). If a short pulse injection is used and a steady-state concentration is never reached, the following formula of Harvey and Garabedian (1991) can be used instead:

$$\alpha = -\frac{d_c\left(\left(\left(1-2\frac{D}{L}\right)\ln(RB)\right)^2-1\right)}{6(1-\theta)D\eta_0}$$

D is the dispersity which should be calculated from inert tracer data. RB is the integrated NM mass recovery obtained from the increasing section of the breakthrough curve (concentration as a function of time at the column outlet) by using modelling software.

The formulae above show that α can only be calculated when d_{50}, effective porosity and/or dispersity are determined. The first formula applies for continuous inputs only, whereas the second formula applies for shorter pulse injection (where a plateau is not reached). d_{50} cannot be obtained from a classical texture analysis, because aggregates are broken up during this analysis. It is recommended that the median aggregate diameter is obtained in the soil suspended in the medium used for the column experiment using e.g. by laser diffraction, static light scattering or disc centrifuge. Alternatively, the grain size of coarser grained soils can be determined using dry stacked sieving.

The determination of α is based on the “clean bed” assumption, which is valid only during the early stages of the deposition process, when low particle loadings are applied and no significant repulsion between particles and porous medium is present (i.e. favourable attachment conditions due to the absence of significant repulsive energy barriers in DLVO interaction profiles). Outside this validity range, more complex mechanisms can influence the particle transport behaviour (e.g. blocking, ripening) (Elimelech et al. 1995; Bianco et al. 2016) and α is not able anymore to properly describe the system. In these cases, the use of α may lead to misinterpretation and misuse of the received data, and more comprehensive numerical models are necessary to perform a reliable quantitative analysis of the results. Here a list of the most commonly used software for simulation of NM transport in porous media is reported:

- STANMOD (STudio of ANalytical MODels - https://www.pc-progress.com/en/Default.aspx?stanmod): analytical model for solute transport with graphical interface; suitable only for simple retention mechanisms; moderate modelling skills are required.
- MNMs 2021 (Micro-and Nanoparticle transport, filtration and clogging Model – Suite - https://areeweb.polito.it/ricerca/groundwater/software/mnms/): specialized numerical model for NM transport with graphical interface; simulation of a wide range of retention mechanisms and complex hydrochemical (e.g. transient ionic strength) and flow (e.g. porous medium clogging, effect of fluid viscosity) conditions; only suitable for saturated porous media; implements specific tools for nanoparticle transport characterisation (e.g. DLVO interaction energy profiles, single collector efficiency calculation); low to moderate modelling skills are required.
- Hydrus-1D (https://www.pc-progress.com/en/Default.aspx?hydrus-1d): numerical model for solute and particle transport with graphical user interface; simulation of a wide range of retention mechanisms; simulation of colloid facilitated transport of solutes; suitable for both saturated and unsaturated porous media; moderate to high modelling skills are required.
- ColloidFit (https://tuceel.tuc.gr/colloidfit): specialized analytical and numerical model for NM transport with graphical interface; simulation of a wide range of retention mechanisms and gravity effect; only suitable for saturated porous media; moderate modelling skills are required.

The overall recovery (mass balance) of NMs should also be determined and reported (as described in paragraph 28).

In addition to the necessary information mentioned in the parent guideline OECD TG No. 312, for accurate interpretation the report should include the parameters listed in chapters 7.1 -7.4.

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5. Terminology, definitions and abbreviations

5.1. Definitions

agglomeration	Process of contact and adhesion whereby dispersed particles are held together by weak physical interactions ultimately leading to enhanced sedimentation by the formation of particles (agglomerates) of larger than colloidal size. In contrast to aggregation where particles held by strong bonds like sinter bridges, agglomeration is a reversible process.
breakthrough	The presence of solute/target substance/NM in the column effluent.
dispersibility	The condition of particular material of being dispersible or a measure to which extent a particular material is dispersible in a dispersing medium or continuous phase. Dispersion stability refers to the ability of a dispersion to resist change in its properties over time.
dissolution	Process under which a substance dissolves.
dissolution rate	The amount of substance dissolved (solute) into a solvent over time.
size	Size of the particles, aggregates or agglomerates is given in micrometres (µm) or nanometres (nm). The method for particle size determination and the character of the particle size average should be reported.

Note: Additional definitions and units relevant for testing NMs under environmental conditions can be found in the OECD TG No. 318 “Dispersion stability of nanomaterials in simulated environmental media” (OECD 2017) and OECD GD No. 318 “Testing of dissolution and dispersion stability of nanomaterials, and the use of the data for further environmental testing and assessment strategies” (OECD 2020).

5.2. Abbreviations

CE	Capillary electrophoresis
DLS	Dynamic light scattering
DLVO	Derjaguin-Landau-Verwey-Overbeek-Theory
DOM	Dissolved organic matter
FFF	Field Flow Fractionation
GD	Guidance Document
HDC	Hydrodynamic chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IEP	Isoelectric point
ILC	Interlaboratory Comparison Test
JEG	Joint WNT-WPMN Expert Groups on Ecotoxicity and Fate of Nanomaterials
K_d	Soil adsorption coefficient
K_f	Freundlich adsorption coefficient
K_{oc}	Organic carbon normalised adsorption co-efficient
k_{OM}	Organic matter normalised distribution co-efficientLimit of detection
LOD	Limit of detection
LOQ	Limit of quantification
MALS	Multi angle light scattering
MWCNT	Multi walled carbon nanotubes
NM	Manufactured nanomaterials
NTA	Nanoparticle tracking analysis
pK_a	Acid dissociation constant
QD	Quantum dots
PVP	Polyvinylpyrrolidone
spICP-MS	Single particle inductively coupled plasma mass spectrometry
TEM-SAED	Transmission electron microscopy, selected area electron diffraction
TEM/SEM EDX	Transmission electron microscopy/scanning electron microscopy energy-dispersive X-ray spectroscopy
TG	Test Guideline
TOC	Total organic carbon

TOF SIMS	Time of Flight - Secondary Ion Mass Spectrometry
WNT	OECD Working Group of the National Coordinators for the Test Guidelines Programme
WPMN	OECD Working Party on Manufactured Nanomaterials
XRD	X-ray diffraction
XRF	X-ray fluorescence

6. Deviations of the GD to TG No. 312

6.1. Overview of modification and additions as guidance in deviation to OECD TG No. 312

TOPIC	OECD TG No. 312	Guidance on NMs
General		
Applicability domain	Test substances (unlabelled or radio-labelled: e.g. ^{14}C) for which an analytical method with sufficient accuracy and sensitivity is available (except chemicals which are volatile from soil and water).	All NMs for which accurate and sensitive analytical strategies are available. Furthermore, following the GD will not produce reliable results for NMs which cannot be sufficiently dispersed such that an adequate amount of test material can be added to the soil column.
Information on the test substance	Solubility in water, solubility in organic solvents, vapour pressure and Henry's Law constant, n-octanol/water partition coefficient, adsorption coefficient (K_d, K_f or K_{oc}), hydrolysis, dissociation constant (pK_a), aerobic and anaerobic transformation in soil.	Chemical composition, primary particle size and size distribution, shape, crystal phase, surface coating or functionalization, zeta potential, isoelectric point, dispersion stability, dissolution behaviour, hydrophobicity.
Reference substance	Use of reference substance with known high leaching behaviour (atrazine or monuron).	Inert tracer that should not interact with the soil medium and the investigated NM, e.g. potassium bromide or sodium nitrate.
Quality criteria	Recovery (70-110 % for non-labelled, and 90-110 % for radio-labelled substances), repeatability and sensitivity of the analytical method.	Recovery (cumulative NM mass in the leachate and the cumulative NM mass in the dissected soil sections) of at least 70 %.

TOPIC	OECD TG No. 312	Guidance on NMs
Detection	"an appropriate analytical method of known accuracy, precision and sensitivity for the quantification of the test substance".	Different options (for metals and metal oxides): 1. NM differentiation from natural background using specific ratios of different metals occurring naturally; 2. NMs containing impurities 3. Labelled materials; 4. Use of alternative soil with lower background of NM in question.
Determination of detection limit	0.01 mg/kg in each soil segment or leachate, or 0.5 % of the applied dose.	Default value not possible. Detection limit needs to be determined for the selected method individually based on the used medium, the NM and background levels.
Test Performance		
Choice of concentrations	Amount of test substance applied to the soil columns should be sufficient to allow for detection of at least 0.5 % of the applied dose in any single segment. For active substances in plant protection products, the amount of test substance applied may corresponds to the maximum recommended use rate (single application).	Concentrations that ensure their detection by appropriate analytical techniques; i.e. to allow discrimination from natural background levels, however amounts should be as low as possible to avoid clogging and agglomeration.
Column specifications	Made of inert material (glass, stainless steel, aluminum, teflon, PVC), 30 cm long	Glass (covered) or stainless steel, 10-20 cm long
No. of columns per experiment	At least duplicates	Strong recommendation for triplicates
Soil texture and composition	5 soils: clay loam, silt loam, loam, loamy sand, loamy sand/sand.	At least 2 soils: loamy sand/sandy loam, loam/silt loam A recommendation for a potential 3rd soil is given.
Guidance on test substance preparation for application	Dissolve in water (deionized or distilled), poorly soluble substances can be used as formulated products or in an organic solvent, solid formulations should be applied in solid from without water.	Application as dispersion is recommended. The test dispersion should be prepared directly from the stock. Homogenous distribution of dispersion over the cross section can be ensured by a thin layer of clean quartz over packed soil.

TOPIC	OECD TG No. 312	Guidance on NMs
Injection and Flow rate	No guidance.	Application of test substance as pulse application (with a maximum of 5 % of the total pore volume of the packed porous matrix) or as a step injection (corresponding to approximately a volume of two to four times of the pore volume). To study dynamic deposition interactions (e.g., blocking or ripening), it can be beneficial to use continuous injection for more than four times of the pore volume. The exact duration of the injection will depend on the experimental conditions, the NM tested and the type of soil. To account for more realistic conditions of NM mobility in soils a low flow rate of 2 $Lm^{-2}h^{-1}$) (corresponds to 0.2 $mLcm^{-2}h^{-1}$) is recommended. To limit test durations, a higher flow rate (e.g., 100 $Lm^{-2}h^{-1}$ for sandy soils) can be applied, however, this will require high pressure deviating from realistic conditions.
Leaching solution/artificial rain	0.01 M $CaCl_2$	0.005 M KCl or NaCl (KNO_3^- for those NMs reacting with Cl^-). The chosen salt should not influence NM stability against agglomeration, transformation or dissolution. Furthermore, the environmental relevance of the chosen salt should be considered, if applicable.
Guidance on sampling of leachate	Leachates should be collected in fractions and their respective volumes are recorded.	Leachates should be sampled every 0.5 pore volumes over a collection period equivalent to twice the number of pore volume injected into the column (e.g., at least 8 times of the pore volume). The optimal material of the recipients in which the leachates are captured depends on the NM studied and should be checked individually.
Pore volume estimation	no	yes
Analytics and Reporting		
Analytics	no guidance	A figure is used to guide the selection of suitable and appropriate analytical methods depending on the needed depth of information.
Number of replicates	At least by duplicate analysis.	strong recommendation for at least triplicates.

TOPIC	OECD TG No. 312	Guidance on NMs
Reporting	Test substance and reference substance (when used), tests soils, test conditions, test results.	Need for additional information: • physicochemical data of the tested NMs and methods used for determination; • time dependent stability and dissolution rate in test media for application to the columns (test dispersion); • physical and chemical properties of the soil used, storage and handling; • sample preparation and application of the NMs to the soil column; • size and zeta potential of the particles in column effluent during breakthrough; • concentration in soil segments and leachate.
Relevant calculations to report	K_{oc}, K_{om}	Particle attachment efficiency α as probability of NMs to get retained onto the soil grain surface. It allows quantitative comparison of the behaviour of different NMs when injected into the same porous medium.

7. Reporting requirements

The following tables describe additional reporting requirements for testing NMs using OECD TG No. 312. These requirements are specific to NMs and understood as addition to the reporting described in the parent TG.

7.1. Minimum particle characterisation parameters to report (for abbreviations see Chapter 5.2) [1]

Parameter	Instrument/Method	Metric to report	Metadata to report
Chemical composition	SEM/TEM-EDX	stoichiometric formula	Acceleration voltage,
	XRF	stoichiometric formula	impurities
	Digestion + ICP-MS	stoichiometric formula	impurities acid used digestion protocol used
	Manufacturer information	stoichiometric formula	URL of manufacturer
(Primary) Particle size distribution[2]	TEM	average corresponding spherical size	grid pretreatment (e.g. air-drying, freeze-drying)
	SEM	average corresponding spherical size	Sample preparation (powder or liquid) Coating applied (carbon or gold)
	XRD	average crystallite size (Sherrer equation)	
	TEM	relative number concentration distribution	grid pretreatment (e.g. air-drying, freeze-drying). particle recognition algorithm (e.g. ImageJ – manual)
	SEM	relative number concentration distribution	Sample preparation (powder or liquid) Coating applied (carbon or gold)

Parameter	Instrument/Method	Metric to report	Metadata to report
	spICP-MS	Number based particle size distribution.	Dilution factor Dilution medium Nebulisation efficiency determination method dissolve/particulate discrimination method and parameter (e.g. 3 x sigma)
Shape	TEM	shape (e.g. sphere, ellipsoid, rod,…) Average dimensions Aspect ratio	grid pretreatment (e.g. air-drying, freeze-drying))
	SEM	shape (e.g. sphere, ellipsoid, rod,…) Average dimensions Aspect ratio	Sample preparation (powder or liquid) Coating applied (carbon or gold)
	Static light scattering	shape (e.g. sphere, ellipsoid, rod,…) Average dimensions	Scattering model applied (e.g. cylinder, sphere, hollow sphere,…) Fit of model
Crystal phase	XRD	crystal phase	resolution
	TEM-SAED	crystal phase	grid pretreatment (e.g. air-drying, freeze-drying)
Surface coating	Raman	Coating molecule Coating length Occupancy (% coverage)	Sample preparation
	TOF-SIMS	Coating molecule Coating length Occupancy (% coverage)	Sample preparation
Concentration in test medium	ICP-MS/ICP-OES	mass concentration (e.g. mg L^{-1})	Dilution factor Dilution medium

Parameter	Instrument/Method	Metric to report	Metadata to report
	spICP-MS	number concentration (e.g. # particles mL^{-1})	Dilution factor Dilution medium Nebulisation efficiency determination method dissolve/particulate discrimination method and parameter (e.g. 3 x sigma)
Dispersion stability	TG 318	see reporting requirements in TG 318	
Dissolution	GD 318	see reporting requirements in GD 318	
Hydrodynamic diameter in the test medium.	DLS	Z-average hydrodynamic diameter polydispersity index.	Applied test concentration
Zeta potential in test medium	Calculation from electrophoretic mobility	Zeta potential	Applied test concentration

[1] Any additional measurements selected from Figure 1 have to be reported, too.

[2] Please consider that an OECD TG on particle size and size distribution of NMs is currently under development (WNT project 1.4).

7.2. Particle stock sample pre-treatment to report

The stock sample is the sample being injected in the soil column.

Pre-treatment action	Parameters to report
Suspending*	Particle concentration dispersed Dispersion medium

	Time between dispersion and analysis
Sonication*	Sonication time Sonication power Sonication volume
(post-sonication) centrifugation	Centrifugation time Centrifugation speed Sample holder height Position of supernatant sampling
(post-sonication) filtration	Filter pore size Filter material
Stirring/shaking	Stirring method (end-over-end, magnetic stirring,…) Stirring speed
dilution	Dilution factor Dilution medium

*****Only valid for NM supplied as powders

7.3. Soil properties to report

The relevant soil properties are presented in the following table.

Soil parameter	**Method(s)**
Soil pH	glass electrode measurement in 0.01 M $CaCl_2$ extract (alternatively 1M KCl could be used)
Total organic carbon	e.g. dry combustion DIN EN 15936:2012-11: Sludge, treated biowaste, soil and waste - Determination of total organic carbon (TOC) by dry combustion
Texture	% clay, % silt, % sand using pipette method
Average grain size	d_{50} determination of a soil suspension using laser scattering or disc centrifuge. Alternatively, stacked sieving of air-dried soil.
Pore water pH	glass electrode measurement of column leachate after equilibration
Pore water conductivity	conductivity measurement in column leachate after equilibration

7.4. Column experiment parameters to report

Column aspect	**Parameters to report**
Column dimensions	length diameter
Flow speed	linear flow speed or Darcy velocity
Medium	Chemical composition major ions and anions pH Conductivity
Effective porosity	as obtained from inert tracer test and modelling
Dispersity	as obtained from inert tracer test and modelling
Top and/or bottom filter	Mesh size Material of filter % recovery of NM during filtration
Time between preparation of stock suspension and column experiment start.	
Attachment efficiency	formulae used to calculate attachment efficiency (see text) Hamaker constant used to calculate single-collector contact efficiency, if applicable.