# DRAFT GUIDANCE DOCUMENT ON TESTING NANOMATERIALS USING OECD TG No. 312 "LEACHING IN SOIL COLUMNS"

The draft Guidance Document was developed by the leads of the WNT project 3.14, Canada and Germany. Previous versions were circulated for inputs amongst the Joint WNT-WPMN Expert Groups on Ecotoxicity and Fate (2018 and 2019) followed by an inter-laboratory comparison test (ILC) to evaluate the benefit of the proposed modifications for NMs and to ensure the comparability of results.

The previous draft versions of this Guidance Document, together with comments received, are available in the TG Expertise site under Nanomaterials Safety Testing [see dedicated section: *Project 3.14 Draft GD Behaviour in Soils Using TG 312 for NMs*].

This is the first draft to be circulated for WNT comments. The WNT is invited to review this draft and provide detail comments by 22nd January 2021.  To supplement the Guidance Document, please note that the report of the Inter-laboratory Comparison Test  (ILC) is also made available in the TG expertise site as follows:

- Report of the ILC for OECD GD312 for Testing NM Final Draft (Nov2020)
    - Annex 1_Interlaboratory Comparison Test Plan
    - Annex 2_Preparation of Stock Suspensions
    - Annex 3_ILC Preparing od Soil Column Dry Packaging
    - Annex4_ILC Graphic soil Column test set up
    - Annex 5_ILC Reporting sheet test performance
    - Annex 6_ILC TG312 Efficiency Calculation
    - Annex 7_ILC rev TG 312 for NM_version comparison test
    - Annex 8_ILC Compilation of Results

The WNT is invited to review this document by **Friday 22nd January 2021**.

# Background

1.     1.      The need for a Guidance Document for testing the behaviour of engineered nanomaterials (NMs)
2. in soils using the OECD TG no. 312 was identified as a priority goal by the OECD's Working Party on
3. Manufactured Nanomaterials (WPMN). On behalf of the WPMN an expert meeting on ecotoxicology and
4. environmental fate of nanomaterials took place in January 2013 in Berlin. During the meeting, it was
5. concluded that the OECD TG No. 312 is generally applicable for the testing of NMs. However, an additional
6. Guidance Document needs to be developed with specification for the testing of NMs (OECD, 2014).  As a
7. follow-up to this conclusion, a Standard Project Submission Form (SPSF) was submitted to OECD and the
8. WNT in November 2016. Following review by National Experts, the SPSF was approved by the WNT-29
9. in April 2017.

10.   2.      In 2017, a preliminary draft guidance was developed by Canadian and German experts, which
11. was further elaborated based on feedback provided by the OECD experts via teleconferences. The first
12. draft was sent to the OECD WNT Expert Group on Environmental Fate of Nanomaterials in June 2018 for
13. commenting.  A 2nd draft version was presented for discussion at the meeting of the Joint WNT/WPMN
14. Expert Groups on Environmental Fate of Nanomaterials and on Ecotoxicity of Manufactured Nanomaterials
15. held in December 2018 in Arona, Italy and open for a round of written comments.

16.   3.      Based on the discussion at the meeting and subsequent comments, it was decided to conduct an
17. inter-laboratory comparison test (ILC) to evaluate the benefit of the proposed modifications for NMs as well
18. as to check if comparability of results is still ensured. The ILC took place from June 2019 to December
19. 2019 followed by the assessment of the analytics by some of the partner laboratories. Results were
20. obtained from seven laboratories for two different nanomaterials (silver, cerium oxide) in two soils featuring
21. different characteristics to enable differentiated leaching behaviour. Results of the ILC and conclusions
22. derived from them for consideration for the draft GD were summarized in a report. Based on the results
23. and experiences from the ILC, further adaptions of the draft GD were made and discussed by the core
24. group, resulting in this draft guidance presented for a first round of comments by the WNT.

# 1. Introduction

4.      It is accepted that the existing OECD Test Guidelines are generally applicable to test NMs, but there are needs for adaptation of certain Test Guidelines or additional Guidance Documents. The OECD Test Guidelines are designed primarily for organic, soluble or readily soluble chemicals. This means that shortcomings in the utilization of these Test Guidelines for testing NMs often relate to 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 that for the investigation of NMs, OECD Test Guidelines should be applied and adapted as appropriate to take into account the specific properties of NMs (OECD 2013).

5.      The need for an OECD Guidance Document (GD) for testing the fate of engineered nanomaterials (NMs) in soils using the OECD TG No. 312 (OECD 2004) was identified during the "Expert Meeting on Environmental Fate and Ecotoxicology of Nanomaterials" which took place in January 2013 in Berlin on behalf of the OECD Working Party on Manufactured Nanomaterials (WPMN). In 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).

6.      For chemicals, it is assumed that mainly thermodynamic processes determine their distribution in the environment because equilibrium is reached in many cases. However, equilibrium partitioning does not apply to undissolved NMs as they do not form solutions but colloidal dispersions that are thermodynamically unstable. Thus, the fate of NMs in the environment is subject mainly to kinetic processes such as (hetero-)agglomeration and sedimentation (Praetorius et al. 2014) as they will never end up in a thermodynamic equilibrium. Thus, methods based on thermodynamic processes are not applicable for reporting on the fate of NMs in environmental matrices. The OECD Test Guideline 106 ("Adsorption-Desorption Using a Batch Equilibrium Method") was developed to report on the fraction of a chemical adsorbed to soil (OECD 2000). However, this Test Guideline is not appropriate for NMs in soils, because it assumes a thermodynamic equilibrium between the dissolved and adsorbed substance. Instead, an adapted version of OECD Test Guideline No. 312 becomes of particular importance for the determination of the behaviour of NMs in soils in a robust and reproducible manner, because this result can be interpreted kinetically and thus has the potential to generate kinetic descriptors that characterize the fate of NMs in soils.

7.      As a follow-up to these conclusions, a Standard Project Submission Form (SPSF) was agreed on within WPMN and was submitted by Canada and Germany to OECD and the Working Group of the National Coordinators for the Test Guidelines Program (WNT) in November 2016. The SPSF was approved by the WNT-29 in April 2017.

8.      Subsequently, in a joint effort, Canadian and German experts started to discuss and elaborate the necessary changes to the OECD TG No. 312 in order to identify which elements of the test guideline might need adaptation within a GD to ensure its applicability to testing NMs. For this aim, international experts were consulted and initial leaching tests with selected NMs (nanoscale silver) and selected soil types (loamy sand (RefeSol 01-A) and silt loam (RefeSol 02-A)) were performed to identify similarities and differences in test results based on test performance. This experimental approach aimed to generate information about which aspects of test set up and performance need a distinct consideration when testing NMs. Additional experts were consulted to support this discussion with data and scientific experience on a national and international basis. Furthermore, scientific findings were considered indicating the need to adjust existing methods to investigate mobility and fate of NMs in soils. Based on the comments received

68 by the WNT expert group on environmental fate and behaviour in June and December 2018, it was decided
69 to conduct an interlaboratory comparison test (ILC) to evaluate the accuracy as well as the benefit of the
70 proposed modifications of the GD for NMs and to check if comparability of the obtained test results is still
71 ensured. The ILC took place from June 2019 until December 2019 plus additional weeks for analytics by
72 some of the partner laboratories. Results were obtained from seven laboratories for two different
73 nanomaterials (silver, cerium oxide) in two soils featuring different characteristics to enable differentiated
74 leaching behaviour and summarized in a report. Subsequently, the draft GD was revised mainly based on
75 the results and experiences with nanosilver and nanoscale cerium oxide from the ILC.

76 9. The aforementioned activities, reviews, discussions and consultation meetings identified a list of
77 aspects for which guidance is needed. These aspects include choice of column, choice of soil, test amount,
78 spiking and application of the test substance, dispersion protocol, test duration, analytics as well as
79 reporting requirements.

80 10. This document provides specific guidance for accurate test preparation, implementation,
81 performance, analysis and reporting using the OECD TG No. 312 for testing the mobility and retention of
82 NMs in different types of soils; however, the guidance is likely also relevant for colloidal materials of greater
83 size ranges, because transport of these materials occurs via the same kinetically dominated processes
84 that determine the fate of NMs. The document informs on necessary modifications and additions to the
85 standard test protocol including preparation and application of the test materials, analytics and data
86 reporting. The modifications and additions proposed in the GD are included in order to generate relevant,
87 accurate and reproducible data on NM retention and mobility in soils. Estimations of parameters such as
88 $K_{oc}$ and $K_{om}$ as presented in the parent TG No. 312 are not applicable for NMs.

89 11. The GD is structured in a way that recommended modifications and additions to the test method
90 and performance as presented in the OECD TG No. 312 are given in accordance with the chapters of the
91 parent TG. An overview of the proposed modification and additions in deviation to OECD TG No. 312 is
92 given in tabular format in Annex 5.2 to this GD.

# 2. Scientific Background

93

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

98 13. In general, the transport of NMs through soil (and other unconsolidated porous media) as well as
99 their fate is governed by (i) the physical and chemical characteristics of the respective NM, including
100 particle size, shape, concentration, surface properties, stabilizing agents used, aging behaviour,
101 aggregation and deposition behaviour, and (ii) by characteristics of the bulk soil and (iii) the soil pore water.
102 In the soil, especially grain size distribution, surface roughness and percentage of clayey material can play
103 an important role, but also moisture content and chemical composition of the medium (e.g. presence of
104 organic matter, iron oxides and hydroxides, oxygen, surfactants, and microorganisms). A third key factor
105 defining NM transport is the pore water chemistry; namely, its chemical composition, ionic strength, pH,
106 the presence of multivalent ions, concentration of dissolved organic matter and colloidal material as well
107 as hydraulic properties such as flow velocity and direction that determine residence time.

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

112 15. In Cornelis et al. (Cornelis et al. 2014) the literature on the fate of NMs in soils and their
113 bioavailability was summarized. Most of the processes determining the fate of colloids in soil also
114 determine the fate of NMs. The main fate-determining processes in solid matrices are leaching of NMs
115 from biosolids (e.g., after contaminated sewage sludge is added to soil), homoagglomeration
116 (agglomeration of NMs with each other – relevant mainly under artificial lab conditions),
117 heteroagglomeration (agglomeration of NMs with unlike particles such as natural colloids or other NMs),
118 deposition (attachment of NMs after they approach a pore wall by Brownian diffusion, direct capture on
119 grain surfaces or sedimentation), straining (physical entrapment of NMs in pore spaces) as well as
120 transport processes in soils. The mobility of NMs in soils is predominantly controlled by salinity, texture,
121 pH, concentration and the nature of mobile organic compounds, degree of saturation, and the presence of
122 macropores. Interactions with solutes, i.e., inorganic (e.g., sulfate, phosphate) and organic molecules such
123 as DOM (dissolved organic matter, e.g., humic and fulvic acids, exudates from soil organisms) which are
124 ubiquitous in soil pore waters significantly alter the physicochemical characteristics and therefore the fate
125 of NMs in the environment. DOM adsorption often renders NM surface potential more negative and
126 increases NM transport because of steric and electrostatic stabilization. However, this may not be
127 considered uniform as DOM adsorption depends on DOM characteristics, NM properties and multivalent
128 cations in soil solution (Degenkolb et al. 2019; Zehlike et al. 2019). Several factors also influence the
129 release of NMs from grain surfaces. In particular, slow diffusion-controlled NM release occurs under
130 constant physicochemical conditions depending on the depth of the NM-grain surface interaction energy
131 minima. Shallow minima (certain roughness conditions) are subject to more rapid release than deeper
132 minima. Only a small fraction of retained NMs may be released under constant physicochemical
133 conditions. Conversely, changes in solution chemistry (decrease in ionic strength or increase in pH), cation
134 exchange (displacement of divalent ions by monovalent ions), or water saturation can produce large
135 episodic release pulses due to alteration of forces and torques controlling retention.

136 16.    Several researchers have investigated the impact of soil and NM properties on NM transport. For
137 example, the transport of PVP-coated AgNP in 11 natural soils (grains <2mm) under saturated conditions
138 was studied by means of column tests (Cornelis et al. 2013). Prior to transport experiments with AgNP,
139 tracer tests using bromide were carried out. HNO3 or NaOH were used to adjust the pH for the respective
140 soil and KNO3 was used as background solution (artificial pore water). AgNP breakthrough was analyzed
141 by applying a model that made use of two-site kinetic attachment–detachment. Results suggest increased
142 deposition of AgNP and/or increased straining after heteroaggregation of AgNP with mobile soil colloids.
143 PVP-coated AgNP can easily interact with natural colloids in soils, which significantly reduces their mobility.

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

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

155 19.    Quevedo and Tufenkji (Quevedo and Tufenkji 2012) studied the transport of two types of quantum
156 dots (QDs) and a nanoplastic in quartz sand and loamy sand over a range of ionic strengths in KCl or
157 CaCl2 solutions. NM deposition rates were at least an order of magnitude greater in the loamy sand than
158 in the quartz sand. Also, the three types of NMs exhibited different transport potential in the loamy sand,
159 likely due to differences in the binding affinities of the nanoparticle surface coatings for specific soil
160 constituents such as clays.

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

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

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

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# 3. Specific guidance on NMs

183 ## 3.1. Applicability of the test

184 23.     The guidance given in this document is applicable for all NMs for which accurate and sensitive
185 analytical strategies are available. Different analytical techniques and strategies might be needed
186 depending on the physicochemical properties of the tested NMs and on the background NMs present in
187 the tested soil. Even though the accuracy of advice given in this GD was experimentally checked to only a
188 limited number of NMs (i.e. nanosilver, cerium oxide), they are supposed to be valid for the current
189 commonly known NMs fulfilling the criteria mentioned. In cases this GD is intended to be followed for NMs
190 strongly deviating in properties currently known, the advices should be carefully checked for applicability.
191 However, this GD is notapplicable to NMs with poor dispersibility or high solubility in aqueous media (as
192 defined in the OECD TG No. 318 (OECD, 2017) or the accompanying guidance document No. 318 (OECD,
193 2020)).

194 ## 3.2. Information on the test substance

195 24.     Annex 5.3 list information on the tested NMs which should be available or determined prior to soil
196 leaching testing for well-informed test performance and adequate interpretation of test results. This
197 information will replace the information needed prior to testing as given in the parent TG No. 312:

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

209 26.     Guidance on how to determine dispersion stability and dissolution rate of the NM in the test
210 medium can be deduced from the OECD TG No. 318 on dispersion stability of NM in simulated
211 environmental media (OECD, 2017) and the  Guidance Document for the testing of dissolution and
212 dispersion stability of nanomaterials, and the use of the data for further environmental testing and
213 assessment strategies (OECD, 2020). These data will support the interpretation of test results and should
214 be considered for testing in parallel.

215 ## 3.3. Reference substance

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

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

231  29.    As injection of the tracer before introducing NMs to the columns presumably will lead to long lasting
232  and laborious test performances and delays in analytical assessments, alternatively, it is proposed to add
233  the tracer to control columns. In case that tracer behaviour is differing in all control columns, it has to be
234  assumed that non-uniform packing also occurred in the test columns and repetition of the test is advisable.
235  It is also possible to inject a tracer/electrolyte solution after the introduction of NMs to evaluate the
236  remobilization of retained NMs (Tufenkji and Elimelech, 2004). In this case any possible effect of the tracer
237  on NM remobilization should be taken into account.

238  ## 3.4. Definitions and units

239  30.    See Annex 5.1 to the Guidance Document.

240  ## 3.5. Quality criteria

241  31.    The parent Test Guideline OECD No. 312 uses recovery ranges as well as repeatability and
242  sensitivity of the analytical method as quality criteria for test conduction. In accordance to the TG, a
243  recovery of at least 70% (for non-labelled NMs) is considered also for NMs. Recovery is understood as the
244  sum of percentage of NM found in the soil segments and the leachate at the end of the experiment. It is
245  acknowledged that the success of recovery of NMs in soil columns strongly depends on many different
246  variables (e.g. particle type, the choice of application and applied amount of the test substance, type of
247  soil used) which may be challenging depending on the NM to be tested and/or the specific test parameters.
248  Therefore, in case the required recovery is not achieved, it is recommended to check different steps and/or
249  elements of test performance for accuracy. These include the analysis of sand layers and tubing used to
250  check for sorption, the stability of the stock dispersion to check for sedimentation as well as the
251  reconsideration of chosen digestion methods. For NMs with high natural background for which recovery of
252  70% will be hard to achieve, labelling is advised while it has to be ensured that labelling will not affect
253  transport behaviour. The use of artificial soil/substrate to exclude natural background should be avoided
254  as the deviation to the recommended soil types is too pronounced.

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

259  33.    Control experiments should be conducted with soil columns (2x) that have previously not been
260  exposed to the tested NM. The purpose of these control experiments is to determine the release of soil
261  colloids (background concentrations of the tested NM).

262  ## 3.6. Considerations on the test method

263  ### 3.6.1. Test system and laboratory equipment

264  34.    Leaching columns should be made of glass or stainless steel as these materials are inert and
265  feature a low likelihood of NM attachment. Utilization of glass columns features the advantage of

266 transparency which allows the user to visually observe the occurrence of air pockets or non-uniform
267 packing of the porous material. However, if NMs are sensitive to photochemical processes light-
268 transmissive glass should be avoided or should be wrapped with aluminium foil to protect them from
269 sunlight. If specific materials are used for the column tubes or at the column outlet (e.g. porous mesh)
270 which cannot be chemically analysed it has to be ensured that no NMs are retained on them. Recovery is
271 dependent on the material at the outlet end itself and on its porosity. Materials with a high enough porosity
272 (but small enough to retain the smallest fraction of soil grains used) are recommended. To avoid
273 attachment of the tested NM to test equipment like tubing and valves, materials made of Teflon are
274 recommended. The same applies to containers that are used to store NM suspensions collected and used
275 during the experiment. However, attachment of NMs to materials may vary (as a function of NM type and
276 any NM coatings) and should therefore be verified individually for every system tested. A blank experiment
277 (no soil, 2 replicates) can be conducted to verify that NMs are not retained on column materials or tubing.

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

283 36.    To prevent soil release from the column into tubing and fittings, it is recommended to place a mesh
284 of an inert material (with pore size smaller than the smallest size fraction of the soil) immediately below the
285 soil (prior to introducing soil into the empty column). Additionally, it is recommended to add a thin layer (~
286 2 mm) of high-purity clean quartz sand (~400 µm grain size) or small high-purity glass beads above the
287 mesh and below the soil.

288 37.    To ensure uniform distribution of water over the column cross-sectional area, it is recommended
289 to add a thin layer (~ 2 mm) of quartz sand (33above the packed soil. The amount of this layer should be
290 as small as possible as sorption of the NM tested cannot be excluded. However, sand layers (or other
291 material) should not be deployed to fill up larger columns in cases of tests which only require a low soil
292 height to avoid effects on NM transport in the sand.

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

299 39.    In order to enhance reliability, at least duplicate leaching columns need to be used for testing, but
300 in general, triplicates are strongly recommended as high variability in NM properties in the different test
301 samples are difficult to exclude, e.g. due to problematic sample preparation.

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

### 3.6.2. Test substance

309 41.    It is recommended to apply the test substance as a dispersion (from the top of the soil). The
310 application can be performed as a pulse application or as a step injection. To study dynamic deposition
311 interactions (e.g., blocking or ripening), it can be beneficial to use continuous injection for more than 4 pore

312  volumes, but it should be considered that a continuous injection can result in relatively high total NM
313  concentrations in the soil that are not environmentally relevant and may therefore lead to misleading
314  interaction mechanisms.

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

322      1.  For geogenically occurring metals or metal oxides, specific ratios between the different metals
323          exist that differ from those of manufactured NMs. These differences in the ratio between NM spiked
324          soil and control soil can be measured and compared (using e.g. ICP-MS). In case ratios are shifted
325          in exposed soils, a distinction of the tested NM from the natural background becomes possible
326          (Gondikas et al., 2014; Praetorius et al., 2017; Montano et al., 2014).

327      2.  Labelled materials might be used to distinguish them from naturally occurring counterparts.

328      3.  An alternative soil featuring a lower background of the NM in question can be selected. In contrast,
329          the use of artificial soil/ / substrate to exclude natural background should be avoided as the
330          deviation to the recommended soil types might be too pronounced.

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

### 3.6.3. Soils

337  44.    The selection of soils should relate to environmental relevance, rather than to properties of the
338  soils promoting leaching of NMs based on their physicochemical properties. For testing the leaching of
339  NMs, at least 2 soils differing in either pH, organic carbon content, clay content and/or texture should be
340  considered. This reduction of soils compared to the OECD 312 is based on reasons of practicability. Soils
341  with high clay content (soil 1, TG 312) tent to block during leaching and sandy soils with high carbon
342  content (soil 5, TG 312) are only limited available.  Main emphasis should be on differences in texture. In
343  order to promote comparability and reproducibility of data, it is recommended that the conditions of
344  parameters of the chosen soils relate to those mentioned in Table 3.1. It has to be noted that the chosen
345  parameters differ from the parameters recommended in the parent TG No. 312 and are aimed for testing
346  explicitly NMs in soils. In case there is a need to investigate the fate of NMs in soils with higher organic
347  carbon content or different texture, this should be tested using a third soil. Soils with high clay content
348  should be avoided. Not only is the hydraulic conductivity of these soils very low, requiring high flow
349  pressures and making saturated column tests difficult, Many NMs tend to attach strongly to clay minerals
350  (Cornelis et al., 2012; Cornelis et al., 2011), resulting in absence of NM breakthrough (Cornelis et al.,
351  2013). Moreover, particle transport in clay-rich soils occurs predominantly in macropores (Ryan and
352  Elimelech, 1996), making experiments with saturated, stacked soil cores unrepresentative for NM transport
353  rates in clay soils in the field.

354

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

| Soil No. | pH value (0.01 M CaCl$_2$) | Organic carbon % | Texture |
|---|---|---|---|
| 1 | 5.0 ± 0.5 | 0.8 – 1.5 | loamy sand / sandy loam |
| 2 | 6.5 ± 0.5 | 1.2 – 2.0 | loam / silt loam |
| 3 | 5.0 ± 0.5 | 3.0 – 4.5 | Loamy sand / sand |

356 *Note*: The soil pH can be measured using the method reported by EN 15933 (Sludge, treated biowaste and soil - Determination of pH) (EN
357 2012), ISO 10390 Soil quality – determination of pH (ISO 2005).

### 3.6.4. Test conditions

359 45.     As a realistic worst-case scenario, a low flow rate of 2-3 L·m-2·h-1) (corresponds to 0.2-0.3 mL·cm-
360 2·h-1) can be used. This shall account for more realistic conditions of NM mobility in soils for which
361 considerably longer residence time is expected and thus avoids an artificial breakthrough of NMs.
362 However, the low flow rate results in long test durations which may be impractical in many cases. Although
363 a higher flow rate (e.g., 100 L·m-2·h-1 for sandy soils) can be applied to limit test duration, it should be
364 considered that this requires high pressure that deviates from realistic environmental conditions and can
365 result in unrealistic conclusions regarding the NM leaching behaviour.

366 46.     As a leaching solution which mimics artificial rain, an aqueous solution of 0.005 M KCl or NaCl is
367 suggested. In case of nanomaterials reacting with Cl (e.g. Ag), suitable anions such as NO3- (KNO3) have
368 to be used. This features a reduction of the recommended concentration of the parent TG OECD No. 312
369 and a change to a monovalent salt. The use of a divalent salt such as CaCl2 will not provide a "worst-case
370 scenario" test as NMs homoaggregates are generally less mobile in the presence of Ca2+. For this reason,
371 we recommend the use of a monovalent salt, such as KCl or NaCl. The chosen salt should not react with
372 the NM, thus e.g. accelerating its dissolution. Moreover, as recommended earlier, the stability of the NM
373 suspension should be measured prior to the column test. Nonetheless, a solution of 0.005 M CaCl2 can
374 also be used to mimic conditions where the soil pore water is dominated by divalent cations (however,
375 cannot be considered as "worst case scenario" conditions).

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

380 48.     In general, columns can be packed with either dry or wet soil. Dry soil is meant to be air-dried soil
381 (preferably between 20-25 °C) while wet soil is adjusted to a certain water content. The decision of dry
382 versus wet packing of columns is of general relevance for testing the behaviour of chemicals in soils and
383 is controversially discussed. In this Guidance Document, it is recommended to preferentially pack the
384 columns using dry soil. While wet packed soil might decrease the extent of air entrapment and water
385 repellency, it is impossible to recommend standardised guidance for adequate water content as water
386 adjustments depend on the physico-chemical properties of the selected soil and need to be individually
387 chosen. As it cannot be excluded that different results will be obtained in dependence of either dry or wet
388 packing, dry packing is suggested as preferred method as a general applicable procedure can be given
389 which will reduce variances in results due to different water adjustments related to wet packing.

390 49.     As mentioned in OECD TG No. 312, equilibration of the soils with the leaching solution should be
391 performed in upflow mode in order to avoid air filled soil pores. In order to mimic the natural conditions of
392 particle transport, leaching of the test substance should be performed in downflow mode. In addition, all

393 solutions and dispersions should be degassed by sonication to avoid introduction of air bubbles into the
394 column. NMs are known to interact strongly with air-water interfaces and air bubbles would thus introduce
395 artefacts in the test. However, as sonication may alter NM dispersion characteristics, devices, duration and
396 energy input need to be chosen carefully.

### 3.6.5. Test performance

398 50.　　At least duplicate leaching columns should be used for testing, but in general, triplicates are
399 strongly recommended (see also Paragraph 39).

400 51.　　The columns are preferably dry packed (see Paragraph 49) with air-dried (preferably 20-25 C) and
401 sieved soil (< 2 mm) up to a height of approximately 10-20 cm. To obtain near uniform packing, the soil is
402 added to the columns in small portions with a ceramic spoon and pressed with a plunger under
403 simultaneous gentle column vibration until the top of the soil column does not sink in further. Near uniform
404 packing is required for obtaining reproducible results from leaching columns. After packing the column, a
405 $CO_2$ flush is recommended to remove all air present in the soil column. The medium for equilibration
406 (leaching solution without NMs) can then be added by flushing up-flow with a degassed aqueous solution.
407 In this way a high degree of water saturation can be achieved.

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

411 53.　　The application of the test substance as dispersion is recommended. It is not recommended to
412 add NM powders to the soil in a realistic emission scenario. Relatively low concentrations are more likely
413 to be found in natural soils. Stock or test dispersions can be prepared using probe sonication (40 W, 10`)
414 using a volume of approximately 50-250 mL, exceptions are fibres or rods which may be damaged as a
415 result of this preparation. Probe sonication allows treating large volumes of dispersion and enables
416 reproducible energy input (compared to e.g. bath sonication). A highly concentrated stock suspension
417 should be avoided as this will promote homoagglomeration and sedimentation in the stock and thus
418 introduce variability in the test dispersions. The sonication step can also be followed by a centrifugation
419 step to remove large, undispersed agglomerates. However, in such a case, quantification of the remaining
420 NM concentration is needed. If technically possible, the test dispersion should be prepared directly in order
421 to avoid errors due to dilution of a stock suspension. If magnetic stirring is used to hold the NM in
422 dispersion, care must be taken to ensure that stirring does not lead to increased agglomeration of the
423 dispersion or modifications of the surface of the NM which may lead to a modified behaviour in the soil
424 column. The occurrence of supernatant of the test dispersion on top of the columns should be avoided. A
425 homogeneous distribution of the dispersion over the cross section of the column should be ensured (by
426 adding a thin layer of clean quartz sand over the packed soil). The application can be performed as a pulse
427 application (with a maximum of 5 % of the total pore volume of the packed porous matrix) or as a step
428 injection (corresponding to 4 pore volumes). To study dynamic deposition interactions (e.g., blocking or
429 ripening), it can be beneficial to use continuous injection for more than 4 pore volumes, but a continuous
430 injection will inevitably add relatively high total NM concentrations in the soil leading to interaction
431 mechanisms that are irrelevant for scenarios of spurious NM emissions to soils leading to low NM
432 concentrations. The exact duration of the injection will depend on the experimental conditions, the NM
433 tested and the type of soil. Stability of the NM dispersion should be verified (e.g., using dynamic light
434 scattering) prior to NM application to the test column. Dissolution of the NMs should also be characterized
435 in the test solution medium (e.g., using ICP-MS or spICP-MS), to determine the fraction of NMs that have
436 been dissolved. For estimation of pore volumes in the columns please refer to see Paragraph 56.

437 54.　　Following introduction of the test substance, the column is purged with the background leaching
438 solution (particle free).

439    55.      The leachates should be sampled every 0.5 ± 0.1 pore volumes over a collection period equivalent
440    to 2x the number of pore volumes injected into the column (at least 8 pore volumes). The optimal material
441    of the recipients in which the leachates are captured depends on the NM studied and should be checked
442    individually prior to the start of the experiment. In the case of metal and metal oxide NMs, polymer materials
443    or even Teflon are preferable instead of glass, because NMs may attach to the latter material. Glass may,
444    however, be a preferable material in the case of hydrophobic NMs such as carbon nanotubes or graphene.

445    56.      Estimation of the pore volume in the columns: The pore volume in the columns is estimated using
446    two separate replicates. The filled and weighed columns are saturated with water in upflow mode (see also
447    paragraph 47). Surplus water is allowed to drain off until the water level equals the soil surface in the
448    column. The column is weighed again. The difference between the two weight measurements (column with
449    air dried soil and column with saturated soil) is used as an indicator of the pore volume. A more exact
450    determination of the effective pore volume may be obtained based on tracer breakthrough data. The pore
451    volume estimate can be calculated:

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

453                                                           with

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

455                                                           $\rho_R$ = grain density (g·cm$^{-3}$)

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

457                                                           $l$ = height of soil column (cm)

458                                                           $r$ = diameter of soil column (cm)

459               The time for 1 pore volume can also be calculated:

460

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

462

463        with

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

465                                                           $PV$  = pore volume (cm³)

466                                                           $r$ = radius of the column (cm)

467        ### 3.6.6. Analytics

468    57.      Mass concentration of the nanomaterials, pH, electrical conductivity, and turbidity should be
469    determined in the leachates. All analyses should be done at least with two replicates, although a higher
470    number of replicates is recommended. Acid digestion of the leachates immediately after they were
471    obtained is highly recommended in the case of metal and metal oxide NMs. NMs may attach to walls of
472    the recipients and/or agglomerate and sediment leading to underestimation of the leached NM
473    concentration. Analysis of leachates can occur without prior digestion of the leachates in some cases, but
474    recovery of the NM mass concentration in non-digested samples should at least be compared with digested
475    samples. In the case of other NMs, such as C-based NMs where digestion destroys the NM material itself,
476    leachate analysis should occur as soon as possible.

477    58.      For analysis of the NMs retained in the soil (depth profile), the upper 10 cm of the column should
478    be sectioned at minimum into 2 cm layers, the rest of the soil in 5 cm layers because for most NMs, only

479 little transport of NM is expected. Care must be taken not to excessively disturb the soil such as to cause
480 release of NMs from the soil grain surfaces (Tufenkji and Elimelech, 2004; Tufenkji and Elimelech, 2005).
481 To avoid broad scattering of data, every soil layer has to be homogenized before sample analysis. These
482 layers should be analysed choosing an appropriate number of replicates of samples per layer in
483 accordance with the diameter of the column (for the recommended minimum number of replicates see 53).
484 In the case of metal and metal oxide NMs, the most common analysis of the retained NM mass involves
485 an acid digestion followed by atomic spectrometry analysis (ICP-MS or ICP-OES). Standard digestion
486 methods applicable to metal analysis in soils such as US EPA method No. 3052 (US EPA, 1996) can be
487 used and involve appropriate blanks and controls for recovery.

488 59.     The analytical method which should be used for the detection of the NMs in the soil and eluate
489 samples is dependent on the type of information which is needed for assessment of the column
490 experiments, e.g. NM mass in the samples, particle number size distribution, agglomeration state, etc.
491 Based on the needed information, standard and/ or more advanced analytical tools can be used to answer
492 these questions. All of these methods have advantages and disadvantages that have to be taken into
493 account. Currently, no universal method for the analysis of NMs in soils exist. We therefore recommend to
494 carefully review the scientific literature and to select the method that best provides the analytical data of
495 interest. Furthermore, the specific requirements for the chemical analysis which will be used have to be
496 taken into account before the experiments are conducted and if standard methods exist they should be
497 applied (e.g., the ISO standard for spICP-MS (ISO, 2017) and Peters et al. 2015 (Peters et al., 2015) or
498 Hadioui et al., 2014 and 2015 (Hadioui et al., 2014; Hadioui et al., 2015) as well as ISO 2018 (ISO, 2018)).
499 Furthermore, the requirements and chosen method must be described in detail, to guarantee that sufficient
500 data interpretation and comparison with other data is possible.

501 60.     Advice for selecting appropriate methods for analysis of NMs in soil samples and leachate as well
502 as parameters which will be addressed by applying these methods is provided in Figure 1. In this figure,
503 relevant methods and corresponding endpoints are listed divided by those which are required in minimum
504 (recovery) with regard to soil column studies (grey boxes) and those which are available for additional
505 analysis depending on the tested NM and/or the individual scope of the study (white boxes). Please
506 consider that the list of methods is non-exhaustive and a selection of methods will depend on the NM under
507 investigation and scientific question to be answered. Valuable scientific literature on determination of the
508 relevant parameters for NM analysis in soil samples and leachates as listed in Figure 1 is available and
509 experiences presented in those references can support the development of a tailored strategy for analysis
510 of the NM in question. With respect to the recommended techniques in Figure 1, it is important to note that
511 once NMs are in soil, some of their properties will alter by interaction with the soil matrix. These properties
512 include agglomeration/aggregation state, surface functionalization, zeta potential, IEP, dissolution kinetics.

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

**Figure 1. Selecting appropriate methods for analysis of NMs in soil samples and leachate**
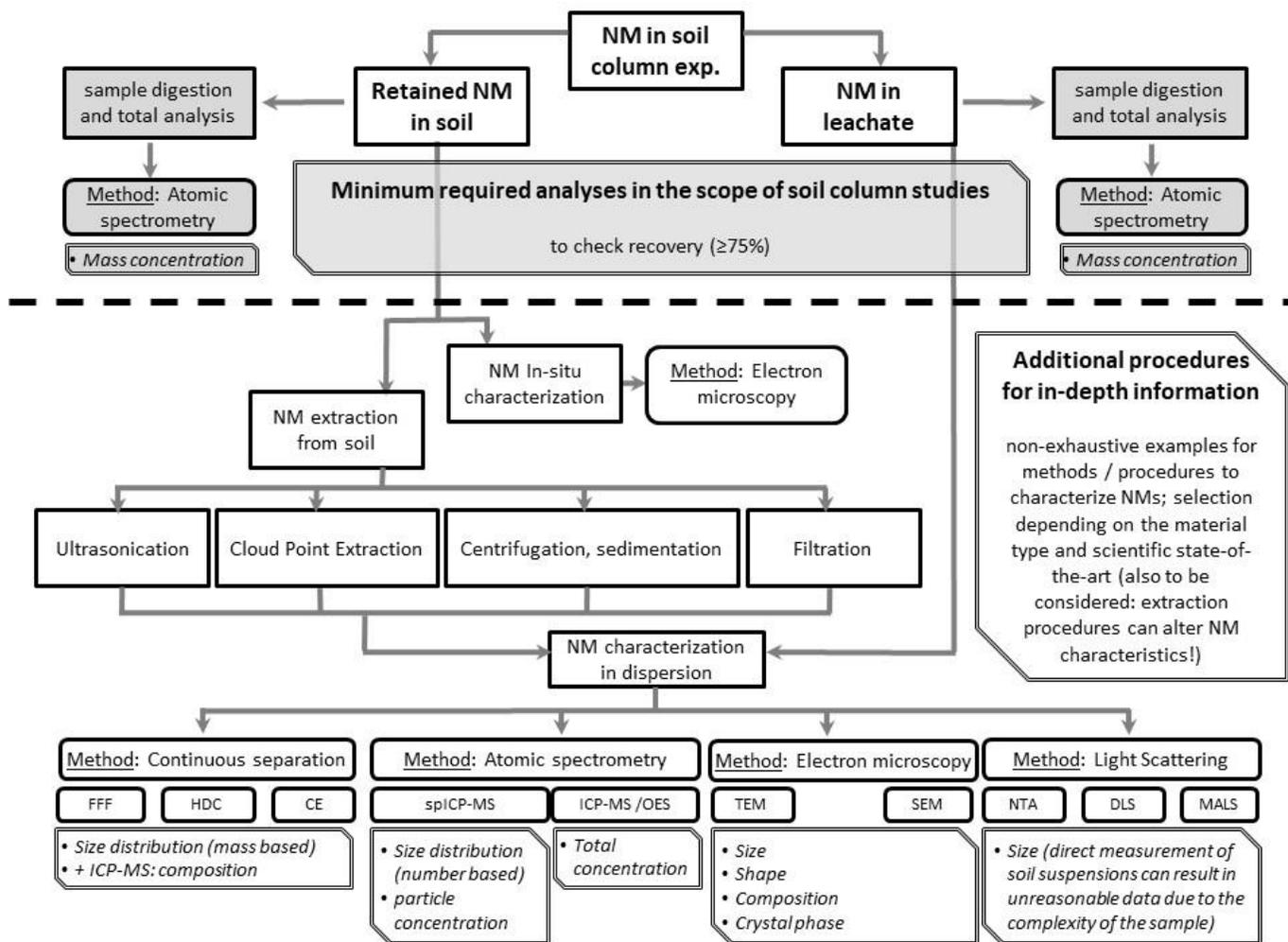
## 3.7. **Data and reporting**

62.     The utilization of the parent TG OECD No. 312 under consideration of this guidance will provide information on retention and mobility. Calculations such as Koc and Kom as presented in the parent TG are not applicable for NMs (Praetorius et al. 2014). Alternatively, calculations of particle attachment efficiency ($\alpha$) can be carried out as per Yao et al. (Yao et al. 1971); Tufenkji and Elimelech, 2004 (Tufenkji and Elimelech 2004). $\alpha$ 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.

63.     A quantitative estimation of $\alpha$ 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, $\theta$ 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. *L* is the length of the column and $\eta_0$ 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 (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.

64.     The formulae above show that $\alpha$ 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.

65.     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 characterization (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.

66. The overall recovery (mass balance) of NMs should also be determined and reported (as described above).

67. In addition to the necessary information mentioned in the parent guideline OECD TG No. 312, for accurate interpretation the report should include the parameter listed in Annex 5.3 – 5.6 including information on minimum particle characterisation (5.3), particle stock sample pre-treatment (5.4), soil properties (5.5) and relevant column parameters (5.6).

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# 5. Annex

## 5.1. Definitions and Units

**agglomerate**    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 appearance of solute/target substance/NM in column effluent.

**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.

**dispersibility**    Is the condition of particular material of being dispersible or a measure to which extent it 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.

68.    Additional definitions and units relevant for testing nanomaterials 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. **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 (except chemicals which are volatile from soil and water). | All NMs for which accurate and sensitive analytical strategies are available. Not applicable to NMs with poor dispersibility in aqueous media (as defined in the OECD TG No. 318). |
| **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 (Kd, Kf or KOC), hydrolysis, dissociation constant (pKa), 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 does not interact with the soil medium, e.g. potassium bromide or sodium nitrate. |
| **Quality criteria** | Recovery(70-110 % for no-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 %. |
| **Detection** | "an appropriate analytical method of known accuracy, precision and sensitivity for the quantification of the test substance". | Different options (for Me and MeOx): 1, NM: differentiation from natural background using specific ratios of different metals occurring naturally; 2, labelled materials; 3, 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 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 | 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. |

| | | |
|---|---|---|
| | substance applied may corresponds to the maximum recommended use rate (single application). | |
| **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<br>A recommendation for a potential $3^{rd}$ 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. A dispersion protocol is provided. 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. |
| **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 2 to 4 pore volumes). To study dynamic deposition interactions (e.g., blocking or ripening), it can be beneficial to use continuous injection for more than 4 pore volumes. The exact duration of the injection will depend on the experimental conditions, the NM tested and the type of soil.<br>To account for more realistic conditions of NM mobility in soils a low flow rate of 2 L/(m²\*h) (corresponds to 0.2 mL/cm²\*h) is recommended. To limit test durations, a higher flow rate (e.g., 100 L/(m²\*h) for sandy soils) can be applied, however, this will require high pressure deviating from realistic conditions. |
| **Leaching solution/artificial rain** | 0.01 M CaCl2 | 0.005 M KCl or NaCl (KNO₃⁻ for those nanomaterials reacting with Cl⁻) |
| **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 2x the number of pore volumes injected into the column (e.g., at least 8 pore volumes). The optimal material of the recipients in which the leachates are captured depends on the NM studied and should be checked individually. |
| **Pore volume** | no | yes |

| estimation | | |
|---|---|---|
| **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** | by duplicate analysis. | strong recommendation for triplicates. |
| **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; size and zeta potential of the particles in column effluent during breakthrough; 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; concentration in soil segments and leachate. |
| **Relevant calculations to report** | Koc, Kom | 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. |

## 5.3. Minimum particle characterisation parameters to report

| Parameter | Instrument/Method | Metric to report | Metadata to report |
|---|---|---|---|
| **Chemical composition** | SEM/TEM-EDS | stoichiometric formula | Acceleration voltage, |
| | XRF | stoichiometric formula | impurities |
| | Digestion + ICP-MS | stoichiometric formula | impurities<br>acid used<br>digestion protocol used |
| | Manufacturer information | stoichiometric formula | URL of manufacturer |
| **Primary particle size** | 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) |
| | 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 | Sample preparation |

| | | Coating length<br>Occupancy (% coverage) | |
|---|---|---|---|
| **Concentration in test medium** | ICP-MS/ICP-OES | mass concentration (e.g. mg L$^{-1}$) | Dilution factor<br>Dilution medium |
| | spICP-MS | number concentration (e.g. # particles mL$^{-1}$) | Dilution factor<br>Dilution medium<br>Nebulisation efficiency determination method<br>dissolve/particulate discrimination method and parameter (e.g. 3 x sigma) |
| **Dispersion stability** | TG 318 | see reporting requirements in TG 318 | |
| **Dissolution** | TG 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 |

## 5.4. Particle stock sample pre-treatment to report

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

| Pre-treatment action | Parameters to report |
|---|---|
| **Suspending\*** | Particle concentration dispersed<br><br>Dispersion medium<br><br>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

5.5. **Soil properties to report**

70.	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** | Eg 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 |

5.6. **Column experiment parameters to report**

**Table 5.5 Relevant column experiment parameters**

| Column aspect | Parameters to report |
|---|---|
| **Column dimensions** | length<br><br>diameter |
| **Flow speed** | linear flow speed or Darcy velocity |
| **Medium** | Chemical composition major ions and anions<br><br>pH<br><br>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<br><br>Material of filter<br><br>% 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)<br><br>Hamaker constant used to calculate single-collector contact efficiency, if applicable. |