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REPORT OF THE INTERLABORATORY COMPARISON TESTING FOR THE GD TO SUPPORT IMPLEMENTATION OF TEST GUIDELINE NO. 312 FOR NANOMATERIAL SAFETY TESTING

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NO. 341

REPORT OF THE INTERLABORATORY COMPARISON TESTING FOR THE GD TO SUPPORT IMPLEMENTATION OF TEST GUIDELINE NO. 312 FOR NANOMATERIAL SAFETY TESTING

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FOREWORD

This document is the report of the inter-laboratory comparison (ILC) testing for the Guidance Document to support implementation of **Test Guideline No. 312** for nanomaterial safety testing. It provides background information on the test performed and the rationale for amendments included in the associated Guidance Document, No. 342 in the OECD Series on Testing and Assessment.

This inter-laboratory report was endorsed in April 2021 by the Working Party of the National Coordinators of the Test Guidelines Programme and is published under the responsibility of the Chemicals and Biotechnology Committee.

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INTERLABORATORY COMPARISON TESTING FOR THE GUIDANCE DOCUMENT TO SUPPORT IMPLEMENTATION OF TEST GUIDELINE NO. 312 FOR NANOMATERIAL SAFETY

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1 Summary

1. The need for a Guidance Document (GD) for testing the behaviour of engineered nanomaterials (NMs) in soils using the OECD TG No. 312[1] was identified as a priority goal by the OECD's Working Party on Manufactured Nanomaterials (WPMN). Thus, in 2016, Canada and Germany proposed a new activity to the OECD Test Guideline Program to develop such a GD to support implementation of Test Guideline (TG) No. 312 for NM safety testing. The inclusion in its Program of Work was approved by the WNT (Working Group of National Co-ordinators of the TGs programme) -29 in April 2017.

2. Draft versions of the GD were presented to the OECD expert group on environmental fate of nanomaterials in June and November 2018. Based on the comments received the leads decided to conduct an interlaboratory comparison test (hereafter ILC) to evaluate the benefit of the proposed modifications of NMs as well as to check if comparability of results is still ensured.

3. Based on a call for contribution, laboratories were identified which indicated their interest in participation. The ILC took place from June 2019 to December 2019 plus additional weeks for analytics by some of the partner laboratories.

4. Results were obtained from seven laboratories for two different nanomaterials in two soils featuring different characteristics to enable differentiated leaching behaviour.

5. The recovery of the added NMs varies significantly between the partners. Either very low (~10%) or high recovery rates (~70 – 100%) were achieved. In case of high recovery, the conclusions were comparable regarding the fate of the added NMs. Important aspects of achieving an acceptable level of recovery were identified and considered for revision of the draft GD.

6. Based on the results and experiences from the ILC several adaptions of the draft GD were made to improve the document.

[1] The Guidance Document is available at the OECD webiste XXX text to be change upon publication

2 Introduction

7. The need for an OECD Guidance Document (GD) for testing the behaviour 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).

8. As a follow-up to this conclusion, 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.

9. 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 need adaptation within a GD to ensure its applicability to test NMs. International experts were consulted in an internal expert group to support this discussion with data and scientific experience. Additional scientific findings were considered indicating the need to adjust existing methods to investigate mobility and fate of NMs in soils.

10. Draft versions of the GD were developed in 2018, which were presented to the WNT expert group for environmental fate and behaviour of NMs in June and November 2018. Valuable comments were received which were used to improve the draft GD. In December 2018 at a meeting of the WNT expert group, scope and content of the draft GD was discussed. The draft GD was seen as quite mature, however an inter- laboratory test comparison was considered as helpful to evaluate the accuracy as well as the benefit of the proposed modifications of the GD for NMs and to check if comparability of the obtained test results is still ensured.

11. A call for contribution to the ILC was sent out by OECD to WNT by end of February 2019. Eleven laboratories indicated their interest in participation. From these laboratories nine laboratories finally participated. These labs either conducted soil column tests according to the draft GD with one or with two nanomaterials in either one soil type or two soil types. Data were received from seven labs from 4 countries, including two industry partners.

12. For the ILC the draft version 3 of the GD was used by the partner laboratories. However, next to the draft GD, additional accompanying documents were prepared to support the testing. These included inter alia an ILC plan describing background and course of the comparison testing as well as additional specification of testing relevant for comparability of test results. Additionally, SOPs for preparation of stock suspensions and column preparation were distributed. Finally, templates for reporting on test performance and results as well as to report on attachment efficiency were developed and distributed.

13. Prior to the start of the interlaboratory testing, a telephone conference (5 June 2019) was conducted with the support of OECD to introduce partner labs to the rationale and concept of the ILC and discuss and finally clarify any issues connected with testing and/or the accompanying documents.

Upon this call, minor revisions were made in the draft GD and the accompanying documents. Version 3 of the draft GD was used for the ILC. After that shipping of nanomaterials and soils was started and final documents were sent out to all participating laboratories.

14. The ILC ran from June 2019 until December 2019 with extension to March 2020 due to internal delays in some of the laboratories. Data were received starting from November 2019 until March 2020. Evaluation started in April 2020. The data were summarized and first observations of the results were shared with the partner labs for discussion. In June 2020 a web conference took place to discuss these observations and to derive needed changes of the draft GD from these observations.

15. The summarized results as well as the derived conclusions for needed changes of the draft GD are included in this report, which were used for the final draft GD.

Participating Laboratories

16. Eleven laboratories from seven countries including two industry laboratories indicated their interest in participation upon the call for contribution by OECD. From these labs seven labs from four countries submitted data to either one or both nanomaterials in either one or both selected test soils.

17. List of participating Laboratories (arranged alphabetically, not identical to the numbering of labs used for data presentation to ensure privacy)

- 3M Center – 3M EHS Laboratory, United States
- BASF SE, Germany
- Federal Institute for Geosciences and Natural Resources (BGR), Germany
- Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany
- Institute of Energy and Environmental Technology (IUTA), Germany
- Korea Institute of Toxicology, Environmental Fate & Exposure Research Group, Korea
- Groundwater Engineering Group, Polytechnic University of Turin, Italy

Chemicals

18. NMs selected for the ILC were nanosilver (NM-300K) and nanosized CeO2 (NM-212) which were both part of the JRC nanomaterials repository (JRC 2016) used for the OECD Sponsorship Programme for testing nanomaterials (OECD 2019). Characterisation data of both NMs are available at JRC webpage (Comero et al. 2011; Singh et al. 2014).

Soils

19. Two soil were selected for the ILC:

I. RefeSol 01A: sandy loam, medium acid, very light humic (sand: 73.1%, silt: 19.8%, clay: 7.0%; Corg: 0.93%, pH ($CaCl_2$) 5.71)

II. RefeSol 02A: silt loam, sub-acid, light humic (sand: 1.7%, silt: 82.6%, clay: 15.6%; Corg: 1,06%, pH (CaCl2) 6.78)

20. Both NMs and soils were kindly provided and distributed by Fraunhofer IME.

3 Documents for the Interlaboratory Comparision

21. Next to the draft GD, version 3, additional documents were provided to partner laboratories to support comparable testing. These included:

i. a comparison test plan,

ii. the SOP in preparation of stock suspensions of the selected NMs,

iii. a SOP how to prepare soil columns

iv. a figure presenting the soil column test set up

v. a sheet to report on test performance and

vi. an Excel sheet to support calculation of attachment efficiency (optional).

22. The last two documents were used by laboratories to document their results. All documents can be found as annexes to this report.

4 Results

Results of soil column testing according to the draft Guidance Document

23. Following data sets on soil column testing according to OECD TG No. 312 and the draft GD (version 3) for nanosilver (NM-300K) and nanosized CeO_2 (NM-212) were obtained by the ILC:

Table 1 Overview of received data sets

	No. of data sets in	
	RefeSol 01A	RefeSol 02A
NM-300K	6	6
NM-212	2	1

24. Results were submitted using the reporting sheet on test performance. Additional experiences during testing were reported in addition. These included e.g. adsorbance to tubings and columns or colouring of eluate.

25. For comparison, results of the partner laboratories were summarized in the tables of this report.

26. Data for nanosilver (NM-300K) were obtained from six partners, while – depending on the used soil type – only one or two data sets were received for nanosized CeO2 (NM-212). Therefore, quantitative comparison of data could only be performed for nanosilver (NM-300K), while comparison of data for nanosized CeO2 (NM-212) was mainly done on a qualitative level.

27. Three to four data sets were received for the tracer, either KBr or NaNO3. The way in which the data were presented varies, however it was able to derive conclusions on the need and choice of tracers.

28. Comparison of leaching behaviour and concentration profile of nanosilver (NM-300K) was only possible using data sets which featured a certain level of recovery (in terms of mass balance). Therefore, it was decided to only consider data sets featuring a recovery of at least 70% of the initial nanomaterial concentrations into the evaluation of data. Differences in recovery ranged from 3 to 100%. For the partner with the lowest recovery, significant sorption on tubings was observed but could not explain the whole extent of sorption.

29. Based on the comparison of these data sets, the differences in leaching behaviour became obvious. While the replicates of the partners were comparable in the most cases, the percent amount in leachate ranged from 0% (partner 2) to 93% (partner 4) for RefeSol 01A and from 0% (partner 3) to 54% (partner 4) for RefeSol 02A.

30. By comparing the different leaching behaviours, potential reasons for the difference were identified and discussed with the partner labs:

31. For testing nanosilver (NM-300K) in RefeSol 01A (Figure 1), partner 6 used a factor 10 higher amount of silica sand above and below the soil with high sorption of nanosilver (NM-300K) (concentration in sand layer above the soil adapted to a layer of 2 cm, concentration in sand layer of the other partners were not reported, but can presumably be neglected). The leaching behaviour and amount of partner 4 differs significantly from those of partners 3, 6, 7. This is presumably due to the different flow direction during the pre-wetting period (downflow for partner 4 instead of upflow).

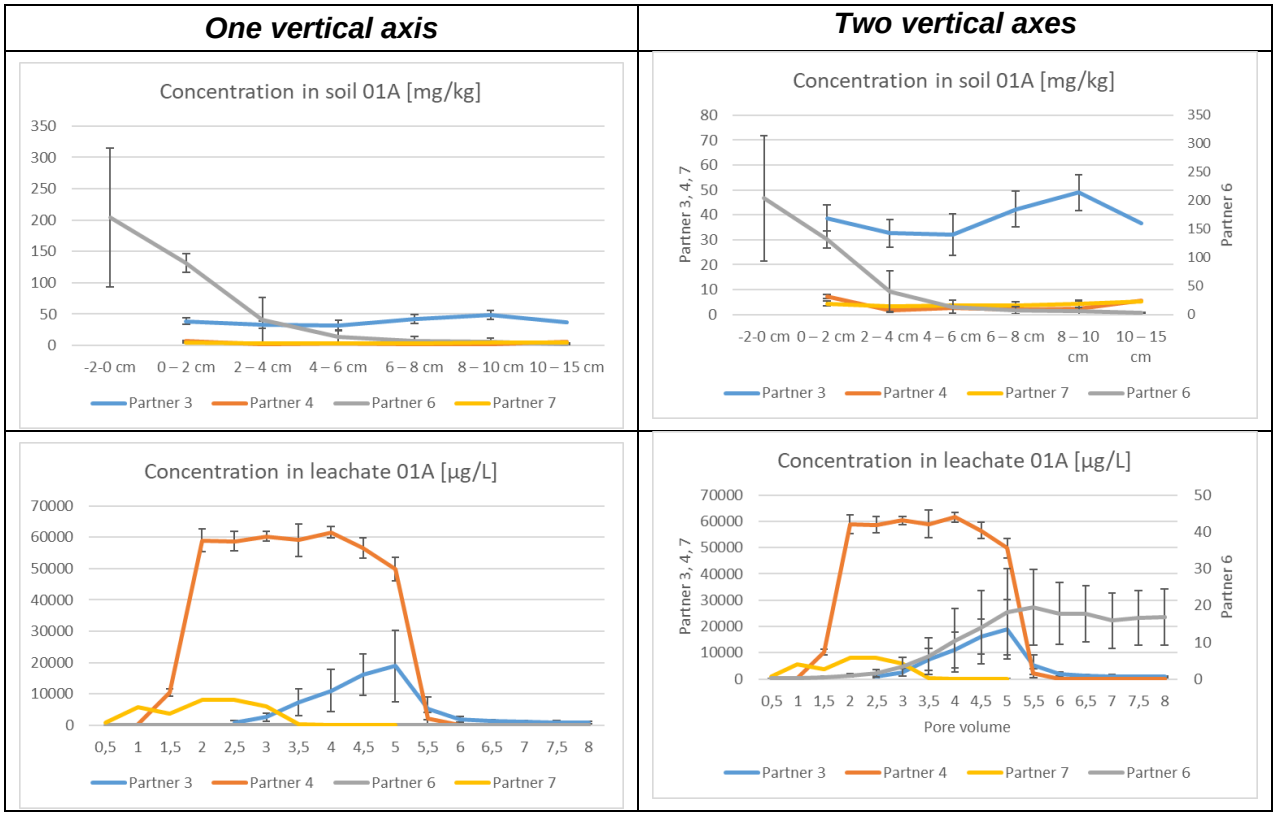


Figure 1: Leaching behaviour and concentration profile of NM-300K determined by the partners with a recovery rate >70% in soil 01A. Left column: all results are presented on one vertical axis for general comparison; right column: two vertical axes are used to show details for every experiment, independent of the absolute data.

32. By reviewing the results obtained for nanosilver (NM-300K) in RefeSol 02A, it became obvious that while silver was mainly found in the upper sand (partner 6) or soil (partner 3) layer, for partner 7, silver distributed in the first 8 cm und for partner 4 leaching in deeper soil layers was observed. The latter results fit together with the leaching data: The leaching behaviour and amount of partner 4 differs significantly from those of partners 3, 6, 7. This is presumably due to the different directions during the pre-wetting period (downflow for partner 4 instead of upflow) resulting in remaining air bubbles.

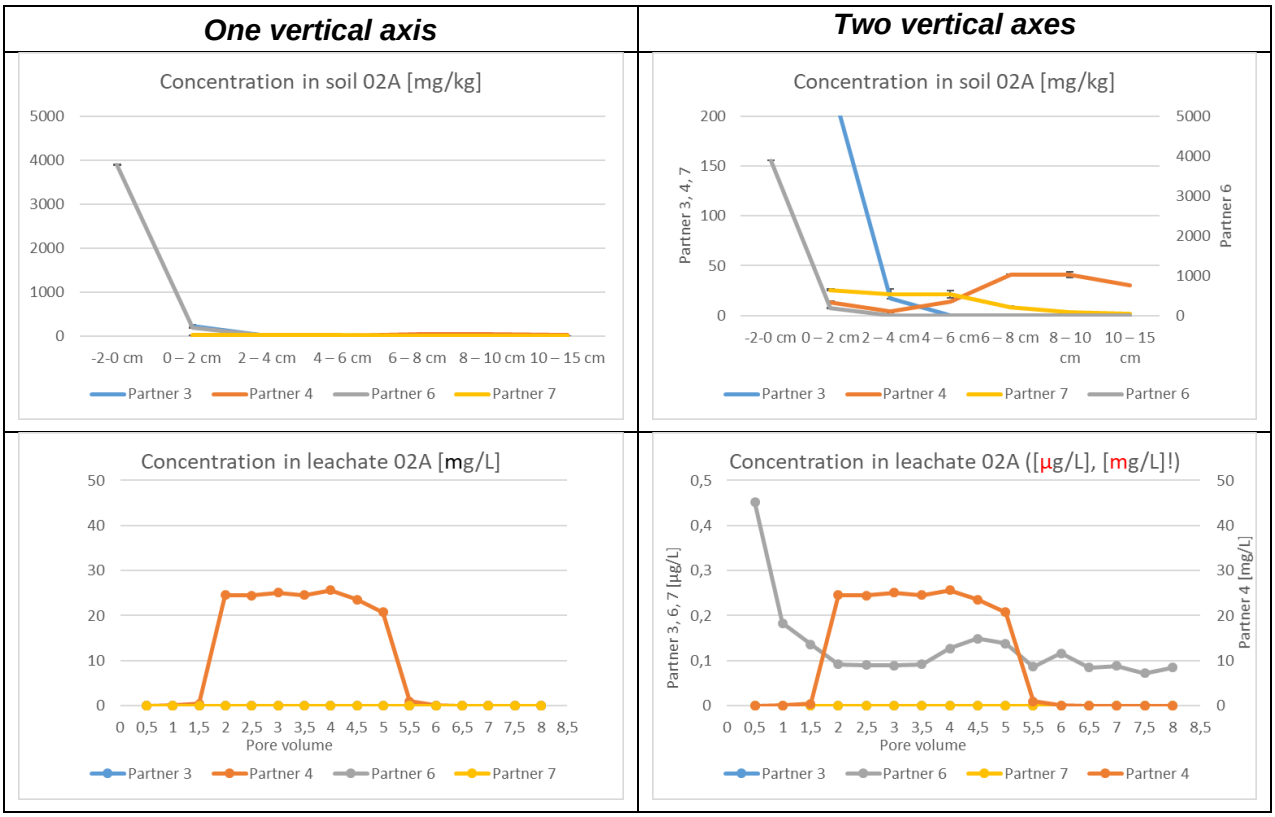


Figure 2: Leaching behaviour and concentration profile of NM-300K determined by the partners with a recovery rate >70% in soil 02A. Left column: all results are presented on one vertical axis for general comparison; right column: two vertical axes are used to show details for every experiment, independent of the absolute data

33. In order to verify that the difference in leaching behaviour detected do not dependent on parameters like the used amount of soil, the chosen pore volume or the amount of introduced nanosilver (NM-300K), a comparison check was made, showing that the differences cannot be explained by these parameters (Table 2). For instance, the ratio of soil and pore volume differs between partners indicating that soil packing was diverse which should influence leaching behaviour. Nevertheless, by comparing with the observed leaching behaviour, there seem to be no obvious relationship explain the differences (e.g. see concentration of the leachate of partner 4). Furthermore, no obvious difference on leaching behaviour was found in deviation of pulse application or continuous application.

Table 2 Overview of variances of pore volume, amount of soil and concentration of silver of the different lab partners

Partner number	Pore volume [cm^3]	Amount of soil per column [g]	Ratio soil/pore volume	NM-300K added per column [mg]	NM-300K [mg/kg]
Soil 01A					
3	96	542 ± 2 (dry matter)	5.7	25	46.1
4	80	382 ± 5 (dry matter)	4.8	19	49.7
6	190	748 ± 3 (dry matter)	3.9	37.5	50.1
7	137	271 ± 2 (dry matter)	2.0	2.3	8.5
Soil 02A					
3	126	449 ± 4 (dry matter)	3.6	19.7	43.9
4	100	318 ± 8 (dry matter)	3.2	16.0	50.3
6	240	624 ± 0 (dry matter)	2.6	31.5	50.4
7	116	233 ± 3 (dry matter)	1.9	3.25	19.9

34. In order to identify if difference of pH in pore water, medium used for dilution / leaching and pH of application dispersion can explain the observed differences in leaching behaviour, these pH values of the different partner labs were compared.

35. A high variability in pH of pore water was observed (01A: 5.9 – 8.2; 02A: 6.5 – 8.4) although variability in pH of medium used for dilution / leaching (5.5 – 6.8) and of application dispersion (5.7 – 7.3) were smaller;

36. ranking of partners:

- *pore water*:

  01A: P4 < P7 < P6 < P1 < P3< P2

  02A: P4 < P1 < P7 ≤ P2 = P6 < P3
- *medium used for dilution / leaching*: P2 < P6 < P3< P1 < P4 < P7
- *application dispersion*: P7 < P2, P4 < P3 (n.d. for P1 and P6)

37. In addition, the potential influence of pore water electric conductivity on the difference in leaching behaviour was checked but only small variance (factor of 2) were found between the labs (Table 3). However, severe differences in the amount of quartz sand used above and below the soil were used which might affected transport behaviour.

Table 3 Comparison of quartz sand above and below the soil, grain size and electric conductivity between partner labs.

quartz sand above and below the soil	**Grain size [mm]**	**Conductivity [µS/cm]**
experiment NM-300K 01-A		
Partner 1: 2 + 2 cm (column ∅ 4 cm) *Assumption: bulk density of 1.5 kg/L → 12 + 12 g*	0.125	717 / 601
Partner 2: ~ 13.5 + ~ 13.5 g	0.3 – 0.8	585±5

Partner 3: 10 + 10 g	0.25 - 0.30	548±47
Partner 6: 150 + 150 g		708
experiment NM-300K 02-A		
Partner 1: 2 + 2 cm (column ∅ 4 cm) *Assumption: bulk density of 1.5 kg/L → 12 + 12 g*	0.125	588 / 603
Partner 2: ~ 13.5 + ~ 13.5 g	0.3 – 0.8	590±5
Partner 3: 10 + 10 g	0.3	530±16
Partner 6: 150 + 150 g		710

38. The recovery rate of nanosized CeO_2 (NM-212) in Refesol 01A was found to be ~ 22% for partner 1 and between 94 and 142% for partner 5. Leaching was found to be 0% for partner 1 and 1.2-3% for partner 5. The difference might be explained by the strong difference in applied amount of nanomaterial, which was nearly 4 times higher for partner 1 than for partner 5.

Results of calculation of attachment efficiency according to the draft Guidance Document

39. The TG OECD No. 312 under consideration of this guidance will provide information on retention and mobility which are usually used to calculate coefficients such as K_{oc} and K_{om}. However, as these are not applicable for NMs, alternative calculation of particle attachment efficiency (α) is recommended in the draft GD. α expresses the probability that NMs will attach when they collide with the soil grain surface. As a consequence, high values of α correspond to high particle retention within the column. An Excel template for calculation of α was distributed to the participating laboratories for optional calculation. Two data sets (partner 2 and 7) with α calculation of nanosilver (NM-300K) were received. The calculated values of α, η_0 and k_a are reported for both soils in table 4 together with the recovery percentage.

40. For RefeSol 01A, experimental results from partner 2 and 7 were very different, with an average recovery percentage at the column outlet of 0% and 56% respectively. Coherently, α values obtained from data analysis for partner 2 (0.00714) were 1 order of magnitude higher than for partner 7 (0.000628).

41. For RefeSol 02A, both partners obtained a very low recovery percentage (0% and 1% respectively). From data analysis, a value of α equal to 0.00152 was estimated by partner 2, while a value of 0.00187 was obtained by partner 7.

42. Partner 7 found higher α values for RefeSol 02A, which is coherent with the lower recovery percentage observed in this soil. The opposite trend was instead observed from partner 2. This discrepancy can be due to the high particle retention observed (0% in both soils): when particle recovery is very low, it gets difficult to estimate a reliable value of α.

43. Because of the heterogeneity and low quantity of data, no general conclusions can be derived from this study.

Table 4 Comparison of modelling results obtained from different partners.

	Refesol 01A				Refesol 02A			
Partner 2	recovery	η_0	α	k_a	recovery	η_0	α	k_a
	0%	1.03	7.14E-03	4.32E-04	0%	2.3	1.52E-03	3.29E-04
	Refesol 01A				Refesol 02A			
Partner 7	recovery	η_0	α	k_a	recovery	η_0	α	k_a
	55%	0.93	6.28E-04	7.90E-06	1%	1.11	1.89E-03	6.26E-05

5 Discussion of obtained results and comparison between laboratories

Soil column testing according to the draft Guidance Document

44. Based on the comparison of the data sets of the partner labs and the observed differences, several discussion points were deduced which need to be considered for a revision of the draft GD based on the observations of the ILC. These discussion points were discussed with the partner labs of the ILC and conclusions were drawn.

45. Considerable different leaching behaviour of one partner (4):

Even though a different flow direction during the pre-wetting period was used by this partner, it was considered that the direction of equilibrium cannot be the only cause of the differing leaching behaviour. It was considered more important to check for other causes, e.g. the filters used (to e.g. avoid air bubbles), the differences in length (and diameter) of columns used. More important than the flow direction during the pre-wetting period is the success and duration of percolation. For this, good and uniform packing is needed (see next discussion point).

46. Description of column preparation:

Even though strong differences in parameters like pore volume, amount of soil etc. were observed, there was no clear indication that these differences cause the differences in leaching behaviour. However, it is obvious that insufficient packing of the columns may lead to air bubbles, preferential flow etc. and thus, will lead to differences in leachate concentrations. Within the ILC, the majority of partners decided to use dry packing. Big sand layer above and below the soil seem to distort results on transport behaviour. If used, the sand layer should be thin and relate to the used diameter of the columns. Some partners of the ILC used different kinds of (plastic) filters/filter membranes. This may lead to a (different, but in any way undesired) retention of NMs in the filters. Filter choice (pore width) will also depend on the way of packing (e.g. wider pores will help air bubbles to escape).

47. Amount of NM added

The amount of applied NM should be as low as possible as high concentrations will enhance clogging, agglomeration etc. and thus, can limit the recovery. To specify an upper limit would be great but is impossible as this will depend on the material, chosen analytics etc.

48. Tracer

Depending on the objective of testing, it might be needed to obtain tracer data (e.g. for modelling, alpha calculations). In some cases, alternative measures to tracer testing might be sufficient.

49. Consideration of a recovery rate as validity criterion

Based on the experiences from the ILC a recovery value is needed for the evaluation of the success of testing and the significance of the received data. The recovery of >70% seems sufficient and is assumed to be generally acceptable.

50. Scope of application

Even though the conclusions for needed revisions of the draft GD base on experiences of a limited number of NM, the envisaged revisions were considered to be valid for NM in general as they are not specific for ion releasing NMs only.

Calculation of attachment efficiency according to the draft Guidance Document

Calculation of α

51. The calculation of α (based on the equation used in the draft GD) is robust independent of the method used – at least for medium α values. It might be instead less robust for tests where the particle breakthrough curve is very low (high attachment and large α values). In these cases, analysis of leachate concentration alone cannot be sufficient for a quantitative analysis of the experimental results, and also concentration profiles in soils should be included in the calculation by applying more complex and comprehensive models. Moreover, the determination of α is generally valid only when the “clean bed” assumption is respected (usually corresponding to low particle loadings, short-time evaluations and absence of significant repulsion between particles and porous medium, i.e. favorable 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 anymore able to properly describe the system. In these cases, an approximate calculation using 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.

52. Grain size is the most relevant parameter affecting NMs transport and calculation of α, and must be known with the highest possible accuracy. Measurements are always needed if not available from reference soil data (e.g. determined according to DIN ISO 112277). Tracer data are to be preferred, for a more reliable interpretation of the results and a more precise estimation of α.

53. In the current situation, a general paragraph on available analytical and numerical models/tools, the prerequisites needed, their benefits and pitfalls for NM seem to be most expedient to allow the assessments of fate of NMs in soils when α cannot be used as a representative parameter.

6 Conclusion for the revision of the draft Guidance Document

54. Based on the experiences of the ILC the following adaption to the draft GD (version 3) were made:

- Consideration of recovery rate (70%) as validity criteria in the draft GD including of advice which steps/element of testing to check if the recoveries were not achieved (e.g. analysis of sand and tubing to check for sorption, stability of the dispersion media to check for sedimentation, appropriate digestion methods). For NMs with high natural background for which recovery of 70% will be hard to achieve, labelling is advised while it has to be ensured that labelling will not affect transport behaviour. The use of artificial soil/substrate should be avoided as the deviation to the recommended soil types is too big.
- Specification of length (as derived from the needed soil height) and diameter of columns in the matter as already described in the GD and TG (length: 10 – 20 cm; ∅ minimum 4 cm), note to avoid air bubbles as they affect the leaching behaviour and advice to check potential effects of used filters on NM transport.
- Presentation of packing of soils either dry or wet, however with advice to prioritise dry packing as a general protocol can be given.
- Note that in case sand layers are used, the amount of sand should be as small as possible as sorption of NMs cannot be excluded, used sand layers or filters should always be checked for effects on NM transport. Sand layers (or other material) should not be used to fill up larger columns with lower soil height. A pre-testing of the potential interaction of filters and sand with the test material in a simplified column test should be added as advice to the draft GD to account for potential influence of material transport and on the recovery rate.
- The relevance of the applied NM amount for successful reliability of leaching results should be highlighted in the draft GD. Furthermore, it should be reported which amount was finally applied to the soil.
- The amount of NM added to the soil should be as low as possible; note that high amount of NMs can result in a reduced leaching (clogging of NMs in the first cm of the soil column).
- Consideration of a paragraph explaining that the recommendations of the draft GD were tested in an ILC and the draft GD was mainly revised based on results obtained with nanosilver (NM-300K) (and nanosized CeO_2 (NM-212)).
- Consideration of a paragraph on calculation of α presenting an overview of existing tools and their advantageous but also limitations depending on the research question.
- These adaptions led to a new draft GD (version 4) for submission to the 1st WNT commenting round.

References

Bianco C, Tosco T, Sethi R. 2016. A 3-dimensional micro- and nanoparticle transport and filtration model (MNM3D) applied to the migration of carbon-based nanomaterials in porous media. Journal of Contaminant Hydrology. 193:10-20

Comero S, Klein C, B. S, Romazanov J, Kuhlbusch TA, Van Doren E, Wick P, Locoro G, Koerdel W, Gawlik B, Mast J, Krug H, Hund Rinke K, Friedrichs S, Maier G, Werner J, Linsinger T. 2011. NM-300 Silver Characterisation, Stability, Homogeneity. https://publications.jrc.ec.europa.eu/repository/handle/JRC60709 , accessed March 2020

Elimelech M, Gregory J, Jia X, Williams RA. 1995. Particle deposition and aggregation: Measurement, modeling, and simulation (Butterworth-Heinemann Ltd.: Oxford)

JRC. 2016. JBianco C, Tosco T, Sethi R. 2016. A 3-dimensional micro- and nanoparticle transport and filtration model (MNM3D) applied to the migration of carbon-based nanomaterials in porous media. Journal of Contaminant Hydrology. 193:10-20.

Comero S, Klein C, B. S, Romazanov J, Kuhlbusch TA, Van Doren E, Wick P, Locoro G, Koerdel W, Gawlik B, Mast J, Krug H, Hund Rinke K, Friedrichs S, Maier G, Werner J, Linsinger T. 2011. NM-300 Silver Characterisation, Stability, Homogeneity. https://publications.jrc.ec.europa.eu/repository/handle/JRC60709 , accessed March 2020.

Elimelech M, Gregory J, Jia X, Williams RA. 1995. Particle deposition and aggregation: Measurement, modeling, and simulation (Butterworth-Heinemann Ltd.: Oxford).

JRC. 2016. JRC Nanomaterials Repository. https://ec.europa.eu/jrc/en/scientific-tool/jrc-nanomaterials -repository, accessed March 2020.

OECD. 2019. Testing Programme of Manufactured Nanomaterials. http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm , accessed March 2020.

Singh C, Friedrichs S, Ceccone G, Gibson P, Jensen KA, Levin M, Goenaga Infante H, Carlander D, Rasmussen K. 2014. Cerium Dioxide, NM-211, NM-212, NM-213. Characterisation and test item preparation. https://publications.jrc.ec.europa.eu/repository/handle/JRC89825 , accessed March 2020.

Annex 1. Interlaboratory comparison plan

Introduction

55. A Inter Laboratory Comparison (ILC) exercise was organized to support the development of the OECD Guidance Document (GD) to support the use the OECD Test Guideline (TG) 312 ("Leaching in soil columns") for testing nanomaterials. The purpose was to verify the significance, appropriateness and comparability of the recommendations and deviations proposed from the original TG 312.

56. The ILC focused on two different nanomaterials (silver and cerium dioxide) in two different soils. Both nanomaterials and soils were provided by Fraunhofer IME, Germany.

57. For conducting the test and reporting the results the following documents were distributed amongst participant laboratories:

- Latest version of the draft GD including its annexes
- The original OECD TG No. 312
- The SOP for dispersion preparation
- The reporting sheet on test performance
- Optional: The Excel sheet to calculate attachment efficiency
- This annex as it provides additional information to conduct the test in the framework of this comparison study (see below)

58. The ILC took place from June to October 2019 with the following participating laboratories:

- Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany
- Swedish Agricultural University, Dept. Soil and Environment, Sweden
- Polytechnic University of Turin, Italy
- Korea Institute of Toxicology, Environmental Fate & Exposure Research Group, Korea
- 3M Center – 3M EHS Laboratory, United States
- Institute of Environmental Sciences, China
- BASF SE, Germany
- Institute of Energy and Environmental Technology (IUTA), Germany
- Federal Institute for Geosciences and Natural Resources (BGR), Germany
- Innovative Environmental Services (IES) Ltd, Switzerland

Reference materials

- Ag NM (NM300K)
- CeO2 (NM 212)

Characterization data:

- Ag NM (NM300K): JRC Report No. JRC60709, 2011: NM-300 Silver characterization, stability, homogeneity: http://publications.jrc.ec.europa.eu/repository/handle/JRC60709
- CeO2 (NM-212): JRC Report No. JRC89825, 2014: Cerium Dioxide, NM-211, NM-212, NM-213. Characterisation and test item preparation: http://publications.jrc.ec.europa.eu/repository/handle/JRC89825

Distribution of nanomaterials:

- In 2 mL subsamples (NM-300K) and 500 mg subsamples (NM-212)

Distribution of soils:

- RefeSol 01A: sandy loam, medium acid, very light humic
- RefeSol 02A: silt loam, sub-acid, light humic

Experimental procedure

Please conduct the tests according to OECD TG No. 312 and by following the guidance given for testing nanomaterials as mentioned in the current version of the draft GD. The draft GD still features some degrees of freedom on how to perform the testing. This is on purpose as the choice of some aspects might depend on the research question, the nanomaterial or available lab equipment. However, for assessing comparability and appropriateness of the draft GD, the way of addressing these aspects in the comparison test needs to be specified. These specifications can be found in the table below. If not stated in the table below, the decision on how to deal any other aspect of the draft GD which feature degrees of freedom is up to the decision of the individual labs.

Table 1: Specification of freely definable aspects in the current draft GD for comparability on the lab comparison test

Paragraph	Issue for potential consideration	Yes/no/no need for agreement (individual lab choice)
24	Tracer	potassium bromide
25/26	KBr injected into the test columns before NM introduction or in parallel in the 3 control columns	KBr to be injected in parallel in the control columns
33	Column diameter (column diameter to soil grain size ratio should be greater than or equal to ~20)	At least 4 cm
33	Column length between 10 and 20 cm	Individual lab choice
34	Mesh of an inert material at the button	Individual lab choice
34	Thin layer (~ 2 mm) of high-purity clean quartz sand (~400 µm grain size) above the mesh and below the soil.	yes
35	Thin layer (~ 2 mm) of high-purity clean quartz sand (~400 µm grain size) above the packed soil	yes

36	2 or 3 replicates of each column?	3
37	Dispersion with or w/o magnetic stirrer	Lab choice (with justification)
38	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) or continuous injection for more than 4 pore volumes	step injection (corresponding to approximately 4 pore volumes)
42	Low flow rate of 2-3 L/(m^2*h) (corresponds to 0.2-0.3 mL/cm^2*h) even though this may end on long test durations	yes
43	Leaching solution: 0.005 M KCl or NaCl	KCl
44	Equilibration upflow or downflow. Upflow is recommended, however downflow is possible, too.	Equilibration upflow
47	Dry packing of soil	Next to dry packing also wet packing is possible for the comparison test. Please make sure to use the reporting sheet on test performance to report on how packing was performed.
49	Check stability of NM dispersion with e.g. DLS	yes
52	Digestion method and analysis?	Lab choice (with justification)
52	Nr. of replicates per layer	3 is preferred
53	Sectioning of soil column: Upper 10 cm in 2 cm layers or more	2 cm
56	Calculation of α as part of the lab comparison	Lab choice (please use the attached Excel Sheet for calculation)
59	Reporting	For requirements see annexes of the GD and the reporting template and add your lab choice with short justification on the issues raised above in this table.

Documentation and presentation of results

- For documenting the test performance please use the reporting sheet. Please also report any deviations or other observations during testing and on individual lab choices using that sheet.

Annex 2. Preparation of stock suspensions

Ag

- Vials with 2 mL of NM-300K (10% Ag) are distributed
- Addition of 8 mL of ultrapure water resulting in a concentration of 200 mg/10mL (20 mg/mL) and shaking per hand
- Final concentration of 50 mg Ag/kg soil (dm) is intended. Mixing of the required amount of stock suspension with 4 pore volumes of ultrapure water
- Continuous stirring of the mixture during application using magnetic bars.

Advice: NM300K bottles needs to be stored in a glove box as soon as they are opened. NM300K is stored in Argon atmosphere and the nanoparticles start dissolving when exposed to oxygen gas. People should thus use the nanoparticles asap after opening.

CeO2

- Vials with NM-212 (500 mg) in powder form are distributed
- A final concentration of 50mg CeO2/kg soil (dm) is intended.
- The Nanomaterial should be dispersed using the following SOP
- Continuous stirring of the mixture during application using magnetic bars is recommended

Suspension preparation for soil experiments

Bringing nanoparticles into suspension is always a challenge and several different protocols are currently used within different studies. However, a dispersion procedure often has to be adapted to the specific material or experiments. Consequently, there is not a unique procedure to be applied, only a compromise of the minimal common base, acceptable for the different investigations.

The aim of this Standard Operating Procedure is the description of the procedure making a stable nanomaterial suspension which is used for leaching experiments in soils for uncoated and not functionalised ENM. An appropriate stability of the ENM suspension is declared as a constant hydrodynamic particle diameter (z.average) and polydispersity index (PDI) with a standard variation ≤ 10 % between the three internal measurements.

Stability criteria are:

Visual observation (no visible sedimentation of the particles)

Size distribution (mean z.average and PDI of the three internal measurements of one sample/analysis or mean D50 values) of the particles in the suspension

Materials

The following materials and chemicals are required:

- Ultrapure water, e.g. HPLC grade water (CAS 7732-18-5) or Millipore-filtered (electrical resistance 18.2 Ω)
- Nanomaterial (solid/powder or in dispersion)
- Clean spatula
- Pipette
- Plastic or glass (for pigment materials glass vials are essential!) vial or beaker glass of suitable size (e.g. 50 mL)

Instruments

Comparable equipment as the mentioned instrument is required:

- Ultrasonication equipment (e.g. Bandelin Sonoplus HD2200 ultrasonic homogenizer 200 Watt rated power, Bandelin Cup Horn BB6)
- Note: The usage and maintenance of the instruments will be not described in this SOP. Please refer to the manual.

Suspension preparation

For preparing the nanomaterial suspension ultrapure water is used. The concentration of the suspension should be adapted based on the application method and volume of the suspension that is added to the soil system. In the end, a concentration of 40 mg Ce / 1kg soil (or 50 mg CeO2 /1kg soil) should be used.

A defined amount of powder is weighted in the vial or beaker glass (a variance of 10 % is accepted). After this a defined amount of the media is carefully added to the material. The vial or beaker glass with the nanomaterial suspension was sonicated with an ultrasonic probe. The probe is dipped into the suspension and placed in the middle of the vial or beaker glass with a distance between horn and bottom of the beaker glass of approximately 1 cm. The suspension is sonicated with a final effective sonication power of 10 W measured by calorimetry (see Taurozzi et al. 2010); a variance of 5 % for the total sonication power is accepted.

For sonication the beaker glass with the suspension is cooled with cold water to minimize the heating of the suspension during the sonication. After use the probe was cleaned with ethanol and afterwards with deionised water. When indicated the stock suspension is diluted to reach the target concentration.

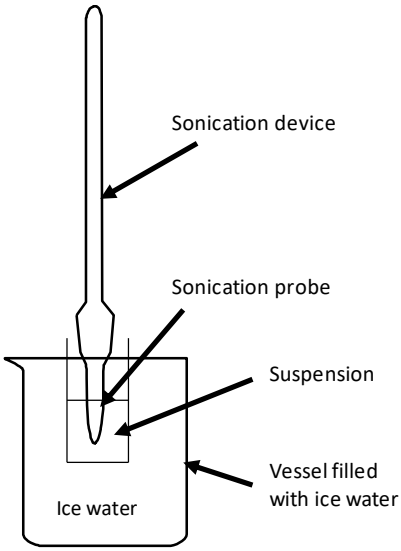


Figure 1: Suspension preparation

* the sonication time must be adapted to the volume of the prepared suspension, diameter of the beaker glass, the concentration of the nanomaterials and the rated power of the ultrasonic instrument.

Safety precautions

Please follow the safety information and regulations of the working laboratory as well of the materials provider. In general handle with care, wear protective clothing and suitable gloves at any time and labelling the material.

Waste disposal

Please follow the disposal advice of the material provider, if available.

Literature

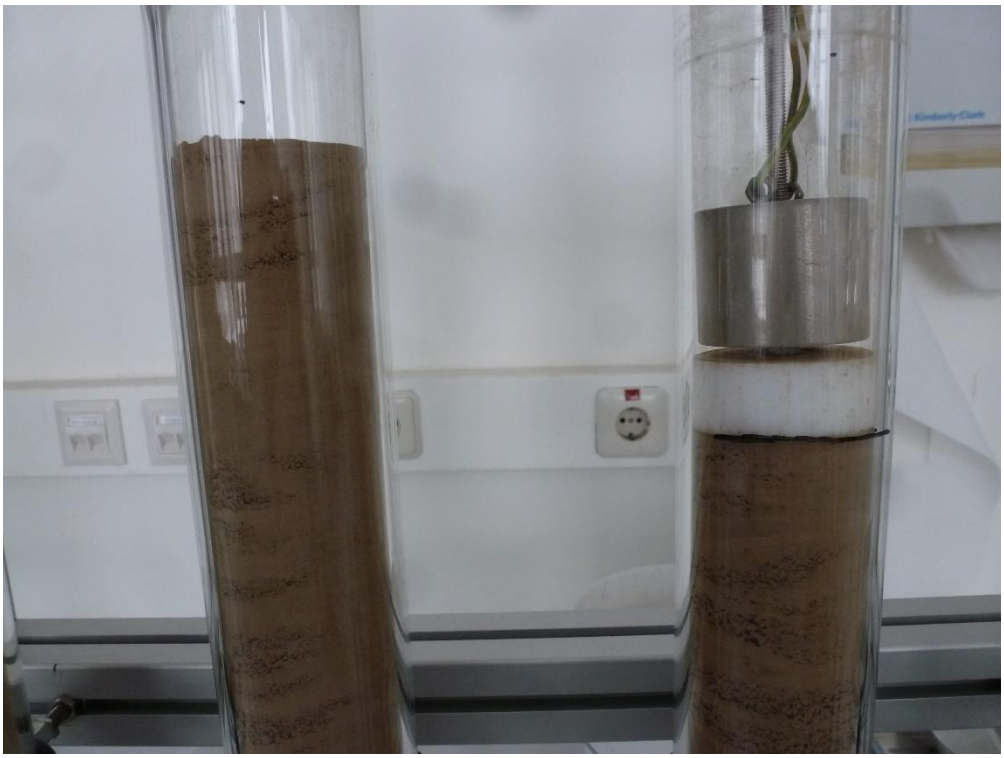
Taurozzi JS, Hackley VA, Wiesner MR (2011) Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment--issues and recommendations Nanotoxicology 5:711-729 doi:10.3109/17435390. 2010. 528846

Annex 3. Preparing of soil columns dry packaging

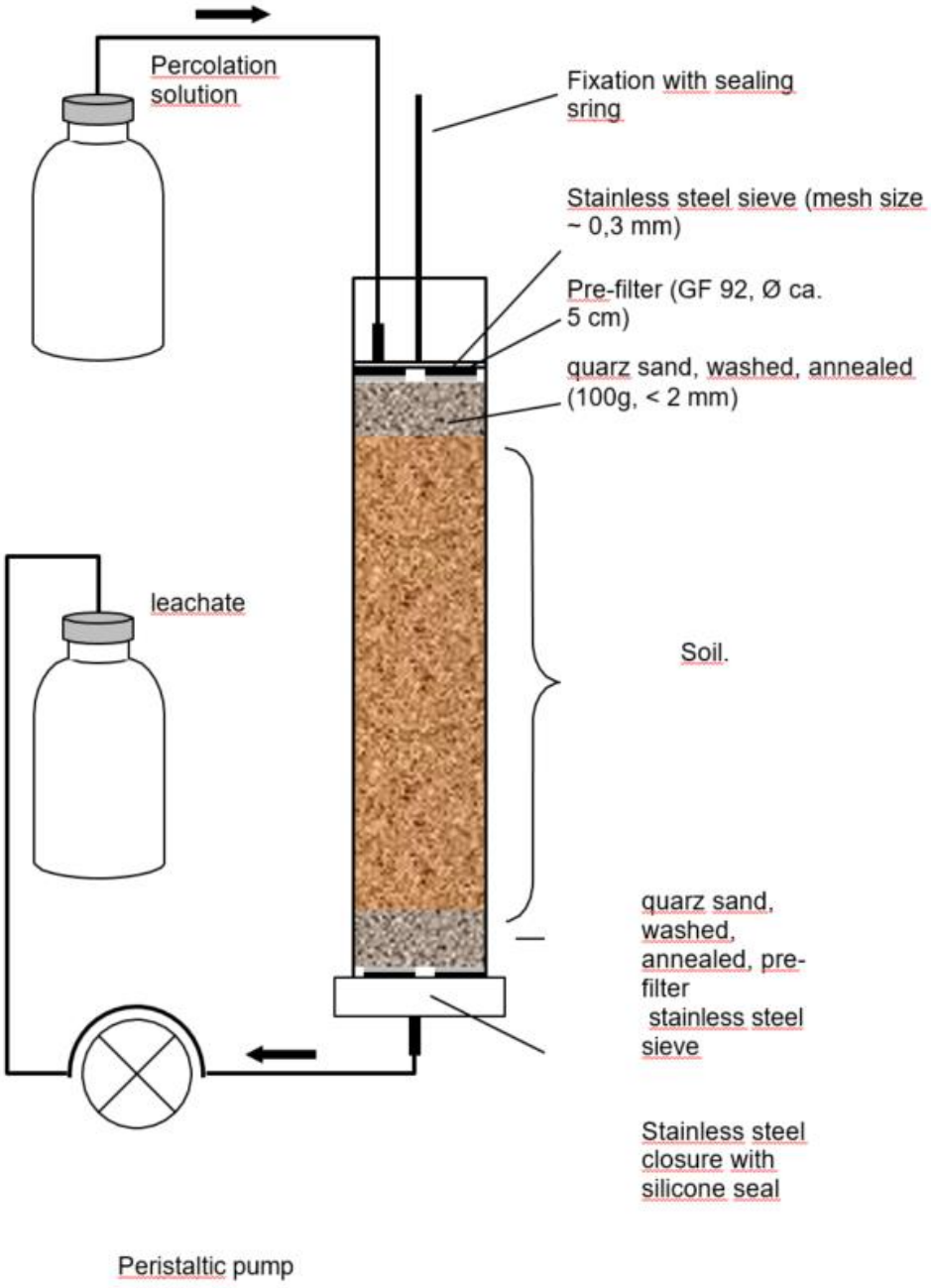
The percolation system consists of glass columns with internal diameters of at least 5 cm and a column height of at least 40 cm. The glass columns are closed at the bottom end with a stainless steel shutter with a silicone gasket. The stainless steel lock contains the feed opening for the percolation solution.

Before assembly, all parts should be cleaned with 0.1 M nitric acid and / or acetone and rinsed with deionised H_20 and then dried. On the stainless steel lock stainless steel screen (mesh size 0.3 mm) and a pre-filter is placed (paper filter, Ø 5 cm).

A layer of quartz sand (layer thickness 2-3 cm) is applied onto the filters. Then the soil material is placed in a layer thickness as required (here: 20 cm). Before filling a determination of dry matter is performed. The filling of the material is split into several individual portions. After filling each individual portion this is compressed. For this purpose, a drop weight of 500 g three times is dropped from 20 cm height on the filled portion. After compaction a filter and a stainless steel screen is used again. The column is sealed by a stamp with seal and a feed opening. See figure: Compaction of soil column.



Annex 4. Graphic soil column test set up



Annex 5. Reporting sheet test performance

Partner:

TESTING NANOMATERIALS USING OECD TG No. 312 “LEACHING in SOIL COLUMNS”

Reporting sheet for Ag

1.1 Information on performance and summarized results

If relevant, please report mean values with standard deviation

Equipment		
Leaching columns	Material	
	Length [cm]	
	Diameter [cm]	
Mesh immediately below the soil	Material	
	Pore size	
Material below the soil	Material	
	Amount	
	Grain size	
Material above the soil	Material	
	Amount	
	Grain size	
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	
	Absorption observed [yes/no]	
	Extent of absorption [%]	
Conditions of experiment - general		
Temperature of incubation [°C]		
Number of replicates		
Duration of the experiment [min]		
Soil type		
Conditions of experiment - soil		
Packing of the soil (dry or wet with detailed description)		
Amount of soil per column [g]	Replicate 1 dry: wet:	
	Replicate 2 dry:	

	wet:	
	Replicate 3 dry: wet:	
Pore water pH	glass electrode measurement of column leachate after equilibration	
Pore water conductivity	conductivity measurement in column leachate after equilibration	
Conditions of experiment – stock suspension, application, leaching		
Preparation of stock dispersion		
Suspending of applied nanomaterials	Dispersion medium	
	Time between preparation of stock suspension and column experiment start	
	Method of suspending (e.g. shaking, …)	
Preparation of application dispersion		
Concentration of NM in application dispersion [mg/L]		
Medium used for dilution and for leaching	Chemical composition	
	pH	
	Conductivity	
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	
	Stirring speed	
Size distribution in application dispersion (DLS)	Z-average hydrodynamic diameter	
	Polydispersity index	
pH of application dispersion		
Information on application and leaching		
Pore volume [cm^3]		
Amount of pore volumes used for application of NM		
Amount of pore volumes without NM used for leaching (background leaching solution)		
Flow rate [$mL/cm^2 * h$]		
Direction of equilibrium (upflow / downflow?)		
Volume of sub-samples (leaching solution)		

Results		
Description of chemical method for total concentration (e.g. ICP-OES/ICP-MS, instrument/measurement parameters (e.g. integration time, number of internal acquisition repetitions, etc), digestion (if performed))		
Recovery of the dissolved ion during ultrafiltration • *Inject a 1 microgram per L solution of Ag(NO3) in the top part of the centrifugal filtration device. This solution is preferably prepared from a ICP standard solution.* • *Centrifuge so that all of this solution is filtered.* • *Measure the Ag concentration in the filtrate (e.g. using ICP-MS or ICP-OES)* • *Recovery equals the ratio between measured and added concentration (in %)*		
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	
	Test end (sampling during last 0.5 pore volume)	
Dissolution (amount of ions determined by ultrafiltration) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	
	Test end (sampling during last 0.5 pore volume)	
Total amount of nanomaterial added to soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial in soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial in leachate [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Recovery [%]	Replicate 1	
	Replicate 2	
	Replicate 3	
Leaching distance of nanomaterial [cm]	Replicate 1	
	Replicate 2	
	Replicate 3	
Formulae used to calculate attachment efficiency		
Hamaker constant used to calculate single-collector contact efficiency, if applicable		

Calculation of α	Replicate 1	
	Replicate 2	
	Replicate 3	

Additional remarks:

In a separate table please provide the amount of NM and volume for every subsample as well as the amount in every analysed soil layer.

1.2 Detailed results: leachate

Total pore volumes of leachate	No of sub-sample	Parameter	Treatment		
			Replikate 1 (column 1)	Replicate 2 (column 2)	Replicate 3 (column 3)
0.5	Sub-sample 1	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1	Sub-sample 2	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1.5	Sub-sample 3	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2	Sub-sample 4	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2.5	Sub-sample 5	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
3	Sub-sample 6	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
3.5	Sub-sample 7	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			

4	Sub-sample 8	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
4.5	Sub-sample 9	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5	Sub-sample 10	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5.5	Sub-sample 11	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6	Sub-sample12	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6.5	Sub-sample 13	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
7	Sub-sample 14	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
7.5	Sub-sample 15	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			

		Size of leached particles (if relevant)			
8	Sub-sample 16	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
8.5	Sub-sample 17	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9	Sub-sample 18	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9.5	Sub-sample 19	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10	Sub-sample 20	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10.5	Sub-sample 21	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11	Sub-sample 22	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11.5	Sub-sample 23	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			

		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
12	Sub-sample 24	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			

1.3 Detailed results: soil

Distance from top	Parameter	Treatment		
		Replikate 1	Replicate 2	Replicate 3
0 – 2 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
2 – 4 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
4 – 6 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
6 – 8 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
8 – 10 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
10 – 15 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
15 – 20 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			

Reporting sheet for CeO_2

1.4 Information on performance and summarized results

If relevant, please report mean values with standard deviation

Equipment		
Leaching columns	Material	
	Length [cm]	
	Diameter [cm]	
Mesh immediately below the soil	Material	
	Pore size	
Material below the soil	Material	
	Amount	
	Grain size	
Material above the soil	Material	
	Amount	
	Grain size	
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	
	Absorption observed [yes/no]	
	Extent of absorption [%]	
Conditions of experiment - general		
Temperature of incubation [°C]		
Number of replicates		
Duration of the experiment [min]		
Soil type		
Conditions of experiment - soil		
Packing of the soil (dry or wet with detailed description)		
Amount of soil per column [g]	Replicate 1 dry: wet:	
	Replicate 2 dry:	

	wet:	
	Replicate 3 dry: wet:	
Pore water pH	glass electrode measurement of column leachate after equilibration	
Pore water conductivity	conductivity measurement in column leachate after equilibration	
Conditions of experiment – stock suspension, application, leaching		
Preparation of stock dispersion		
Suspending of applied nanomaterials	Particle concentration dispersed (only for CeO_2)	
	Dispersion medium	
	Time between preparation of stock suspension and column experiment start	
Sonication	Sonication time	
	Sonication power	
	Sonication volume	
Preparation of application dispersion		
Concentration of NM in application dispersion [mg/L]		
Medium used for dilution and for leaching	Chemical composition	
	pH	
	Conductivity	
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	
	Stirring speed	
Size distribution in application dispersion (DLS)	Z-average hydrodynamic diameter	
	Polydispersity index	
pH of application dispersion		
Information on application and leaching		
Pore volume [cm^3]		

Amount of pore volumes used for application of NM		
Amount of pore volumes without NM used for leaching (background leaching solution)		
Flow rate [mL/cm^2 * h]		
Direction of equilibrium (upflow / downflow?)		
Volume of sub-samples (leaching solution)		
Results		
Description of chemical method for total concentration (e.g. ICP-OES/ICP-MS, instrument/measurement parameters (e.g. integration time, number of internal acquisition repetitions, etc), digestion (if performed))		
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	
	Test end (sampling during last 0.5 pore volume)	
Dissolution (amount of ions determined by ultrafiltration) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	
	Test end (sampling during last 0.5 pore volume)	
Total amount of nanomaterial added to soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial in soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial in leachate [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Recovery [%]	Replicate 1	
	Replicate 2	
	Replicate 3	
Leaching distance of nanomaterial [cm]	Replicate 1	
	Replicate 2	
	Replicate 3	
Formulae used to calculate attachment efficiency		

Hamaker constant used to calculate single-collector contact efficiency, if applicable		
Calculation of α	Replicate 1	
	Replicate 2	
	Replicate 3	

Additional remarks:

In a separate table please provide the amount of NM and volume for every subsample as well as the amount in every analysed soil layer.

1.5 Detailed results: leachate

Total pore volumes of leachate	No of sub-sample	Parameter	Treatment		
			Replikate 1 (column 1)	Replicate 2 (column 2)	Replicate 3 (column 3)
0.5	Sub-sample 1	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1	Sub-sample 2	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1.5	Sub-sample 3	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2	Sub-sample 4	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2.5	Sub-sample 5	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			

3	Sub-sample 6	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
3.5	Sub-sample 7	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
4	Sub-sample 8	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
4.5	Sub-sample 9	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5	Sub-sample 10	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5.5	Sub-sample 11	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6	Sub-sample12	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6.5	Sub-sample 13	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			

		Size of leached particles (if relevant)			
7	Sub-sample 14	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
7.5	Sub-sample 15	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
8	Sub-sample 16	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
8.5	Sub-sample 17	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9	Sub-sample 18	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9.5	Sub-sample 19	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10	Sub-sample 20	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10.5	Sub-sample 21	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			

		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11	Sub-sample 22	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11.5	Sub-sample 23	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
12	Sub-sample 24	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			

1.6 Detailed results: soil

Distance from top	Parameter	Treatment		
		Replikate 1	Replicate 2	Replicate 3
0 – 2 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
2 – 4 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
4 – 6 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
6 – 8 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
8 – 10 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
10 – 15 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
15 – 20 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			

Reporting sheet for the tracer (e.g. KBr, NaNO3)

The tracer shall be added to the control columns. These columns provide information on the background concentration of Ag and Ce and on the reproducibility of the packing procedure.

1.7 Information on performance and summarized results

If relevant, please report mean values with standard deviation

Equipment		
Leaching columns	Material	
	Length [cm]	
	Diameter [cm]	
Mesh immediately below the soil	Material	
	Pore size	
	% recovery of NM during filtration	
Material below the soil	Material	
	Amount	
	Grain size	
Material above the soil	Material	
	Amount	
	Grain size	
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	
	Absorption observed [yes/no]	
	Extent of absorption [%]	
Conditions of experiment - general		
Temperature of incubation [°C]		
Number of replicates		

Duration of the experiment [min]		
Soil type		
Conditions of experiment - soil		
Packing of the soil (dry or wet with detailed description)		
Amount of soil per column [g]	Replicate 1 dry: wet:	
	Replicate 2 dry: wet:	
	Replicate 3 dry: wet:	
Pore water pH	glass electrode measurement of column leachate after equilibration	
Pore water conductivity	conductivity measurement in column leachate after equilibration	
Conditions of experiment – stock suspension, application, leaching		
Preparation of stock dispersion		
Suspending of applied tracer	Dispersion medium	
	Time between preparation of stock suspension and column experiment start	
Preparation of application dispersion		
Concentration of tracer in application dispersion [mg/L]		
Medium used for dilution and for leaching	Chemical composition	
	pH	
	Conductivity	
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	
	Stirring speed	
pH of application dispersion		
Information on application and leaching		
Pore volume [cm^3]		
Amount of pore volumes used for application of NM		
Amount of pore volumes without NM used for leaching (background leaching solution)		

Flow rate [mL/cm^2 * h]		
Direction of equilibrium (upflow / downflow?)		
Volume of sub-samples (leaching solution)		
Results		
Description of chemical method for total concentration (e.g. ICP-OES/ICP-MS, instrument/measurement parameters (e.g. integration time, number of internal acquisition repetitions, etc), digestion (if performed))		
Total amount of tracer added to soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of tracer in soil [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of tracer in leachate [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Total amount of tracer (soil + leachate) [mg]	Replicate 1	
	Replicate 2	
	Replicate 3	
Recovery [%]	Replicate 1	
	Replicate 2	
	Replicate 3	
Leaching distance of tracer [cm]	Replicate 1	
	Replicate 2	
	Replicate 3	

Additional remarks:

In a separate table please provide the amount of NM and volume for every subsample as well as the amount in every analysed soil layer.

1.8 Detailed results: leachate

Total pore volumes of leachate	No of sub-sample	Parameter	Treatment		
			Replikate 1 (column 1)	Replicate 2 (column 2)	Replicate 3 (column 3)
0.5	Sub-sample 1	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1	Sub-sample 2	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
1.5	Sub-sample 3	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2	Sub-sample 4	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
2.5	Sub-sample 5	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
3	Sub-sample 6	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
3.5	Sub-sample 7	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			

		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
4	Sub-sample 8	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
4.5	Sub-sample 9	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5	Sub-sample 10	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
5.5	Sub-sample 11	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6	Sub-sample12	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
6.5	Sub-sample 13	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
7	Sub-sample 14	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
7.5	Sub-sample	Volume of leachate [mL]			
		Concentration of NM [µg/L]			

	15	Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
8	Sub-sample 16	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
8.5	Sub-sample 17	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9	Sub-sample 18	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
9.5	Sub-sample 19	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10	Sub-sample 20	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
10.5	Sub-sample 21	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11	Sub-sample 22	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
11.5	Sub-	Volume of leachate [mL]			

	sample 23	Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			
12	Sub-sample 24	Volume of leachate [mL]			
		Concentration of NM [µg/L]			
		Amount in subsample [µg]			
		Zeta potential (if relevant)			
		Size of leached particles (if relevant)			

1.9 Detailed results: soil

Distance from top	Parameter	Treatment		
		Replikate 1	Replicate 2	Replicate 3
0 – 2 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
2 – 4 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
4 – 6 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
6 – 8 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
8 – 10 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
10 – 15 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			
15 – 20 cm	Concentration of NM [µg/kg]			
	Amount in soil [µg]			

Annex 6. Attachment efficiency calculation

Please refer to the community site: https://community.oecd.org/docs/DOC-189538 (link to be replaced once published.

Annex 7. Compilation of results from the partner laboratories

Overview on testing contributions

Refesol 01A

Lab* / NM	1	2	3	4	5	6	7
NM300K (Silver)	x	x	x	x		x	x
NM212 (Cerium oxide)	x				x		
Tracer (KBr or NaNO3)	x	x			x		x

* numbering according to the sequence of submission of data

Refesol 02A

Lab* / NM	1	2	3	4	5	6	7
NM300K (Silver)	x	x	x	x		x	x
NM212 (Cerium oxide)	x						
Tracer (KBr or NaNO3)	x	x					x

* numbering according to the sequence of submission of data

Ag – 01-A

Column

	Partner lab	1	2	3	4	6	7
Leaching columns	Material	Glass	Glass	Glass	Glass	Glass	Quartz glass
	Length [cm]	25	20.6±0.1	15 (soil height; column: 30 cm)	12 (soil height; column: 22 cm)	20	21.5
	Diameter [cm]	4	4.25	5	5	5.5 cm	4.14
Mesh immediately below the soil	Material	Paper and glass wool	Polypropylene (PP)	Filter paper	Stainless Steel	steel	-
	Pore size	Additional information available	105 µm	4 µm	300 µm		-
Material below the soil	Material	Quartz sand	Quartz sand	Quartz sand	Quartz Sand	quartz sand	Glass beads (Merck, Darmstadt, Germany)
	Amount	2 cm	≈13.5 gr	10 g	2 mm thick layer	150 g	50.5 cm^3
	Grain size [mm]	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	0.250-0.300	0.400 – 0.800		5 mm
Material above the soil	Material	Quartz sand	Quartz sand	Quartz sand	Quartz Sand	quartz sand	Glass beads, Sigma-Aldrich
	Amount	2 cm	≈13.5 gr	10 g	2 mm thick layer	150 g	50.5 cm^3
	Grain size	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	0.250-0.300	0.400 – 0.800		5 mm
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	yes	Yes	yes	Yes	no	Yes
	Absorption	yes	No	no	No		No

	observed [yes/no]						
	Extent of absorption [%]	Not quantifiable as only part was recovered, total 100 µg/L recovered from a piece of tubing from both replicates and 2 magnet bars from both columns	-	1.15	0 %		0

Conditions of experiment – general

	1	2	3	4	6	7
Temperature of incubation [°C]	20 ±2	21 - 22	23	22.5	20	22.5
Number of replicates	2	3	3	3	4	4
Duration of the experiment [min]	5,760 /96 h	28,800 / 480 h	7680 / 128 h	9,600 / 160 h	11,500 / 192 h	12,960 / 216
Soil type	01A	01-A-05	sandy loam	Loamy Sand, RefeSol 01-A	01A	Cambisol

Conditions of experiment - soil

		1	2	3	4	6	7
Amount of soil per column [g]	Replicate 1 dry: wet:	290.7 366.0	dry: 472 wet: 583	543.08	378	Dry: 745.4 Wet: 750.0	268.9 309.6
	Replicate 2 dry: wet:	291.7 363.8	dry: 472 wet: 580	539.61	381	Dry: 745.4 Wet: 750.0	272.6 313.8
	Replicate 3 dry: wet:	--	dry: 472 wet: 583	544.22	387 g	Dry: 745.4 Wet: 750.0 Replicate 4 = 3	271.6 312.7 Replicate 4: 269.2 / 309.9
Pore water pH	glass electrode measurement of column leachate after equilibration	7.3/7.3	8.20±0.05	7.93±0.14	5.87	7.1 – 7.2	6.8
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]	717 601	585±5	548±47.03	Not Measured	708	448

Preparation of stock dispersion

		1	**2**	3	4	6	7
Suspending of applied nanomaterials	Dispersion medium	NM300K aliquot made up to 1 L in 0.005 M $NaNO_3$	water	DW	18.32 MΩ electronics grade water from a specially designed water purification system that stores the purified water under a bed of nitrogen	water	Bidistilled water
	Time between preparation of stock suspension and column experiment start	30 min	1 hour	1 h	N/A	30 min	120 h
	Method of suspending (e.g. shaking, …)	Stirring at 40 rpm	Shaking + probe sonication	Shaking	N/A	shaking	sonication

Preparation of application dispersion

		1	2	3	4	6	7
Concentration of NM in application dispersion [mg/L]		18 mg AgNM/L (measured value)	63.74	69.41±0.72	60 mg/L	4.2	HNO_3 digestion, Thermo-ICP-MS: 7.39 ± 0.05 (n = 5) Filtration (0.45 µm), acidification, by ICP-OES: 7.09 ± 0.06 (n = 3)
Medium used for dilution and for leaching	Chemical composition	5 mM $NaNO_3$	5 mM $NaNO_3$	5 mM KNO_3	Ultra Pure Water (UPW) for Dilution, 5 mM KCl for Leaching	5 mM KCl	Bidistilled water 5 mmol/l NaNO3
	pH	6.3	5.5	6.04	UPW = 6.15, 5mM KCl = 6.55	5.8	Leaching 5.52
	Conductivity	543 µS/cm	570 µS/cm	676	Not Measured	710	Leaching: 558
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	magnetic stirring	Magnetic stirring	magnetic stirring	Magnetic Stirring	Magnetic stirring	No stirring
	Stirring speed	40 rpm	250 rpm	100rpm	100 rpm		---
Size distribution in application dispersion (DLS)	Z-average hydrodynamic diameter	99.8 ±24.62	50±3 nm	43.21±5.07	17nm Particle size as measured by single particle inductively-coupled plasma mass spectrometry		42 ± 14 nm (n = 4)
	Polydispersity index	0.24 ±0.03	0.44±0.05	0.53±0.09	Unknown		0.49 ± 0.02 (n = 4)
pH of application dispersion		--	6.5	7.13±0.01	6.45		5.68 ± 0.14 (n = 3

Information on application and leaching

	1	2	3	4	6	7
Pore volume [cm^3]	73.7 ±2.3	100	96	80	190	137.3 ± 6.6
Amount of pore volumes used for application of NM	4	3.8	3.66±0.12	4	0.05 (pulse application)	2.2
Amount of pore volumes without NM used for leaching (background leaching solution)	3	7.3	3.63±0.18	8	8	2.8
Flow rate [$mL/cm^2 * h$]	0.3	0.3	0.28±0.01	0.306	0.333	0.238
Direction of **equilibrium** (upflow / downflow?)	upflow	Upflow	Upflow (i) equilibration of the soils with the leaching solution was performed in upflow mode; (ii) columns were leached with leaching solution at least two pore volumes)	Downflow	upflow	upflow
Volume of sub-samples (leaching solution)	40.7 ±2.1mL	47±14	43.72±1.68 ml	40 mL/sub-sample	20 mL	About 40 ml per percolation step (12 h).

Results

		1	2	3	4	6	7
Recovery of the dissolved ion during ultrafiltration [%]		90	35.3	107	Not measured	Not measured	
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	Z-average: 297.9 ±35.7 PDI: 0.5±0.2 Z-potential (mV): -15.9±3.2	50±3 nm	43.21±5.07 nm	17 nm Particle size as measured by single particle inductively-coupled plasma	Not measured	36 nm

					mass spectrometry		
	Test end (sampling during last 0.5 pore volume)	Z-average: 493.3 (rep 1) ; 1984 (rep.2:) PDI: 0.7 Z-potential (mV): -12.6±5.3	84±2 nm	42.34±7.05 nm	17 nm Particle size as measured by single particle inductively-coupled plasma mass spectrometry	Not measured	32 nm
Dissolution in the test media at start and end of application [mg/L]	Test start (sampling during 1st 0.5 pore volume)	<0.0005 (after ultracentfugation)	0.013	3.22±0.05	Not measured	Not measured	Data from ICP-OES C (Ag+) = 0.424 mg/l C (Ag+) = 5.63%
	Test end (sampling during last 0.5 pore volume)	0.097 (rep 1) <0.0005 (rep 2) (after ultracentfugation)	0.027	3.42±0.05	Not measured	Not measured	Data from ICP-OES and Thermo- ICP-MS C (Ag^+) = 0.523 mg/l C (Ag^+) = 7.26%
Total amount of nanomaterial added to soil [mg]	Replicate 1	6.1 mg Ag/column	34.59	24.44	18.8	37.5	2.261 (V*c_median_Thermo-ICP- MS = 7.39 mg/l * 0.306 l)
	Replicate 2	5.7 mg Ag/column	34.96	24.52	19.0	37.5	2.267
	Replicate 3	--	33.47	26.78	19.0	37.5 Replicate 4: 37.5	2.262 Replicate 4: 2.220
Total amount of nanomaterial in soil [mg]	Replicate 1	0.89 mg (in 0-10 cm)	3.59	21.53	1.34	32.85	0.907 (Aqua regia digestion_(ARD)_Agilent-ICP-MS)
	Replicate 2	0.15 mg (in 0-10 cm)	4.43	18.98	1.27	25.88	1.109
	Replicate 3	--	4.47	21.63	1.34	29.04 Replicate 4: 38.14	1.059 Replicate 4: 1.031
Total amount of nanomaterial in leachate [mg] *(italic values in brackets:*	Replicate 1	0.018 *(2%)*	1.20e-4 *(0.003%)*	1.34 *(5.9%)*	16.4 *(92.1%)*	11.38 *(30.3%)*	1.270 (BT recovery1 = 56.2%)
	Replicate 2	0.0002 *(0.13%)*	7.93e-5 *(0.002%)*	4.29 *(18.4%)*	16.6 *(92.8%)*	5.39 *(14.4%)*	1.250 (BT recovery[1] = 55.2%)

percentage of added total amount)	Replicate 3	--	5.02e-5 *(0.001%)*	3.29 *(13.2%)*	15.8 *(92.4%)*	16.84 *(44.9%)* Replicate 4: 22.90 *(77.3%)*	1.206 (BT recovery[1] = 53.3%) Replicate 4: 1.234 (BT recovery[1] = 55.6%)
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	0.90	3.59	22.87	17.8	32.858	2.177
	Replicate 2	0.15	4.43	23.27	17.9	25.885	2.359
	Replicate 3	---	4.47	24.92	17.1	29.058 Replicate 4: 38.158	2.266 Replicate 4: 2.265
Recovery [%]	Replicate 1	14.9	10.4	93.6	94.3	87.6	96.3
	Replicate 2	2.7	12.7	94.9	94.1	69.0	104.1
	Replicate 3	---	13.4	93.1	89.9	77.5 Replicate 4: 101.8	100.2 Replicate 4: 102.0
Leaching distance of nanomaterial [cm]	Replicate 1	> 10	7	> 15	12	10	> 14
	Replicate 2	> 10	7	> 15	12	15	> 14
	Replicate 3	---	7	> 15	12	15 Replicate 4: 15	> 14
Formulae used to calculate attachment efficiency			$\alpha=-2/3*d_c/(1-\varepsilon)*L*\eta_0)*Ln(C/C_0)$		Formula provided by GC		Formula provided by GC
Hamaker constant used to calculate single-collector contact efficiency. if applicable			5.36E-20 J [2]		1.29 E-19 Joules		$2.37 * 10^{-21}$ J
Calculation of α	Replicate 1		η_0 1.03				0.000590 (BT recovery2 = 56.5%)
			α 0.0069				
	Replicate 2		η_0 1.03				0.000614 (BT recovery2 = 55.5%)
			α 0.0071				

[2] Taghavi et at., 2013 (10.1021/es400692r; Environ. Sci. Technol. 2013, 47, 8499−8507).

	Replicate 3		η_0	1.03				0.000648 (BT recovery2 = 53.7%) Replicate 4: 0.000621 (BT recovery = 54.9%)
			α	0.0074				
Overall result	**Partners with a recovery of >90% indicate leaching in a loamy sand; percentage in leachate and leaching distance can vary.**							
			•	•	•	•		

Ag – 02-A

Column

		1	2	3	4	6	7
Leaching columns	Material	Glass	Glass	Glass	Glass	Glass	Quartz glass
	Length [cm]	25 cm	15	15 (soil height; column: 30 cm)	12 (soil height; column: 22 cm)	20	22.0
	Diameter [cm]	4 cm	4.25	5	5	5.5 cm	4.14
Mesh immediately below the soil	Material	Paper and glass wool	Polypropylene (PP)	Filter paper	Stainless Steel	steel	-
	Pore size	Additional information available	105 µm	4 um	300 µm		-
Material below the soil	Material	Quartz sand	Quartz sand	Quartz Sand	Quartz Sand	quartz sand	Glass beads (Merck, Darmstadt, Germany)
	Amount	2 cm	≈13.5 gr	10 g	2 mm thick layer	150 g	53.8 cm^3
	Grain size	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	0.250 – 0.300	0.4 – 0.8		5 mm
Material above the soil	Material	Quartz sand	Quartz sand	Quartz Sand	Quartz Sand	quartz sand	Glass beads, Sigma-Aldrich
	Amount	2 cm	≈13.5 gr	10 g	2 mm thick layer	150 g	53.8 cm^3
	Grain size	0.125	0.3-0.8 (d_{50}=0.58)	0.250 – 0.300	0.4 – 0.8		5 mm
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	yes	No	yes	Yes	no	Yes
	Absorption observed [yes/no]	yes	No	no	No		No

	Extent of absorption [%]	Not quantifiable as only part was recovered, total 692 µg/L recovered from a piece of tubing from replicate 2 and 2 magnet bars from both columns	-	1.15	0		No

Conditions of experiment - general

	1	2	3	4	6	7
Temperature of incubation [°C]	20 ±2	21 - 22	23	22.5	20	22.5
Number of replicates	2	3	3	3	4	4
Duration of the experiment [min]	6960 min/116 h	28,800 / 480 h	10080 / 168 h	12,100 / 202 h	11,500 / 192 h	13680 / 228 h
Soil type	02A	02-A-02	silt loam	Silt Loam, RefeSol 02-A	02-A	Luvisol

Conditions of experiment - soil

		1	**2**	**3**	**4**	**6**	**7**
Amount of soil per column [g]	Replicate 1 dry: wet:	376.6 275.0	282 379	443.85	326.8	624.3 630.0	234.1 271.1
	Replicate 2 dry: wet:	369.4 271.8	282 379	450.85	312.0	624.3 630.0	233.1 269.9
	Replicate 3 dry: wet:	--	282 379	451.45	313.8	624.3 630.0 Replicate 4: 624.3 630.0	232.5 269.3 Replicate 4: 233.1 270.0
Pore water pH	glass electrode measurement of column leachate after equilibration	7.3/7.3	7.7±0.5	8.44±0.16	6.53	7.7	7.6
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]	588 µS/cm 603 µS/cm	590±5	530±15.87	Not Measured	710	362.3

Preparation of stock dispersion

		1	**2**	3	4	6	7
Suspending of applied nanomaterials	Dispersion medium	NM300K aliquot made up to 1 L in 0.005 M $NaNO_3$	water	DW	N/A	water	Bidistilled water
	Time between preparation of stock suspension and column experiment start	35 min	1 hour	1 h	N/A	30 min.	96 h
	Method of suspending (e.g. shaking, …)	Stirring at 40 rpm	Shaking and probe sonication (12 min-40 W)	Shaking	N/A	shaking	Sonication

Preparation of application dispersion

		1	2	3	4	6	7
Concentration of NM in application dispersion [mg/L]		13.7	36.26	44.45±2.13	40	4.2	8.96 ± 0.10 (n = 3) 7.51 ± 0.36 (n = 4)
Medium used for dilution and for leaching	Chemical composition	AgNM in 0.005 M $NaNO_3$	5 mM $NaNO_3$	5 mM KNO3	Ultra Pure Water (UPW) for Dilution, 0.005 M KNO3 for Leaching	0.005M KCl	5 mM $NaNO_3$
	pH	6.4	5.5	6.04	UPW = 6.15, 0.005M KNO3: 6.41	5.8	5.52
	Conductivity	Not measured	570 µS/cm	676	Not Measured	710	558 µS/cm
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	magnetic stirring	Magnetic stirring	magnetic stirring	Magnetic Stirring		No stirring
	Stirring speed	40 rpm	250 rpm	100rpm	100 rpm		
Size distribution in application dispersion (DLS)	Z-average hydrodynamic diameter	139.5 (unfiltered sample); 43±4 (filtered sample)	46±1 nm	43.78±1.60	17nm Particle size as measured by single particle inductively coupled plasma mass spectrometry		54 ± 2 nm (n = 3)
	Polydispersity index (PDI)	0.18	0.465±0.050	0.53±0.03	---		0.45 ± 0.06 (n = 3)
pH of application dispersion		Not measured	6.3	7.13±0. 01	6.45		5.70 ± 0.09 (n = 3)

Information on application and leaching

	1	2	3	4	6	7
Pore volume [cm^3]	99.6 ±2.8	85.3	126	100	240	116.4 ± 2.3
Amount of pore volumes used for application of NM	4 PV	4.4	3.74±0.13	4	0.05 (pulse application)	3.4
Amount of pore volumes without NM used for leaching (background leaching solution)	3 PV	8.4	3.70±0.20	8	8	3.1
Flow rate [$mL/cm^2 * h$]	0.3	0.265	0.28±0.01	0.306	0.333	0.261 ± 0.005 (n = 4)
Direction of equilibrium (upflow / downflow?)	upflow	Upflow	Upflow	Downflow	upflow	upflow
Volume of sub-samples (leaching solution)	46.05 ±9.2mL	46.3±3.3	58.63±2.76 ml	50 mL/sub- sample	20 mL	About 42 ml per percolation step (12 h).

Results

		1	2	3	4	6	7
Recovery of the dissolved ion during ultrafiltration [%]		90	35.3	**107**	No information		
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	Z-average: 144.9 ±58.2 (unfiltered samples); 43±4 (filtered samples) PDI: 0.6±0.3 Z-potential (mV): -14.7±1.7	46±1 nm	43.78±1.60 nm	17nm Particle size as measured by single particle inductively coupled plasma mass spectrometry		54 nm
	Test end (sampling during last 0.5 pore volume)	Z-average: 274.3 ±188.3 (unfiltered) PDI: 0.9±0.2 Z-potential (mV): -20.7±0.2	75±2 nm	43.81±6.93 nm	17nm Particle size as measured by single particle inductively coupled plasma mass spectrometry		45 nm
Dissolution in the test media at	Test start (sampling	<0.0005 (after	0.010	1.93±0.20	Not measured		Data from

start and end of application [mg/L]	during 1st 0.5 pore volume)	ultracentrifugation)					ICP-OES C (Ag+) = 0.56 ± 0.04 mg/l C (Ag+) = 6.93 ± 0.57%
	Test end (sampling during last 0.5 pore volume)	<0.0005 (after ultracentrifugation)	0.017	2.11±0.18	Not measured		Not measured
Total amount of nanomaterial added to soil [mg]	Replicate 1	4.5 mg Ag/column	26.10	19.70	16.0	31.5	3.250
	Replicate 2	5.1 mg Ag/column 18.6 mg Ag /kg dry soil (13 mg/L AgNP)	25.84	20.18	15.9	31.5	3.092
	Replicate 3	--	25.68	20.76	15.4	31.5 Replicate 4: 31.5	3.160 Replicate 4: 3056
Total amount of nanomaterial in soil [mg]	Replicate 1	0.46 mg (in 0-10 cm)	8.24	18.65	7.09	15.1	3.018
	Replicate 2	0.62 mg (in 0-10 cm)	9.49	15.69	7.70	23.16	3.114
	Replicate 3	--	6.66	19.50	6.98	25.07 Replicate 4: 24.16	3.050 Replicate 4: 3.076
Total amount of nanomaterial in leachate [mg] *(italic values in brackets: percentage of added total amount)*	Replicate 1	0.29e-3 *(0.06 % of total)*	0.515e-3 *(0.006% of total)*	0.00 *(0% of total)*	8.53 *(54.7% of total)*	0.27 *(0.9%)*	*0.012 (BT recovery[1] = 0.37%)*
	Replicate 2	1.03e-3 *(0.17 %)*	0.255e-3 *(0.003%)*	0.00 *(0%)*	8.77 *(53.1%)*	0.16 *(0.5%)*	*0.010 (BT recovery[1] = 0.32%)*
	Replicate 3	--	3.31e-4 *(0.004%)*	0.00 *(0%)*	7.92 *(53.2%)*	0.24 *(0.8%)* Replicate 4: 0.24 *(0.8%)*	*0.010 (BT recover[1] = 0.30%)* *Replicate*

							4: 0.011 (BT[1] recovery = 0.35%)
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	0.47	8.24	18.65	15.6	15.1	*3.030*
	Replicate 2	0.62	9.49	15.69	16.5	23.16	*3.124*
	Replicate 3	---	6.66	19.50	14.9	25.07 Replicate 4: 24.16	*3.059* *Replicate 4:* *3.087*
Recovery [%]	Replicate 1	3.3	31.56	94.65	97.7	47.9	*93.2*
	Replicate 2	4.7	36.74	77.73	103.6	73.5	*101.1*
	Replicate 3	---	25.92	93.89	96.8	79.6 Replicate 4: 76.7	*96.8* *Replicate 4: 101.0*
Leaching distance of nanomaterial [cm]	Replicate 1	*2 – 4*	3	2-4	12	10	*>14*
	Replicate 2	*2 – 4*	2	0-2	12	10	*>14*
	Replicate 3	---	2	2-4	12	10 Replicate 4: 8	*>14*
Formulae used to calculate attachment efficiency			$\alpha=-2/3*d_c/(1-\varepsilon)*L*\eta_0)*Ln(C/C_0)$		Formula provided by GC		Formula provided by GC
Hamaker constant used to calculate single-collector contact efficiency. if applicable			$5.36E^{-20}$ J		$1.29\ E^{-19}$ Joules		$2.37*10^{-21}$ J
	Replicate 1		η_0 2.3				0.0024

Calculation of α			α	0.0014				(BT recovery^2 = 0.34%)
	Replicate 2		η_0	2.3				0.0025 (BT recovery^2 = 0.28%)
			α	0.0015				
	Replicate 3		η_0	2.3				0.0025(BT recovery^2 = 0.28%) Replicate 4: 0.0024 (BT recovery^2 = 0.31%)
			α	0.0016				
Overall results	**Extent of leaching differs; nearly no leaching and breakthrough in a silt loam**							
		•	•		•	•	•	

CeO_2 – 01-A

Column

		1	5
Leaching columns	Material	Glass	glass
	Length [cm]	25 cm	15 & 25
	Diameter [cm]	4 cm	4
Mesh immediately below the soil	Material	Paper and glass wool	Nylon
	Pore size	Additional information provided	
Material below the soil	Material	Quartz sand	Quartz sand
	Amount	2 cm	30 g
	Grain size	0.125 mm (63%)	0.4-0.6
Material above the soil	Material	Quartz sand	Quartz sand
	Amount	2 cm	30 g
	Grain size	0.125 mm	0.4-0.6
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	no	no
	Absorption observed [yes/no]	no	
	Extent of absorption [%]	Not calculated	

Conditions of experiment - general

	1	5
Temperature of incubation [°C]	20 ±2°C	Not measured
Number of replicates	2	3
Duration of the experiment [min]	5760 min/96 h	11520 / 192 h
Soil type	01A	01A

Conditions of experiment - soil

		1	5
Amount of soil per column [g]	Replicate 1 dry: wet:	305.2 380.0	Dry: 250.9
	Replicate 2 dry: wet:	296.5 374.7	Dry: 240
	Replicate 3 dry: wet:	Not used	Dry: 239
Pore water pH	glass electrode measurement of column leachate after equilibration	Not determined	Not measured
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]	Not determined	321 +- 31 µS

Preparation of stock dispersion

		1	**5**
Suspending of applied nanomaterials	Particle concentration dispersed	Not determined	12,5mg/130ml
	Dispersion medium	Deionised water- 20 mL, rest for making upto 1 L in 0.005 M NaCl	Water)
	Time between preparation of stock suspension and column experiment start	30 to 35 min	immediatly
	Method of suspending (e.g. shaking, …)	16 min, at 10 W, 20 mL	60sec; 50W; 560ml

Preparation of application dispersion

		1	**5**
Concentration of NM in application dispersion [mg/L]		43.88 mg/L	96
Medium used for dilution and for leaching	Chemical composition	CeO2+5 mL D.I water to make a paste and made upt0 20 mL stock suspension with D.I. After ultrasonication, made up to 1L with 0.005M NaCl and ultrasonicate again for 10 minutes in a ultrasonication bath.	0.005 M KCl
	pH	6.1	
	Conductivity	509 µS/cm	
Stirring of application dispersion during	Stirring method (magnetic stirring,…)	magnetic stirring at 50 rpm	

application (if relevant)	Stirring speed		
Size distribution in application dispersion (DLS)	Z-average hydrodynamic diameter	205.2 (after 5 µm filtration:189.5)	Directly after dispersion 191nm PDI 0,14 - monomodal; after 80min 482 nm PDI 0,23; after 100min 548 PDI 0,25 bimodal with larger particles (D50 for all three measurements 224nm +- 20nm)
	Zeta Potential (mV)	38 (after 5 µm filtration: -)	--
	Polydispersity index	0.30	0,14 0,23 0,25
pH of application dispersion		6.1	Not measured.

Information on application and leaching

	1	**5**
Pore volume [cm^3]	76.5 ±2.4	66.2
Amount of pore volumes used for application of NM	4 PV	2
Amount of pore volumes without NM used for leaching (background leaching solution)	2 PV	
Flow rate [$mL/cm^2 * h$]	2-3	3.3 ml/m²h
Direction of equilibrium (upflow / downflow?)	upflow	Downflow
Volume of sub-samples (leaching solution)	39.2 ±1.6mL	

Results

		1	5
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	Z-average: 279.5 ±8.7 PDI: 0.3±0.01 Z-potential (mV): -25.2±4.3	Not tested
	Test end (sampling during last 0.5 pore volume)	Z-average: 539.3±41.7 PDI: 0.7±0.1 Z-potential (mV): -13.2±2.2	Not tested
Total amount of nanomaterial added to soil [mg]	Replicate 1	46.29 mg/ kg dw (14.13 mg in column)	12.5
	Replicate 2	45.13 mg/ kg dw (13.38 mg in column)	12.5
	Replicate 3	--	12.5
Total amount of nanomaterial in soil (including sand filter at the inlet) [mg]	Replicate 1	3.18 mg (in 0-10 cm)	17.54
	Replicate 2	2.85 mg (in 0-10 cm)	12.28
	Replicate 3	--	11.54
Total amount of nanomaterial in leachate [mg] *(italic values in brackets: percentage of added total amount)*	Replicate 1	0.00093 *(0 %)*	0.241 *(1.9 %)*
	Replicate 2	0.00093 *(0 %)*	0.376 *(3.0 %)*
	Replicate 3		0.149 *(1.2 %)*
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	3.19	17.781
	Replicate 2	2.85	12.656
	Replicate 3	---	11.689
Recovery [%]	Replicate 1	22.5	142
	Replicate 2	21.3	101
	Replicate 3	---	94
Leaching distance of nanomaterial [cm]	Replicate 1	---	10
	Replicate 2	---	10

	Replicate 3	---	10
		• LOQ: 0.95 µg/L, S.D: 0.01 µg/L • LOD:1.01 µg/L	

CeO_2 – 02-A

Column

		1
Leaching columns	Material	Glass
	Length [cm]	25 cm
	Diameter [cm]	4 cm
Mesh immediately below the soil	Material	Paper and glass wool
	Pore size	Siehe Anhang
Material below the soil	Material	Quartz sand
	Amount	2 cm
	Grain size	0.125 mm (63%)
Material above the soil	Material	Quartz sand
	Amount	2 cm
	Grain size	0.125 mm
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	no
	Absorption observed [yes/no]	no
	Extent of absorption [%]	Not calculated

Conditions of experiment - general

	1
Temperature of incubation [°C]	20 ±2°C
Number of replicates	2
Duration of the experiment [min]	5760 min/96 h
Soil type	02A

Conditions of experiment - soil

		1
Amount of soil per column [g]	Replicate 1 dry: wet:	273.5 369.7
	Replicate 2 dry: wet:	267.4 378.9
	Replicate 3 dry: wet:	 ---
Pore water pH	glass electrode measurement of column leachate after equilibration	7.6 8.3
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]	831 µS/cm 762 µS/cm

Preparation of stock dispersion

		1
Suspending of applied nanomaterials	Dispersion medium	Deionised water- 20 mL, rest for making upto 1 L in 0.005 M NaCl
	Time between preparation of stock suspension and column experiment start	40 min
	Method of suspending (e.g. shaking, …)	16 min, at 10 W, 20 mL

Preparation of application dispersion

		1
Concentration of NM in application dispersion [mg/L]		43.7 mg/L
Medium used for dilution and for leaching	Chemical composition	CeO2+5 mL D.I water to make a paste and made upt0 20 mL stock suspension with D.I. After ultrasonication, made up to 1L with 0.005M NaCl and ultrasonicate again for 10 minutes in a ultrasonication bath.
	pH	5.2
	Conductivity	536 µS/cm
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	magnetic stirring at 50 rpm
	Stirring speed	
Size distribution in application dispersion (DLS)	Z-average hydrodynamic	316.2

	diameter	
	Polydispersity index	0.41
pH of application dispersion		

Information on application and leaching

	1
Pore volume [cm^3]	103.9 ±10.8
Amount of pore volumes used for application of NM	4 PV
Amount of pore volumes without NM used for leaching (background leaching solution)	2 PV
Flow rate [$mL/cm^2 * h$]	2-3
Direction of equilibrium (upflow / downflow?)	upflow
Volume of sub-samples (leaching solution)	39.0 ±2.0mL

Results

		1
Stability (measured with DLS) in the test media at start and end of application	Test start (sampling during 1st 0.5 pore volume)	Z-average: 1502.5 ±174.2 PDI: 1.1±0.1 Z-potential (mV): -3.7
	Test end (sampling during last 0.5 pore volume)	Z-average: 573.0±35.0 PDI: 0.8±0.1 Z-potential (mV): -1.8±2.4
Total amount of nanomaterial added to soil [mg]	Replicate 1	52.36 mg/ kg (14.32 mg in column)
	Replicate 2	48.60 mg/ kg (13.00 mg in column)
	Replicate 3	--
Total amount of nanomaterial in soil (including sand filter at the inlet) [mg]	Replicate 1	7.87 mg (in 0-10 cm)
	Replicate 2	7.90 mg (in 0-10 cm)
	Replicate 3	--
Total amount of nanomaterial in leachate [mg]	Replicate 1	0.0003
	Replicate 2	0.0002
	Replicate 3	
Total amount of nanomaterial (soil + leachate) [mg]	Replicate 1	7.87
	Replicate 2	7.90
	Replicate 3	---
Recovery [%]	Replicate 1	54.9
	Replicate 2	60.8
	Replicate 3	---
Leaching distance of nanomaterial [cm]	Replicate 1	---
	Replicate 2	---
	Replicate 3	---

Tracer KBr / $NaNO_3$ – 01-A

Column

		1 - KBR	2 - $NaNO_3$	5	7 – $NaNO_3$
Leaching columns	Material	Glass	Glass	Glass	
	Length [cm]	25	20.6±0.1	25	
	Diameter [cm]	4	4.25	4	
Mesh immediately below the soil	Material	Paper and glass wool	Polypropylene (PP)	Nylon	
	Pore size	Additional information available	105 µm		
Material below the soil	Material	Quartz sand	Quartz sand		
	Amount	2 cm	≈13.5 gr	Quartz sand	
	Grain size [mm]	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	30	
Material above the soil	Material	Quartz sand	Quartz sand	0.4-0.6	
	Amount	2 cm	≈13.5 gr	Quartz sand	
	Grain size	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	30	
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	No		0.4-0.6	
	Absorption observed [yes/no]	No		No	
	Extent of absorption [%]	No			

Conditions of experiment – general

	1 - KBR	**2 - $NaNO_3$**	**5**	**7- $NaNO_3$**
Temperature of incubation [°C]	20 ±2	21 - 22		
Number of replicates	1	3	1	
Duration of the experiment [min]	5760 min/96 h	28,800 / 480 h		
Soil type	01A	01-A-05	01A	

Conditions of experiment - soil

		1 - KBR	**2 - $NaNO_3$**	**5**	**7- $NaNO_3$**
Amount of soil per column [g]	Replicate 1 dry: wet:	295.0 369.5	dry: 472 wet: 583	Dry: 243.8	
	Replicate 2 dry: wet:	---	dry: 472 wet: 580		
	Replicate 3 dry: wet:	--	dry: 472 wet: 583		
Pore water pH	glass electrode measurement of column leachate after equilibration	7.7	8.20±0.05		
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]	316	585±5		

Preparation of stock dispersion

		1 - KBR	**2 - $NaNO_3$**	5	7**- $NaNO_3$**
Dissolution of tracer	Dispersion medium	Deionised water	water	Water	
	Time between preparation of stock suspension and column experiment start	15 min		immediately	

Preparation of application dispersion

		1 - KBR	**2 - $NaNO_3$**	**5**	**7- $NaNO_3$**
Concentration of tracer in application dispersion [g/L]		1.2		2475 µS	
Medium used for dilution and for leaching	Chemical composition		5 mM $NaNO_3$	0.005 MKCl	
	pH	5.5	5.5		
	Conductivity	1316 µS/cm	570 µS/cm		
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	magnetic stirring			
	Stirring speed	40 rpm			
pH of application dispersion		5.5			

Information on application and leaching

	1 - KBR	**2 - $NaNO_3$**	**5**	**7- $NaNO_3$**
Pore volume [cm^3]	74.5	100	66.2	
Amount of pore volumes used for application of NM	4	3.8	2	
Amount of pore volumes without tracer used for leaching (background leaching solution)	3	7.3		
Flow rate [$mL/cm^2 * h$]	0.3	0.3	3.8 ml/m^2h	
Direction of equilibrium (upflow / downflow?)	upflow	Upflow	Downflow	
Volume of sub-samples (leaching solution)	40.5 ±1.2mL	47±14		

Results

		1 - KBR	2 - $NaNO_3$		5	7
Total amount of tracer added to soil [mg]	Replicate 1	258.6 Br^-				
	Replicate 2	---				
	Replicate 3	--				
Total amount of tracer in soil [mg]	Replicate 1	37.7 Br^-				
	Replicate 2	---				
	Replicate 3	--				
Total amount of tracer in leachate [mg]	Replicate 1	184.9 Br^-				
	Replicate 2	---				
	Replicate 3	--				
Total amount of tracer (soil + leachate) [mg]	Replicate 1	222.5 Br^-				
	Replicate 2	---				
	Replicate 3	---				
Recovery [%]	Replicate 1	86				
	Replicate 2	---				
	Replicate 3	---				
Leaching distance of nanomaterial [cm]	Replicate 1	---				
	Replicate 2	---				
	Replicate 3	---				
Calculation of porosity (ε) and dispersivity (D) using supporting excel file	Replicate 1		ε (-)	0.409		
			D (m^2/s)	1.4e-8		
	Replicate 2		ε (-)	0.408		
			D (m^2/s)	2.72e-8		

	Replicate 3		ε (-)	0.427		
			D (m^2/s)	3.02e-8		
Calculation of α	Replicate 1		η_0	2.3		
			α	0.0014		
	Replicate 2		η_0	2.3		
			α	0.0015		
	Replicate 3		η_0	2.3		
			α	0.0016		
Remarks					Measurement of conductivity; no results presented	Please refer to the former section for detailed data of the tracer breakthrough. After the percolation of AgNM-300K, 5 mmol/l NaNO3 was percolated through the columns as a tracer. Therefore, the pore volumes were calculated by the tracer induced changes of the electrical conductivity in the columns 3 - 6 • Volume of water added to the column: pore volume calculated from tracer breakthrough (column 3 = 137.10 cm^3, column 4 = 135.64 cm^3, column 5 = 130.37 cm^3, column6 = 146.20 cm^3) • Dead time tracer: death volume of the tubing • D50: 160 µm (Makselon *et al*., 2017) • Hamaker constant: $2.37*10^{-21}$ J (Makselon *et al*., 2017)

Tracer KBr / $NaNO_3$– 02-G

Column

		1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Leaching columns	Material	Glass	Glass	
	Length [cm]	25	15	
	Diameter [cm]	4	4.25	
Mesh immediately below the soil	Material	Paper and glass wool	Polypropylene (PP)	
	Pore size	Additional information available	105 µm	
Material below the soil	Material	Quartz sand	Quartz sand	
	Amount	2 cm	≈13.5 gr	
	Grain size [mm]	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	
Material above the soil	Material	Quartz sand	Quartz sand	
	Amount	2 cm	≈13.5 gr	
	Grain size	0.125 (63%)	0.3-0.8 (d_{50}=0.58)	
Tubing	Recovery / adsorption of nanomaterial to tubing tested [yes/no]	No	No	
	Absorption observed [yes/no]	No	No	
	Extent of absorption [%]	No	No	

Conditions of experiment – general

	1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Temperature of incubation [°C]	20 ±2	21 - 22	
Number of replicates	1	3	
Duration of the experiment [min]	6960 min/116 h	28,800 / 480 h	
Soil type	02A	02A	

Conditions of experiment - soil

			1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Amount of soil per column [g]	Replicate 1	dry: wet:	275.3 378.6	dry: 282 wet: 379	
	Replicate 2	dry: wet:	---	dry: 282 wet: 379	
	Replicate 3	dry: wet:	--	dry: 282 wet: 379	
Pore water pH	glass electrode measurement of column leachate after equilibration		7.3	7.7±0.5	
Pore water conductivity	conductivity measurement in column leachate after equilibration [µS/cm]		385	590±5	

Preparation of stock dispersion

		1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Dissolution of tracer	Dispersion medium	Deionised water	Deionized water	
	Time between preparation of stock suspension and column experiment start	15 min	1 h	

Preparation of application dispersion

		1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Concentration of tracer in application dispersion [g/L]		1.2		
Medium used for dilution and for leaching	Chemical composition		5 mM $NaNO_3$	
	pH	5.5	5.5	
	Conductivity	1316 µS/cm	570 µS/cm	
Stirring of application dispersion during application (if relevant)	Stirring method (magnetic stirring,…)	magnetic stirring		
	Stirring speed	40 rpm		
pH of application dispersion		5.5		

Information on application and leaching

	1 - KBR	2 - $NaNO_3$	7 - $NaNO_3$
Pore volume [cm^3]	103.3	85.3	
Amount of pore volumes used for application of NM	4	4.4	
Amount of pore volumes without tracer used for leaching (background leaching solution)	3	8.4	
Flow rate [$mL/cm^2 * h$]	0.3	0.265	
Direction of equilibrium (upflow / downflow?)	Upflow	Upflow	
Volume of sub-samples (leaching solution)	46.8 ±0.8mL	46.3±3.3	

Results

		1 - KBR	2 - $NaNO_3$		7 - $NaNO_3$
Total amount of tracer added to soil [mg]	Replicate 1	247.3 Br^-			
	Replicate 2	---			
	Replicate 3	--			
Total amount of tracer in soil [mg]	Replicate 1	45.6 Br^-			
	Replicate 2	---			
	Replicate 3	--			
Total amount of tracer in leachate [mg]	Replicate 1	220.3 Br^-			
	Replicate 2	---			
	Replicate 3	--			
Total amount of tracer (soil + leachate) [mg]	Replicate 1	265.6 Br^-			
	Replicate 2	---			
	Replicate 3	---			
Recovery [%]	Replicate 1	107.4			
	Replicate 2	---			
	Replicate 3	---			
Leaching distance of nanomaterial [cm]	Replicate 1	---			
	Replicate 2	---			
	Replicate 3	---			
Calculation of α	Replicate 1		η_0	2.3	
			α	0.0014	
	Replicate 2		η_0	2.3	
			α	0.0015	
	Replicate 3		η_0	2.3	

			α	0.0016	
Remarks					Please refer to the former section for detailed data of the tracer breakthrough. After the percolation of AgNM-300K, 5 mmol/l $NaNO_3$ was percolated through the columns as a tracer. Therefore, the pore volumes were calculated by the tracer induced changes of the electrical conductivity in the columns 11 -14.