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Advanced Materials: Methodological adaptation needs for safety assessment

NanoCarriers Workshop Report

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The Environment, Health and Safety Division publishes free-of-charge documents in twelve different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; Safety of Manufactured Nanomaterials;** and **Adverse Outcome Pathways.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (<https://www.oecd.org/en/topics/chemical-safety-and-biosafety.html>).

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

The Organisation for Economic Co-operation and Development (OECD), through its Working Party on Manufactured Nanomaterials (WPMN), has played a pivotal role in advancing international cooperation on the safety of manufactured nanomaterials and advanced materials. Since its establishment, WPMN has provided a global platform for harmonizing approaches to the testing, assessment, and regulatory preparedness of nanomaterials. Building on this foundation, the WPMN has expanded its scope to address advanced materials (AdMa) (OECD, 2023a).

The OECD's work on advanced materials reflects a proactive response to evolving scientific and regulatory challenges. To support this, WPMN has initiated case studies as practical tools for exploring regulatory implications and identifying knowledge gaps. Recent examples include NanoCarriers, 2D Titanium Carbide Mxenes, and Graphene Related Materials. These efforts underscore the importance of method harmonization, robust characterisation, and the development of guidance documents tailored to advanced materials.

Through collaborative workshops, stakeholder engagement, and the publication of technical reports, WPMN fosters a Safer and Sustainable Innovation Approach (SSIA), aiming to integrate safety and sustainability to foster safety and innovation. This foreword acknowledges the contributions of member countries, experts, and partners who have supported these initiatives, ensuring that regulatory frameworks remain fit-for-purpose in the face of rapid technological progress.

As advanced materials continue to shape the future of science and industry, the OECD WPMN remains committed to promoting international convergence, reducing duplication of efforts, and enabling responsible innovation that protects human health and the environment.

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Executive summary

The OECD Working Party on Manufactured Nanomaterials (WPMN) organised a second workshop on NanoCarriers¹, which was held on 5–6 November in Vienna with the possibility to follow online. This workshop focused on methodological challenges and opportunities identified in testing the potential hazard and risks of NanoCarriers. Accordingly, the workshop addressed the needs and possibilities for NanoCarriers characterisation and analysis as well as specific adaptation and development needs for appropriate testing for hazards and risks of NanoCarriers.

NanoCarriers, which enable encapsulation or attachment of active ingredients to inorganic, organic, hybrid materials, or supraparticles, offer significant potential across diverse application areas, such as cosmetic, pharmaceutical and agriculture as they allow targeted transport, protection and release of the active ingredient. However, their unique properties raise important questions regarding safety, sustainability, and regulatory preparedness.

Participants contributed insights on scientific advancements, regarding method development in the areas of analytics, physical chemical characterisation and environmental and human health safety assessment. In addition, recent developments in utilizing NanoCarriers in different application areas were presented.

Discussions emphasised the need for robust risk assessment methodologies for NanoCarriers and NanoCarrier systems (including the active ingredient). Such methodologies need to reflect changes in behaviour, fate, kinetics and effects of the active ingredient due to the encapsulation in or attachment to the carrier. Focus of the discussion was on methodological approaches for investigating relevant environmental fate descriptors, such as mobility, release, and degradation. However, such information is also highly relevant for assessing human health implications of NanoCarrier systems (e.g. a shift in toxicokinetics of the active ingredient) with regards to areas such as pharmaceuticals, cosmetics, and food supplements, and considering conditions that mimic those body fluids, such as blood, sweat or gastrointestinal fluids. Reliable safety testing of NanoCarriers and NanoCarrier systems was highlighted as a prerequisite for effective environmental and human health protection.

Session I: Introduction and Background

Bernd Giese welcomed the participants on behalf of the project team and BOKU University. He pointed out that this meeting on NanoCarriers is an expert workshop of the OECD Working Party on Manufactured Nanomaterials (WPMN). The workshop was made possible by project funding from the German Federal Ministry for the Environment, Climate Action, Natural Conservation and Nuclear Safety (BMUKN), which is dedicated to testing strategies necessary for assessing the fate and effects of NanoCarriers in the environment. Bernd Giese emphasised that the topic of this workshop is of great urgency in view of a global increase in chemical consumption, which has long been decoupled from global population growth, and in view of the increasing diversity of chemicals, which is also reflected in the growing activity in the field of NanoCarriers.

OECD WPMN activities on advanced materials

Mar Gonzalez, OECD, France

The OECD Secretariat presented an overview of OECD Work on Nanomaterials and Advanced Materials. Since 2004, the OECD has played a leading role in developing internationally harmonized approaches for the safety assessment of nanomaterials. This work has included the creation of testing guidelines, guidance documents, and collaborative projects to address the unique properties and potential risks of engineered nanomaterials. Building on this foundation, the OECD expanded its scope in 2021 to include Advanced Materials and developed a *working description on Advanced Materials*² to align the understanding and scope of its work. The WPMN also recognizes their relevance in innovation and sustainability, which present both opportunities and challenges for regulatory systems.

The rapid pace of development and complexity of these materials highlight the need for a strategic approach to their governance. Such an approach should align with OECD approaches for chemical safety and best international practices.

Consequently, the OECD Working Party on Manufactured Nanomaterials (WPMN) initiated a series of case studies on advanced materials (such as NanoCarriers³, 2D Titanium Carbide MXenes⁴, Graphene Related Materials⁵) to better understand their characteristics, applications, and potential implications for human health and the environment. These case studies aim to identify critical information needs, data gaps, and methodological challenges that regulators may face when assessing such materials. By examining real-world examples, WPMN seeks to anticipate emerging issues and develop practical approaches that support regulatory preparedness. This work is essential to ensure that innovation in advanced materials aligns with OECD principles for chemical safety and international best practices, enabling harmonized and proactive governance across jurisdictions.

Background and aim of the workshop

Doris Völker & Kathrin Schwirn, UBA, Germany

Doris Völker representing UBA as co-organising institution as well as member of the steering group on advanced materials of the OECD WPMN, introduced to the background and objectives of the 2nd OECD WPMN Workshop on NanoCarriers.

The 1st workshop of OECD WPMN on NanoCarriers⁶ was held as an online meeting in June 2023. NanoCarrier were chosen as a case study as an example for an AdMa with many different types and application areas.

The Early4AdMa tool of OECD⁷ was used to identify knowledge gaps and signals of possible concern regarding safety, sustainability and regulatory coverage of NanoCarrier in combination with respective cargo. Breakout groups were held that allowed a dedicated discussion on three selected NanoCarrier examples from the application areas of cosmetics (skin care), plant protection (fungicide), and pharmaceutical (chemotherapeutic).

Step 5 of the Early4AdMa was used to evaluate the most relevant topics of concern and data gaps, and from that, provided needs for actions for different stakeholders. Lessons learned from the previous OECD workshop on NanoCarriers revealed that while toxicity as well as fate of the active ingredient is usually well known, a lot of knowledge gaps remain with regard to the toxic potential of the respective carrier deployed, and how the carrier influences fate and effect of the active ingredient.

It was also highlighted, that there is the need for test methods that allow a differentiated investigation of the NanoCarrier itself, and how it influences fate and effect of its cargo. This need built the frame for the 2nd OECD WPMN workshop on NanoCarriers. This workshop focused on methodological challenges and opportunities identified in testing the potential hazard and risks of NanoCarriers. That includes the needs and possibilities for NanoCarrier characterisation and analysis as well as specific adaptation and development needs for appropriate testing for hazard and risk of NanoCarriers. The speaker highlighted that one session of the workshop will include a discussion on specific outcomes of a research project executed by BOKU which investigates methodological approaches to investigate the environmental fate of NanoCarriers based on three relevant fate descriptors. The results of the workshop are summarised in this document, and it includes recommendations for further methodological adaption needs to appropriately investigate environmental fate of NanoCarriers and will become part of the final report to the research project⁸.

Introduction on NanoCarriers and project goals

Florian Part, BOKU, Austria

The speaker provided an introduction to NanoCarrier, offering a comprehensive overview of the application areas and proposing a novel categorisation framework⁹. The objectives of the project led by BOKU were also outlined.

The example of precision farming has already demonstrated that NanoCarriers can be used very effectively. Entire NanoCarrier systems are designed to enable the targeted delivery and release of active ingredients such as pesticides or micronutrients, which often have hydrophobic properties^{10,11}. In addition, NanoCarrier systems prevent rinsing off, washing out and possible groundwater contamination by providing targeted delivery via controlled release mechanisms for improved plant health management. Future nano-enabled precision agriculture could benefit from using NanoCarrier systems for targeted delivery of agrochemicals.

Additionally, controlled release mechanisms could improve soil fertility and mineral balance. NanoCarrier systems can be applied to the epigeal part of plants, where the active ingredients are passively absorbed into the plant via stomata or wounds, or transported in the soil and absorbed through the hypogeal part of plant roots. This innovative approach can achieve higher efficiency of NanoCarrier systems, implying a lower total amount of active ingredients used compared to conventional agricultural techniques. As there is currently little information on NanoCarrier systems in the environment and no harmonised methods for assessment are available^{12,13}, our research project aims to investigate appropriate methods for selected types of NanoCarrier systems to test their behaviour and fate in freshwater systems at lab scale. Knowledge and methodological gaps regarding environmental fate must be closed before such innovative applications are used on a large scale on agricultural land. Therefore, our NanoCarrier project has the following objectives:

- Three representative NanoCarrier types are purchased and the material safety data sheets are screened regarding their physicochemical properties.
- Search for existing test methods that are suitable to test the behaviour and fate of NanoCarriers in the aquatic environment.
- Perform and, if necessary, adapt OECD (2017) Test No. 318 Dispersion Stability of Nanomaterials in Simulated Environmental Media¹⁴ and other test guidelines to test their applicability regarding the environmental endpoints of mobility, unintended release and degradability.

Session II: NanoCarriers for agriculture

Calcium phosphate nanoparticles for agriculture: an opportunity for stable, scalable, and environmentally friendly NanoCarriers

Alessandra Azzali, Universidad de Granada, Spain

Nanotechnology has emerged as a potential solution to address the inefficiencies of traditional agrochemicals by enabling the design of smart and tunable nanomaterials with controlled kinetics and target-specific release. Calcium phosphate nanoparticles (CaP), mimicking the bone mineral, exhibit outstanding properties. These include inherent biocompatibility and biodegradability, superior adsorption capacity of organic molecules, high capacity to host a large variety of cationic (e.g., K^+ , Mg^{2+} , Zn^{2+} , etc.) and anionic substitutions (e.g., NO_3^-). In addition, pH-dependent solubility provides a controlled and gradual release of the ionic constituents (Ca, P and dopants) and adsorbed molecules upon partial dissolution in (slightly acidic) aqueous media. These nanoparticles have been extensively investigated in the field of nanomedicine and, more recently, have been proposed for applications in nanoagriculture. They have been considered for use as fertilisers¹⁵, biofortifying agents¹⁶, and herbicides¹⁷. Their synthesis is scalable and can be conducted in a cost-effective manner. Studies assessing their ecotoxicity are currently in progress.

Naturally derived NanoCarriers for sustainable agriculture

Joseph Edward Govan, Universidad de Chile, Chile

Conventional agriculture has become increasingly unsustainable due to greater contamination of the environment linked to the use of agrochemicals and other agricultural practices. More sustainable practices such as organic agriculture can greatly improve the environmental impact of agriculture but there are issues with lower yields which can affect their viability.

Nanotechnology provides a potential solution to this problem. The conversion of naturally derived materials, as used in organic farming, into nanomaterials can greatly improve their efficiency in a range of agricultural applications.

As an example, organic coatings for fruit conservation were presented. Natural products such as e.g. bee propolis can have anti-microbial properties but might at the same time have strong flavors that also change the flavor of the treated fruits. Encapsulating propolis into nanoemulsions (e.g. with natural polymers such as Alginate) can reduce the flavor but keep their preservative effect. Therewith, the shelf life of several fruits can be extended without the use of artificial preservatives nor with the strong flavors associated with the natural agents.

The use of nanotechnologies based on organic farming agents can help create a future form of farming that is both highly productive and truly sustainable.

RNAi-based NanoCarriers for plant defenses and pathogen resistance

Aline Koch, Universität Regensburg, Germany

The presentation addressed RNA and NanoCarriers in plant protection from the perspective of benefits and risks and to close research gaps.

RNA-based active ingredients represent an innovative class of crop protection agents, with the first commercial products already available¹⁸. A key challenge lies in translating laboratory proof-of-concept into effective field applications, given the rapid degradation of RNA by environmental factors and cellular nucleases. Efficient uptake by plants and target organisms is also critical for efficacy. NanoCarriers are being developed to overcome environmental, structural and cellular barriers, thereby enhancing RNA stability and bioavailability. While NanoCarrier-based formulations offer clear advantages, they also raise concerns regarding their potential toxicity and environmental impact, which remain poorly understood. In contrast, unformulated RNA is generally considered to be low risk due to its low stability and subsequent rapid degradation in the environment and within organisms. Therefore, regulatory evaluation of RNA–NanoCarrier formulations is an active area of research to inform future approvals. Notably, NanoCarriers may not always be necessary for effective field application. Two examples of this are chitosan-based microgels for delivering RNA to lepidopteran pests and alginate 'attract-and-kill' capsules for targeting stink bugs. These developments highlight the potential of RNA-based crop protection and the associated regulatory considerations, emphasizing the need for ongoing research into safety and environmental fate.

Session III: Methods related to human health assessment

Standard development and toxicity delivery of NanoCarriers

Youngsam Kim, KIST Europe, Germany / Korea

This presentation introduced recent advancements in the establishment of NanoCarrier standards and toxicity assessment in the Republic of Korea. The main topics included the development of certified reference materials, the generation and characterisation of environmentally derived nanoplastics and nanoliposomes, and the evaluation of their biological effects in cellular, aquatic, and mammalian systems.

In addition, a high-throughput physiological assessment method using *D. magna* was presented, demonstrating its sensitivity in detecting sublethal toxicity of nanomaterials. These methodologies are expected to contribute to the development of internationally applicable nano-standard materials and evaluation techniques.

Absorption, Distribution, Metabolisation & Excretion – a risk-based approach on the impact of pharmaceutical nanomaterials on patient and environment welfare

Sam Moré, DendroPharm GmbH, Germany

Polymer nanomaterials are built from the bottom-up. Thus, their inherent toxicity might be lower than those of nanomaterials which are manufactured by milling or chemical precipitation of typically larger granular substances and who exhibit a larger chemically reactive surface when compared to their non-nano counterparts.

Polymer NanoCarriers for pharmaceutical use can change the biodistribution of drugs and are able to reduce the amount of active pharmaceutical substances in both patient and environment. To access the risks of pharmaceutically used nanomaterials, analyzing and defining the actual distribution of NanoCarrier particles is necessary as the Absorption, Distribution, Metabolism and Excretion in living organisms can be influenced by the size distribution. Methods of DLS are compared to a MALS-SEC analysis. Both literature studies and ADME data from preclinical evaluation can support a risk-based assessment of environmental impact.

NanoCarrier case study in Nams4Nano

Andrea Holzwarth, BfR, Germany

This presentation introduced the planned EFSA NAMS4NANO Project LOT 3 / Case Study 1: NanoCarrier loaded with a pesticidal active substance.

Nanopesticides have already started to appear on the market¹, but still more research is needed to maximise their benefits while minimizing potential adverse impacts. The most advanced technology are NanoCarriers to improve the properties of existing pesticide active ingredients. NanoCarriers enable new pesticide functionalities, such as controlled release, increased stability, enhanced attachment/penetration resulting in a greater efficacy that also allows reducing the doses of the active ingredient and/or the application rates.

Next generation NanoCarriers may also allow for targeted and/ or stimuli-responsive delivery. The focus of this case study will be on the formulation of a known active ingredient into a polymer-based NanoCarrier, which is regarded as the most realistic and advanced scenario. As an active ingredient, azoxystrobin (CAS: 131860-33-8) was selected. The overall objective of this case study is to develop an Integrated Approach to Testing and Assessment (IATA) for NanoCarriers with known active ingredient utilizing to the extent possible NAMs.

Characterisation of NanoCarriers for food applications

Elke Walz, Max Rubner-Institut, Germany

Organic NanoCarriers such as liposomes, phytosomes, cyclodextrin complexes, and polymeric nanoparticles are increasingly studied in food-related applications to encapsulate bioactive compounds. These carriers can enhance stability, control the release of functional ingredients, and improve bioavailability. Although these carrier systems are well established in medical formulations and are increasingly applied in dietary supplements, their use in food products remains limited to date. Reliable analytical characterisation of such systems in complex food matrices, however, remains highly challenging. Matrix components—ranging from simple electrolytes to proteins, lipids, and polysaccharides—may interact with the carrier surface, affect dispersion behaviour, and interfere with measurement signals, thus complicating data interpretation.

This contribution outlines central analytical challenges in the characterisation of organic NanoCarriers, using curcumin-loaded systems as model systems. After introducing the main classes of carrier materials (lipid-based, polymer-based, inorganic, and hybrid systems) and their representative examples, the focus is placed on the applicability and limitations of current nanoparticle characterisation methods. Dynamic light scattering (DLS) is discussed regarding its sensitivity to dispersion conditions and sample polydispersity, while cryo-scanning electron microscopy (cryo-SEM) provides complementary insights into structural diversity but limited information on different size fractions. Thermal analysis by differential scanning calorimetry (DSC) is applied to differentiate between physical mixtures and true molecular complexes.

The results demonstrate that careful sample preparation—including optimised dispersion, deagglomeration, and stabilisation—is critical to obtain representative data without altering carrier structures. Finally, the need for harmonized analytical strategies and potential adaptation of existing OECD

¹ For an example, see e.g. <https://www.vivecrop.com/technology>

guidelines is highlighted to ensure the reliable assessment of organic carrier systems in food-related matrices.

Session IV: Methods related to environmental fate investigations

Some reflections on the environmental fate and effect profiles of NanoCarriers and nano-enabled pesticides

Willie Peijnenburg, RIVM & Leiden University, Netherlands

Recent insights into the environmental behaviour and biological effects of NanoCarriers used in nano-enabled pesticide formulations are discussed. Drawing on multiple studies, the complexity of evaluating these materials and the need for case-by-case assessments is emphasised.

Context and Rationale

NanoCarriers (e.g., sepiolite, bentonite, TiO₂ nanoparticles) are increasingly used to improve pesticide delivery, targeting, and stability. However, their impacts on the environmental persistence, bioavailability, and toxicity of the chemicals (pesticides) they carry, are not yet fully understood. Therefore, there is a growing research interest, as reflected in the increasing number of publications on nano-enabled formulations.

Environmental Fate Considerations

Once released into aquatic environments, both spatial and temporal fate must be considered:

- Carrier persistence and toxicity may influence exposure duration.
- Carriers can modulate the availability and degradation of the active substance.
- Effects on bioavailability are particularly relevant for assessing environmental risk.

Case Study 1 – Eugenol-Loaded Sepiolite exposure in outdoor mesocosms

Mesocosm experiments showed that eugenol at initial concentrations of 20–200 µg L⁻¹ significantly affected aquatic invertebrates. Freely dissolved eugenol concentrations declined below detection within 48 h, yet persistent reductions in taxa abundance were observed. The NanoCarrier formulation (sepiolite-based) exacerbated and prolonged ecological impacts compared to non-nano eugenol.

Case Study 2 – TiO₂-Based Carbendazim Formulations in outdoor mesocosms

Comparisons between carbendazim alone and nTiO₂-coated carbendazim revealed that the carrier itself did not significantly contribute to most of the additional effects, emphasizing the context-specific nature of nano-formulation impacts. The responses of zooplankton and macroinvertebrates varied across taxa and time.

The main conclusions from this presentation included that:

- The behaviour and impacts of nano-enabled formulations cannot be generalised.
- Some carriers can enhance toxicity, while others may have negligible effects.
- Taxon-specific and species-specific sensitivities are critical for risk assessment.
- Evaluation of nano-formulations should include carrier toxicity and environmental persistence.

Consequently, continued experimental and ecological studies are needed to support regulatory decisions.

Development of OECD test guidelines to assess the dispersion stability and solubility of nanomaterials in simulated environmental media²

Frank van der Kammer, Universität Wien

Nanomaterial dissolution and agglomeration are two of the four major transformation processes nanomaterials undergo in the environment. For environmental risk assessment, it is of utmost importance to quantify these processes in environmental media. The OECD started early to address problems which occur when nanomaterials' risks are assessed with methods developed for conventional chemicals. Activities were directed at the development of test guidelines and guidance documents for the (homo-) agglomeration and dissolution of nanomaterials. Currently, also test protocol for nanomaterial hetero-agglomeration and (abiotic) transformation is developed.

The presentation looked into the typical processes which a test guideline undergoes during the development phase, the (informed) simplifications that were needed to transfer complex environmental reactions into a test system that can be implemented and validated. The presentation also provided an overview of the current activities related to test guideline development and validation.

² The work presented by Frank von der Kammer, was co-authored with Boris Meisterjahn, Andreas Gondikas, Philipp Kozin, Junfeng Liu, Antonia, Praetorius, Fazel A. Monikh, Melanie Vital, Boyoung Song and Lucie Stetten

Session VI: Methods related to environmental investigations

Analytics, fate, ecotoxicity and risk assessment of NanoCarriers and the challenges associated with both environment and human health¹⁹

Melanie Kah, University of Auckland, New Zealand

The presentation addressed challenges associated with the exposure assessments of NanoCarriers.

The application of nanotechnology in agriculture results in the release of engineered nanoparticles made of a wide variety of chemicals, with different shapes and sizes. Of particular interest are organic NanoCarrier systems that can be loaded with one or several pesticides or fertilisers. NanoCarrier systems can improve the performance of agrochemicals by increasing their bio-uptake by the target pest or reducing unwanted losses by leaching or volatilisation. Well-designed NanoCarrier systems can contribute to reducing the amount of agrochemicals required to fertilise and protect crops. While this sounds very promising, the use of NanoCarriers can also have unintended consequences, and a careful risk assessment is required before their placement on the market. NanoCarriers impact the fate of their cargo and may increase the uptake by non-target organisms or increase their persistence.

The NanoCarrier itself may be of concern if it persists for an extended period of time and accumulates in soil or food products. The fate and effects of NanoCarriers remain largely unknown, not only due to the absence of current regulatory requirements but also because of analytical challenges.

This presentation is based on the experience gained from a portfolio of NanoCarriers made from lignin, zein, polyacrylate, and poly-ε-caprolactone. During the talk the applicability of methods that were designed to track inorganic nanoparticles were discussed. Furthermore, alternative analytical approaches to detect quantify and characterize NanoCarriers when studying their fate on plants, soil and water were evaluated. Finally, challenges associated with NanoCarrier labelling and the use of model NanoCarriers were presented.

Results from the NanoCarrier project

Florian Sitzwohl & Christian Zafiu, BOKU, Austria

Results on mobility

The presentation reports the results on adaptations of a mobility test based on the OECD (2017) Test No. 318 “Dispersion Stability of Nanomaterials” for three different NanoCarriers. One commercial (Cavacurmin®) and two mature experimental NanoCarriers (Plentrance™ and Dendrosol™) made from organic molecules with traceable carriers were investigated. Adaptions and considerations towards the

usage of test tubes and UV-VIS as analytical methods were reported. UV-VIS represents only the alternative method of the OECD test guideline, while ICP-MS is recommended, because the guideline was designed for inorganic nanomaterials. However, the majority of NanoCarriers are made from organic compounds, which cannot be detected by ICP-MS without further introduction of traceable atoms (e.g. isotopes). Mobilities could be determined based on the characteristic absorption band of the cargo over a period of 6 h. Similar as in OECD (2017) Test No. 318, eighteen different aqueous media were tested (varying CaNO_3 , pH and addition of Natural Organic Matter (NOM)) that serve as model matrices for about 90% of surface water conditions. The results showed that Cavacumin® exhibited medium mobility, while the other tested NanoCarriers were highly mobile, when the calculation methods of the OECD guideline were applied in the same way as for nanomaterials. However, the determination of the mobility by UV-VIS was restricted to the mobility of the traceable cargo, or NanoCarrier-cargo complex, as only the cargo was detectable by the method.

Results on release of active ingredients

Due to the lack of existing appropriate methodology to investigate the release behaviour of the active ingredient from a carrier, a method was applied following the principles of OECD (2017) Test No. 318 and a draft OECD test guideline to determine the dissolution behavior of nanomaterials. Similar to the mobility test, the same aqueous buffer systems were used, whereas the release was also tested at three different temperatures (4°C, 25°C, 30°C), to cover a broad range of surface water conditions, however excluding the addition of NOM. Different to the mobility test, the samples were agitated, to allow a homogeneous distribution of the NanoCarriers in the matrix and eventually trigger faster release, reducing the need for extended incubation times. The differentiation between released and bound cargo was made by centrifugation, as other methods like filtration or dialysis risk either clogging or material loss by adsorption, take too much time or cannot be performed at larger scale to separate the high quantities of samples. In these experiments, only the supernatant was analyzed by UV-VIS after incubations (0, 1, 2, 3, 4, 5, and 6 h). As for the mobility endpoint, release was measured via detection of the cargo. The behaviour of the NanoCarriers was very different. Cavacumin showed a rising absorbance with longer incubation times, indicating release of the cargo and separation of the NanoCarrier, while the protein based NanoCarrier Plentrance™ decreased in absorption, which is interpreted as agglomeration of the NanoCarrier caused by the agitation. The third NanoCarrier Dendrosol™ showed only small releases. The tests showed that all investigated parameters modified the release behaviour of individual NanoCarrier systems.

Results on biodegradation of NanoCarriers

Biodegradation tests were made according to the established OECD (1992) Test No. 301 Ready biodegradability²⁰ that describes six different experimental procedures. The test was made for Cavacurmin® according to the variant F, the respirometry test determining the consumption of O_2 over a period of 28 days in activated sewage sludge. This type of test requires information on the composition and quantity of the NanoCarrier and the cargo, which is necessary to calculate the theoretical O_2 demand for mineralisation. The experimental setup requires at least 3 different samples, the NanoCarrier-cargo complex, the empty NanoCarrier and the cargo alone. The results on the biodegradation of Cavacurmin® showed that biodegradation was >99% after 28 days confirming biodegradability, while the cargo (curcumin) achieved only ca. 55% biodegradability, which may be associated with the low solubility of the cargo alone. Surprisingly, the empty NanoCarrier material cyclodextrin exhibited a lower degradation degree of 78%, which shows that biodegradation cannot be predicted by determining only the cargo and NanoCarrier alone but must be made also for the NanoCarrier systems, as synergistic effects can modulate biodegradation behaviour.

Session VII: Breakout groups and discussions on the needs for methodological adaptations

This session discussed the experimental procedures and findings for environmental fate descriptors for NanoCarriers or NanoCarrier systems (i.e., including the active ingredient) based on the project findings presented by BOKU under “*Introduction on NanoCarriers and project goals*”. This active session covered the discussion on adaptation needs of the methodological approach to enable appropriate environmental fate testing of NanoCarriers.

Breakout group discussions were conducted in the format of a World Café on-site, with three thematic tables attended by three different groups. Each discussion round was scheduled for 20 minutes, with a set of guiding questions for each fate descriptor (endpoint). Three discussion rounds were scheduled and participants changed tables for each round. The following thematic tables were made available to stakeholders for discussion:

- Table 1: Endpoint mobility (on-site and online)
- Table 2: Endpoint release (on-site and online)
- Table 3: Endpoint degradation (on-site and online)

For online participants, a virtual whiteboard was provided. The results of the World Café discussions and virtual whiteboard notices are presented below.

Fate descriptor Mobility

A. Do the proposed adaptations to OECD (2017) Test No. 318 provide a sufficient basis for assessing the mobility of NanoCarrier systems in surface waters?

The groups attending discussions which focused on testing the mobility of NanoCarriers under simulated environmental conditions, generally considered that the OECD (2017) Test No. 318 provides a sufficient basis to investigate the mobility of a NanoCarrier and a whole NanoCarrier system. Still, mobility should be considered together with information about release of cargo to avoid misinterpretation of the measured data. The complexity of the NanoCarrier increases the analytic effort for testing with this test guideline. Challenges can also arise when investigating hydrophobic active ingredient and should therefore be further investigated. And whether dispersion stability as determined with OECD TG 318 sufficiently reflects all aspects of mobility remains an open question. Solubility and solvation interactions play an important role in dispersion stability.

B. What other adaptations and analytical techniques would you suggest?

Much can be learned from the analytical characterisation of nanomaterials or micro- and nanoplastics for necessary adaptations. It is proposed to extend the measurements over longer periods and to pursue a multi-stage approach, starting with the most unfavourable and realistic case. To avoid misinterpretation, further investigations into the partitioning behaviour of NanoCarrier system (NanoCarrier together with the active ingredient) are necessary. Signal interference can influence the results. In addition, the influence of solvation on the measurements must be considered. For simultaneously measuring of release during mobility testing, HPLC and filtration can be used. Multiangle light scattering (MALS) can be used as a complementary method to quantify particle number concentration. However, analytic methods depend on the specificity of NanoCarrier and therefore, it is a case-by-case decision.

C. What other environmentally relevant conditions should be investigated?

Adaptations could be made with regard to testing mobility with different environmental conditions. Different temperatures, UV radiation from sunlight, different aquatic media (plain rainwater, marine waters, groundwater) and the presence of agrochemicals such as fertilizers were suggested for further conditions, which should be considered when developing test protocols. Furthermore, the transport and loss of NanoCarriers in porous media (e.g. soil or sediments) are also important areas for investigation due to potential agricultural applications. Here the leaching potential of test substance and transformation products should be investigated. Further research is required into the partition behaviour of the NanoCarrier system, as well as mobility assessment in marine and groundwater systems. A few hours of testing may be insufficient to investigate the long-term mobility of the NanoCarrier, so the timeframe of the investigation should be extended.

D. What should be the main areas of research and standardisation in the future?

It was recommended to investigate into the application range of the method to find out if the method can be applied properly to other NanoCarrier system. Furthermore, the possibilities for simplification must be investigated.

Apart from its application in agriculture, the three fate descriptors – mobility, release of the active substance and biodegradability – are also highly relevant for assessing human health exposure with regards to areas such as pharmaceuticals, cosmetics, and food supplements, it is essential to consider conditions that mimic those of the gastrointestinal tract, lung lining or sweat.

Fate descriptor Release

A. Do the proposed adaptations to OECD (2017) Test No. 318 provide a sufficient basis for assessing the release from a NanoCarrier system in surface waters?

This descriptor is particularly relevant for the assessment of the environmental fate of the active ingredient, as it determines the release of the active ingredient from the NanoCarrier under simulated environmental conditions. It could provide information to predict release dynamic in terms of the time, and space.

The group discussing the testing of the release of cargo from the NanoCarrier under simulated environmental conditions considered OECD TG 318 appropriate for the development of suitable tests for the release of active ingredient. The results can help to identify new hotspots relevant to the environmental exposure of active ingredient, which could be useful information for environmental risk assessment.

B. What other adaptations and analytical techniques would you suggest?

The current methodology is based on centrifugation as separation technique, followed by UV/VIS measurement of the resulting supernatant to determine the amount of released cargo. Centrifugation is critical, e.g., for phytosomes or solubilizers and small NanoCarrier might not be separated by centrifugation. Alternative techniques for separating released cargo from the carrier that were discussed include filtration. However, it was highlighted that this could result in clogged filters and burst carriers, leading to high losses or bad recovery. Ultracentrifugation (UC) could be an option, but it is expensive and may not be widely available in laboratories. In addition, UC could result in particle fusion. Further techniques for separating NanoCarriers from released active ingredient include Field flow fractionation (FFF), size exclusion chromatography (SEC), dialysis, two phase density-gradient centrifugation using colloidal silica, and immobilisation of the carrier followed by a washing step. However, a more in-depth analysis is needed to identify the potential pitfalls of these techniques for separating the released cargo fraction.

UV/VIS detection, as specified in the adapted test strategy, assumes that all NanoCarriers are present in the pellet while only released cargo is measured in the supernatant. It was highlighted that an orthogonal method is needed to verify this assumption. For example, DLS could be used to investigate whether the supernatant is free of carrier, or the released cargo could be measured directly, for example, by using a fluorescence quencher as a surrogate for the actual cargo. However, it needs to be clarified which characteristics such a surrogate would need to mimic the actual cargo appropriately.

C. What other environmentally relevant conditions should be investigated?

The participants agreed that parameters like pH, ionic strength and temperature are those that will highly determine release of cargo from the carrier under environmental conditions. These are the parameters that are currently covered by the proposed protocol based on OECD (2017) Test No. 318. A further relevant parameter might be UV light. Regarding the investigation of mobility, the influence of solvation processes should also be examined in release measurements. The results of the release should be communicated to experts in (eco-)toxicity testing (e.g., focus on chronic effects if the release is slow).

D. Which conditions are most important for evaluating a burst release?

A burst release of cargo from the carrier was considered unlikely. Usually, release will happen slowly and will change with changing environmental parameters as those mentioned above. However, the rate of release will always depend on the composition of the NanoCarrier system and how carrier and cargo are associated to each other. Also burst release depends on the composition of the individual NanoCarrier system. Relevant environmental conditions may be pH, temperature, the presence of ions, UV radiation by sunlight and how fast these conditions change. A burst is considered more likely with fast changes of environmental conditions. Dialysis is recommended as a gentler separation method, particularly when burst release is expected. Burst release can be caused by mechanical stress (e.g., during shaking or centrifugation). In addition, burst release may be triggered by residual organic solvents or enzymes. This raises the question of what methods could be used to investigate this in detail.

E. What should be the main areas of research and standardisation in the future?

The release of active ingredient into matrices such as soils and sediments, as well as the chosen timeframe, are important research areas and factors for future studies. Further research is needed to better understand the influence of sample preparation and separation techniques.

Fate descriptor Degradation

A. Is OECD Test No. 301 F sufficient for evaluating the ready biodegradability of NanoCarrier systems, and thus their degradation and persistence?

Discussions highlighted that we could learn from advances in the analysis of polymers and microplastics in terms of degradation testing. Experience gained from simulation tests should also be utilized. Nanomaterial- or NanoCarrier-specific guidance should be developed to determine which method (A-E) of OECD 301 test series is most relevant.

B. What other adaptations and analytical techniques would you suggest?

The entire NanoCarrier system must be tested. It is important to determine degradation of which component (i.e. NanoCarrier, active ingredient or NanoCarrier systems) is being measured and to what extent. OECD Test No. 301 F is generally suitable, but requires some adjustments, e.g., a spiking method.

C. Do we need a new, nano-specific test guideline?

OECD Test No. 301 F requires a large quantity of testing material (in the double-digit gram range). During the early development and 'pre-marketing' phase, only very small quantities of the test substances are often available. Consequently, so-called 'micro-testing' approaches for very small quantities of NanoCarrier/NanoCarrier systems must be developed. A test guideline specific for nanomaterials to cover biodegradation is not needed, as available TGs cover this endpoint well. However, a nano-specific annex to the guidance document would be helpful.

D. Which matrices are most relevant for investigating the degradability/persistence of NanoCarrier systems in the environment?

Participants stated that that water, soil and sediment are all relevant considering the fate of pollutants in general.

E. What should be the main areas of research and standardisation in the future?

Since the persistence of degradation products can contribute to the hazard potential of NanoCarrier system applications, the degradation products should be specified. The influence of UV radiation and temperature on degradation under environmental conditions should be investigated in more detail, as they could provide valuable information for environmental risk assessment. For all three fate descriptors, the need for valid grouping approaches for NanoCarriers was discussed to help avoiding testing every NanoCarrier type individually. However, alongside the general challenges associated with grouping complex chemicals, the question arose as to whether grouping should be established for NanoCarrier themselves, or for the whole carrier system including the corresponding active ingredient. The need for a reference NanoCarrier was also highlighted, which would greatly improve the applicability of the proposed test methods.

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Annex A. Workshop Programme

Needs for methodological adaptation for safety assessment of NanoCarriers

Second Expert Workshop of the OECD Working Party on Manufactured Nanomaterials (WPMN) on NanoCarriers, November 5-6, 2025, in Vienna / Austria or Online

First day		
November 5, 2025, from 12:00–18:30		
11:30 – 12:00	Arrival (open doors) with coffee	
Session I: Overview and background (15min talks)		
12:00 – 12:10	Opening	Bernd Giese, BOKU, Austria
	Welcome address by the Vice Rector for Research and Innovation	Christian Obinger, BOKU, Austria
12:10 – 12:25	OECD WPMN activities on advanced materials	Mar Gonzalez, OECD, France
12:25 – 12:40	Background and aim of the workshop	Doris Völker & Kathrin Schwirn, UBA, Germany
12:40 – 12:55	Introduction on NanoCarriers and project goals	Florian Part, BOKU, Austria
12:55 – 13:05	10min break	
Session II: NanoCarriers for agriculture (20min talks + 5min of discussion)		
13:05 – 13:30	Calcium phosphate nanoparticles for agriculture: an opportunity for stable, scalable, and environmentally friendly NanoCarriers	Alessandra Azzali, Universidad de Granada, Spain
13:30 – 13:55	Naturally derived NanoCarriers for sustainable agriculture	Joseph Edward Govan, Universidad de Chile, Chile
13:55 – 14:20	RNAi-based NanoCarriers for plant defenses and pathogen resistance	Aline Koch, Universität Regensburg, Germany
14:20 – 14:45	Plentrance: Improving uptake and reducing use of agrochemicals in foliar crop sprays	Alberto Natali, Hudson River Biotechnology, Netherlands

14:45 – 15:10	25min coffee break	
Session III: Methods related to human health assessment (20min talks + 5min of discussion)		
15:10 – 15:35	Standard development and toxicity delivery of NanoCarrier	Youngsam Kim, KIST Europe, Germany / Korea
15:35 – 16:00	Absorption, Distribution, Metabolization & Excretion – a risk-based approach on the impact of pharmaceutical nanomaterials on patient and environment welfare	Sam Moré, DendroPharm GmbH, Germany
16:00 – 16:25	NanoCarrier case study in Nams4Nano	Andrea Holzwarth, BfR, Germany
16:25 – 16:50	Characterization of NanoCarriers for food applications	Elke Walz, Max Rubner-Institut, Germany
16:50 – 17:00	10min break	
Session IV: Methods related to environmental fate investigations (20min talks + 5min of discussion)		
17:00 – 17:25	Some reflections on the environmental fate and effect profiles of NanoCarriers and nano-enabled pesticides	Willie Peijnenburg, RIVM & Leiden University, Netherlands
17:25 – 17:50	Development of OECD test guidelines to assess the dispersion stability and solubility of nanomaterials in simulated environmental media	Frank van der Kammer, Universität Wien
Session V: Plenary discussion		
17:50 – 18:30	Plenary discussion about the challenges that will arise on the topics covered on Day 1	Moderation by Bernd Giese, BOKU, Austria
18:30	Drinks and snacks	

Second day of the expert workshop on NanoCarriers		
November 6, 2025, from 9:00–13:00		
Session VI: Methods related to environmental investigations (15-20min talks + 5min of discussion)		
9:00 – 9:05	Welcome back	
9:05 – 9:30	Challenges associated with the exposure assessment of NanoCarriers	Melanie Kah, University of Auckland, New Zealand Florian Sitzwohl & Christian Zafiu, BOKU, Austria
9:30 – 9:45	Results from the NanoCarrier project on mobility	
9:45 – 10:00	Results on release of active ingredients	
10:00 – 10:15	Results on biodegradation of NanoCarriers	
10:15 – 10:30	Open questions about our NanoCarrier project	
10:30 – 10:50	20min coffee break	
Session VII: Breakout groups and discussions on the needs for methodological adaptations		
10:50 – 11:00	Explanation of World Café	
11:00 – 12:10	World café & breakout groups for online participants: lessons learned on adaptation needs and feasibility, recommendations for next steps. Expert input is required for: • Table 1: Endpoint mobility • Table 2: Endpoint release • Table 3: Endpoint degradation	
12:10 – 13:00	Reporting back incl. wrap-up and closing of the NanoCarrier workshop	Florian Part & Bernd Giese, BOKU, Austria

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