Bundesinstitut für Risikobewertung

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NAMs: More than just "alternatives to animal testing"

New Approach Methodologies for health risk assessment of nanomaterials

Currently, assessing health risks of food and feed, chemicals and consumer products, in many cases still requires results from animal studies. However, scientists around the world continue to work in accordance with the so-called 3R principle on replacing animal experiments (Replacement) and, where this is not (yet) possible, to reduce the number of animals (Reduction) and their suffering in experiments (Refinement) as far as possible. New Approach Methodologies (NAMs) that utilise a variety of technologies and methods such as cell cultures, (bio)chemical tests and computer simulations play a central role in this context.

In the research project NAMS4NANO, funded by the European Food Safety Authority (EFSA), scientists from 10 research institutes collaborated under the coordination of the German Federal Institute for Risk Assessment (BfR) to systematically analyse the existing NAMs for application in health risk assessment of nanomaterials (NM). They identified more than 260 individual NAMs, several of which could already provide reliable results to support NM risk assessment. Nevertheless, many of these NAMs have so far only been used to a limited extent in the context of risk assessments, as the majority of them have not yet been validated and are hence not available as test guidelines (TGs) of the Organisation for Economic Co-operation and Development (OECD). The development and adoption of OECD TGs is a time and resource intensive process.

Therefore, the NAMS4NANO consortium also proposes an initial concept for how the existing NAMs could be integrated more rapidly in risk assessments. The researchers suggest the introduction of an accelerated recognition procedure for qualification of existing NAMs, whereby an EFSA expert panel could examine the regulatory readiness of individual NAMs. If the available data on specific NAMs are sufficient to prove their reliability and relevance, they could be recognised as valid to enable their use for well-defined, specific questions related to NM risk assessment in the food and feed sector. Also, this so-called "qualification" allows to

better guide the method development, as in the process of qualification recommendations can be made for the further optimisation of NAMs that are not yet sufficiently mature. In this way, new methods can be applied much faster than before, at least in certain integrated risk assessment procedures for selected areas and contexts-of-use, where they can help to further reduce the number of animal experiments.

Nevertheless, the NAMS4NANO team emphasises that animal testing in risk assessment is unlikely to be completely replaced by NAMs in the near future. For the foreseeable long term, certain aspects and questions will continue to require investigations in a living organism.

1 New Approach Methodologies (NAMs)

New Approach Methodologies (NAMs) include a variety of novel approaches such as *in silico*, *in chemico*, *in vitro* and *ex vivo* methods, aiming to replace animal tests. *In silico* methods rely on computer simulations and mathematical models to predict, for example, the effect or the distribution of certain substances in the organism. *In chemico* methods investigate chemical reactions, for example to study the reaction or interaction behaviour of a substance. *In vitro* methods use cell cultures, i.e. cells that are cultivated in an artificial environment, such as a Petri dish, outside the organism. *Ex vivo* methods utilise tissue taken directly from a living organism to study effects of chemicals outside the organism. Animal studies, on the other hand, are referred to as *in vivo* methods.

Although the term NAMs has been widely used recently, there is no harmonised/ binding definition. Therefore, as a first step, the NAMS4NANO consortium has developed a working definition for the term New Approach Methodologies (NAMs). This working definition comprises NAMs for hazard and exposure assessment. Moreover, specifically for nanomaterials (NM), physiochemical characterisation methods should also be considered as NAMs.

2 Use of NAMs in the risk assessment of nanomaterials

NAMs have great potential to fundamentally improve the current practice of risk assessment. The use of NAMs is particularly obvious for the assessment of NMs. This is because a huge amount of data usually already exists on the corresponding non-nanoscale substances, to a large extent from high-quality animal studies. However, there are often numerous NM variants of a given substance that differ in certain physicochemical properties such as shape, size/size distribution or surface chemistry. This virtually infinite number of variants urgently requires new approaches to assess their safety. It is not efficient to assess every single NM variant for all conceivable health effects using conventional animal studies. Moreover, certain aspects of NM risk assessment can technically be much better realised with NAMs than in animal experiments, for example the investigation of NM transport by using biological models

that mimic human body barriers or studies on NM uptake in specific cell models. Therefore, NAMs can play an important role in NM risk assessment to avoid additional, nano-specific animal studies wherever possible. In other cases, NAMs are important as an orientation aid in order to conduct the necessary animal studies in a more targeted manner and to plan them efficiently to keep the number of animal studies to an essential minimum.

However, the advantages of NAMs are not limited to ethical considerations. In many cases, NAMs are much simpler and more flexible to use than animal experiments. They provide larger amounts of data more rapidly. For instance, they allow to investigate different cell models or different parameters in parallel or, in some cases, in combination. Furthermore, NAMs allow important insights into the underlying toxicity mechanisms. This could make them more efficient and informative for risk assessment than animal studies. In addition, in some cases the results of animal studies cannot be transferred to humans due to the physiological species differences while NAMs rely on human cell models. In many respects, NAMs therefore offer the potential to significantly improve the current practice of risk assessment, which is implied by the term next generation risk assessment.

3 Regulatory readiness of existing NAMs

There are already a number of validated and officially recognised NAMs for conventional chemicals. In contrast, most NAM-based instruments and tools for NMs have not yet even reached the status of validation. There are a few exceptions, such as some ISO or ASTM standards (for example those for the assessment of cell viability of cells following NM treatment). Therefore, especially for NMs, much more efforts and resources are needed to adequately develop and validate NAMs for nano-specific assessments.

Already validated and officially recognised NAMs, which are available for chemicals as OECD test guidelines (TGs), specifically for the areas of genotoxicity, phototoxicity, skin or eye irritation/corrosion and skin sensitisation, in most cases can also be used for NM risk assessment, provided that the necessary nano-specific adaptations have been considered, as explained in the report of the NAMS4NANO consortium.

However, the majority of NAMs for NMs are currently still at the research and development stage. Nevertheless, some of them could already be suitable for supporting the risk assessment of NMs, especially in so-called integrated or tiered approaches, even if they are not yet validated and regulatory accepted.

4 Qualification of NAMs as a supplement to validation

Despite the enormous progress that has been made in the development of NAMs in numerous research projects in recent decades, their regulatory use is still limited and does not correspond their scientific development. One of the biggest obstacles is the fact that standardisation and validation are highly time and resource –intense processes.

For widespread use in risk assessment, validation and recognition of the method as an OCED TG is necessary. If no TG exists, the corresponding methods can also be used for regulatory risk assessments but only to a limited extent. Furthermore, assessments that contain data from non-standardised test methods requires a very time-consuming and complex case-by-

case evaluation. As many OECD TGs for the risk assessment of NMs have not yet been adapted to nano-specific characteristics or are still in the process of being adapted, any risk assessment for NMs is currently a very complex and time-consuming endeavour.

Establishing an OECD TG is a formal and time-consuming process, but it enables that the data obtained in accordance with a TG will be recognised internationally and can be used in regulatory procedures in different countries. To date, only a few NAMs have been validated and established as OECD TGs for implementation in a regulatory context. It usually takes several years before a new method is recognised as an OCED TG.

The NAMS4NANO consortium was commissioned by EFSA to propose a framework for a qualification system for chemical risk assessment in the food and feed sector in order to accelerate the regulatory use of NAMs. The NAM4NANO consortium therefore proposed an initial concept for the design of NAMs qualification system, which has been published in a separate document. The authors suggest that a panel of experts could be established by EFSA to assess the regulatory readiness of individual NAMs for specific applications. If the available data is sufficient to prove the reliability and the relevance of the method, the respective test method could be recognised within a narrowly defined scope of application (“context-of-use” concept). This so-called "qualification" would be limited to NAMs in integrated procedures for narrowly defined areas of application. Comparable qualification systems already exist for the research and development of drugs.

The consortium also describes assessment criteria to evaluate the regulatory readiness of the NAMs. Of central importance is a detailed description of the entire test method in all individual steps, preferably in the form of standard operating procedures (SOPs), covering the set-up of the NAMs, its application and evaluation phase. In addition, the scientific validity, i.e. the reliability (robustness) and the suitability (relevance) for the application context, must be demonstrated, for which a somewhat less stringent procedure was proposed compared to OECD TGs. The NAMS4NANO consortium suggests to introduce such a qualification system initially only to support the risk assessment of NMs, in very close alignment with the existing EFSA guidance documents on the risk assessment of NMs (https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2021.6768 and https://www.efsa.europa.eu/en/efsajournal/pub/6769). However, the approach might also be interesting for other NAMs in other contexts. The document published here is an interim version that is initially intended to facilitate a broader discussion among experts and interest groups. Therefore, interested stakeholders are invited to provide feedback and comments on the proposed concept.

5 Background to the NAMS4NANO research project:

The Federal Institute for Risk Assessment (BfR) has been involved in the development and use of animal-free research methods for many years. The German Centre for the Protection of Laboratory Animals (Bf3R) is based at the BfR.

The collaborative research project NAMS4NANO is a project funded by the European Food Safety Authority (EFSA) established under the call "NAMS4NANO - Integration of New Approach Methodologies results in chemical risk assessments (case studies addressing nanoscale considerations (GP/EFSA/MESE/2022/01))". The consortium is coordinated by the German Federal Institute for Risk Assessment (BfR). In addition, experts from various partner

organisations from Italy (Istituto Superiore di Sanità, ISS), Belgium (Sciensano), France (French Agency for Food, Environmental and Occupational Health & Safety, ANSES), the Netherlands (Dutch National Institute for Public Health and the Environment, RIVM and Wageningen Food Safety Research, part of Wageningen University and Research, WFSR) and Luxembourg (Luxembourg Institute of Science and Technology, LIST) are also working on the project. Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) is involved via a subcontract and the Singapore Food Agency (SFA) is participating as an international partner. Furthermore, the Joint Research Centre (JRC) of the European Commission is involved in the project.

The project is scheduled in total for 4 years. The overarching aim is to gain a more profound understanding of the opportunities, challenges and remaining uncertainties when using NAMs in the risk assessment of NM. The project comprises several sub-projects. In the first sub-project, two documents were published as a first interim result after one year of work: a review article on the currently available NAMs for NMs and a detailed proposal for the introduction of a qualification system for NAMs, the latter as a preliminary report being open for public comments. Both documents are published by EFSA but do not constitute an official opinion of EFSA.

In a second sub-project (also led by the BfR), selected/ prioritised NAMs are currently being tested in risk assessment case studies. In a third sub-project (led by ISS), individual methodologies will be further developed.

Publications:

Review of New Approach Methodologies for Application in Risk Assessment of Nanoparticles in the Food and Feed Sector: Status and Challenges
https://doi.org/10.2903/sp.efsa.2024.EN-9008

Proposal for a qualification system for New Approach Methodologies (NAMs) in the food and feed sector: example of implementation for nanomaterial risk assessment
https://doi.org/10.2903/sp.efsa.2024.EN-8826

Further information on nanomaterials on the BfR website:

Health risk assessment of nanomaterials
https://www.bfr.bund.de/en/health_risk_assessment_of_nanomaterials-30439.html

Nanomaterials Research
https://www.bfr.bund.de/en/nanomaterials_research-10431.html

FAQ Nanomaterials: Tiny particles mediate manifold properties:
https://www.bfr.bund.de/en/nanomaterials__tiny_particles_mediate_manifold_properties-8568.html

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry for Food and Agriculture (BMEL). It advises the Federal Government and the States ("Laender") on questions of food, chemical and product safety. product safety. The BfR conducts its independent research on topics that are closely linked to its assessment tasks.

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