Global Coalition for Regulatory Science Research

EUROPEAN FOOD SAFETY AUTHORITY

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THEME 1 | Qualification system supporting the use of NAMs in food

New Approach Methodologies (NAMs) can be considered in a broad context, covering *in silico*, *in vitro*, and *in chemico* methods, which in the area of nanomaterials are also connected to aspects related to their physicochemical characterisation. For the assessment of nanomaterials, EFSA's Nano Guidances (EFSA Scientific Committee, 2021a and 2021b) describe the use of NAMs as part of Integrated Approaches to Testing and Assessment (IATA) for evaluating nano-specific toxicokinetic and toxicodynamic considerations. In parallel, the EFSA Roadmap on NAMs (Escher et al., 2022) identified, among others, the area of data integration (i.e. the use of IATAs to integrate NAMs with existing *in vivo* information while promoting harmonised data reporting) as an area for which the development of an EFSA system for "fit-for-purpose validation"[1] of methods could enhance the practical use of NAMs in the risk assessment. In the field of medicinal products, the use of scientifically valid but not yet validated NAMs can be accepted for a specific use or in a specific context in a process called "qualification" (EMA[2], US FDA[3]). The experience of EMA and US FDA can support the implementation of a qualification system in the EFSA remit and as such can facilitate the regulatory use of results from non-standardised methods in the food and feed sector, provided that specific criteria are defined for regulatory acceptance for different problem formulations. A harmonization of criteria used for the qualification of a NAM would also facilitate the "*one substance one assessment approach*" aiming to support the transparency of safety assessments across relevant legislative frameworks. Based on the recommendations of the EFSA Strategy for 2027 and EFSA Roadmap on NAMs, EFSA has funded projects to promote the use of NAM-based IATA to fill data gaps in the risk assessment practice, integrating the available toxicological information with newly generated NAM-based studies developing proof-of-concept case studies. Some examples in the field of nanomaterials are the EFSA NAMs4NANO and NANOCELLUP Projects, established through partnership with EU Member State organisations. The EFSA NAMs4NANO Project aims to develop a "Qualification system for NAMs for EFSA's risk assessment" and, in parallel, a number of proof-of-concept case studies. The EFSA NANOCELLUP Project is an example of a recently finalised case study that aimed to design a NAM-based IATA to fill data gaps encountered during the risk assessment practice. Although the ultimate goal would be to establish a system for broader use of NAMs for risk assessments in general, nanotechnology is used as a starting point being an area in which the implementation of novel approaches is promising for several reasons, such as the general unavailability of suitable OECD Test Guidelines.

On this basis, this Workshop was organised to discuss the experience and lessons learnt from the qualification systems in place at EMA and US FDA and to consider the possible implementation of a similar system within EFSA's remit, finally aimed to promote the use of reliable and relevant but not yet validated NAM-based tools and resulting data to fill gaps in the risk assessment process for nanomaterials.

[1] Definition of "Fit-for-purpose validation" from the EFSA Roadmap on NAMs (Escher et al., 2023): *A process based on scientifically sound principles by which the relevance and reliability of a particular method or process are established for a specific purpose.*
[2] https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-advice-protocol-assistance/qualification-novel-methodologies-medicine-development-0
[3] https://www.fda.gov/media/144891/download

EMA and US FDA presented the criteria for the "qualification" and the most important aspects for ensuring regulatory acceptance of NAMs were discussed. The decision of regulatory acceptance of NAMs not (yet) incorporated in testing guidelines but useful for regulatory decision making, should be based on the evaluation of the proposal advanced by the applicant against specific criteria. First, the '*context of use'* should be considered as a key aspect. It defines the circumstances under which the data derived from a particular NAM can be used in the assessment of the product under evaluation as well as limitations and uncertainties. In other words, the context of use dictates the criteria that should be evaluated in order to demonstrate the confidence in that method for a specific problem formulation. Such criteria are related to the definition of the test method, biological and toxicological relevance, and reliability/robustness[4,5]. It was however highlighted that acceptance criteria should not be too prescriptive. The use of NAMs is method and context dependent and general criteria should be used by applicants adapting them to the specific question to be answered from a regulatory perspective.

Another aspect of fundamental importance to foster the qualification of methodologies is to ensure collaboration between researchers and regulatory agencies early in the development phase. For example, EMA exchanges with applicants 'qualification advice' on the data needed to reach regulatory acceptance[2]. US FDA actively cooperates with researchers to establish particular NAMs and ensures that the developed tools and data respond to the specific purpose (e.g. qualification of NAMs for biomarkers, qualification of NAMs for microphysiological systems (MPS) for mechanistic risk assessment). In this regard, a platform for consultation/exchange has been established to invite developers to present their technologies as well as with the aim of promoting capacity building on the topic by sharing free of charge training courses[6].

Lastly, the ultimate goal and benefit of a qualification system was discussed. It was highlighted that the qualification of NAMs should aim to promote the use of the best science available to increase safety of products on the market. When NAMs cannot be used as stand-alone methods to solve risk assessment questions, they should be used to promote targeted testing in animals resulting in a reduction of animal experimentations. As a result, this would foster modernisation of the risk assessment process while promoting 3Rs principles.

With its NAMs4NANO Project, EFSA aims to develop a qualification system focused on nano-specific assessments as a first example of implementation. In fact, in the area of nanotechnologies, work is still ongoing to adapt the current OECD TG for the assessment of nanoparticles, and in most of the cases, validated methods are not available. Furthermore, some studies, such as the identification of nanoparticles inside cells and tissues, are technically easier to implement using *in vitro* methods than *in vivo* studies. This is the case of some materials of carbonaceous nature which are difficult to be traced and detected *in vivo*. In this regard, EFSA launched in 2020 its EFSA NANOCELLUP Project as a proof-of-concept case study aimed to design a NAM-based IATA for addressing data gaps in the assessment of hazards associated to nanocellulose oral exposure[7]. Cellulose was already assessed by EFSA (EFSA ANS Panel, 2017) and the Panel concluded that there was no need to establish a numerical Acceptable Daily Intake

[4] https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-principles-regulatory-acceptance-3rs-replacement-reduction-refinement-testing-approaches_en.pdf
[5] Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup, 2023. Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies. Available at: https://ntp.niehs.nih.gov/sites/default/files/2023-08/VWG%20Report%20Draft_for%20public%20comment_08Aug2023.pdf
[6] https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda
[7] https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2023.EN-8258

due to lack of absorption and toxicity. From this starting point, the aim of the Project was to use NAMs to address whether the nanosize nature of cellulose had any impact on its bioavailability and possible toxicity. In the absence of 'validated' *in vitro* methods, 'valid' methods covering the different endpoints were used, taking into account the recommendations from international bodies (i.e. OECD, EURL ECVAM) and the EFSA SC Guidance on Nano - Risk Assessment (EFSA Scientific Committee, 2021a and 2021b). The experimental work for this case study was developed through a collaborative effort among EU Member States (i.e. Italy, Belgium, France) and the European Commission Joint Research Centre (EC JRC). As a result, this case study showed the ability of nanocellulose to enter into systemic circulation, demonstrating that, in some cases, NAMs can be qualified as best available methodologies to target specific questions of regulatory relevance. From this example, it was discussed that proof-of-concept case studies should be considered as the way forward to build confidence and experience in the practical use of NAMs in the risk assessment. While producing useful information to fill data gaps observed during risk assessments, case studies can also serve as a collaborative platform between researchers and risk assessors, from which experience can be gain and blockers towards full implementation of NAMs can be identified.

The elements discussed in this Workshop will be considered by EFSA during the development of a qualification system for NAMs in the context of the EFSA NAMs4NANO Project with the final aim to promote harmonisation with ongoing international efforts on the topic.

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THEME 2 | Challenges and ways forward to investigate micro and nanoplastics

Microplastics and nanoplastics (MNPs) are particles that are formed predominantly from degradation of bulk plastic waste, such as plastic bottles, grocery bags, and many other consumer products, with a portion that are intentionally manufactured. There is evidence on human exposure to MNPs through environment, food, sea food, water, and beverages; however, analytical methods and methodologies for accurate quantitative assessment of these MNPs of various sizes, shapes, and chemical compositions in these complex matrices is lacking in the scientific literature for hazard and risk assessment by regulators. Especially, data on nanoplastics (less than a micron in size dimensions) is lacking due to the additional challenges in measuring sub-micron particles and speciation from environment and tissue matrices.

This brief workshop focused on characterization of MNPs in food, sea food, feed, and other complex matrices. Even though the procedures for few standard pristine microplastics identification are well established, the knowledge gaps are in the analytical methods of unknown MNP mixtures from complex matrices, and especially for nanoplastics. Data on pristine polymers may be different from those from degraded/oxidized/reduced materials from nature, in addition to chemical contaminants that bind to MNPs in the environment.

Consider, for example, if one has to measure MNPs presence in sea food for monitoring purposes and quantify different unknown MNPs to assure the safety of food we consume. How does one go about isolating the complex unknown MNP mixtures from different parts of sea food (fish, for example) without compromising the integrity of the polymers. MNPs of various compositions, sizes, shapes, additives and other chemical components may be present at different abundance levels in sea food depending on the source. The biodistribution of microplastics may be different from nanoplastics, and they may pose a different immunological or toxicological threat. Considering real-world samples, and not model compounds such as polystyrene latex beads, metal containing species or radiolabelled models, what practical methodologies are available for measuring and monitoring of these contaminants of emerging concern? Acid, base and enzymatic digestion methods all have their advantages and limitations in isolating different MNPs, and one has to consider keeping the integrity of the species for eventual hazard identification, exposure and risk assessment.

During this Workshop, the currently available analytical methods and other research needs to address the above knowledge gaps for the assessment of MNPs were discussed.

As a starting point, the key importance of having a clear estimation of exposure to MNPs was discussed as key factor to enable realistic (eco)toxicological studies and risk assessment. Primary obstacles in this respect are the analytical challenges for the identification of suitable reference materials (including test materials as well as controls), sample preparation (e.g. isolation from aqueous and complex (tissue) matrices; separation and fractionation methods) and analysis techniques for qualitative and quantitative characterisation. These challenges are related to the fact that MNPs are very different from engineered nanomaterials. Environmental relevant nanoplastics of anthropogenic origin are more similar to natural colloids due to their irregular

shape, organic and non-homogeneous nature. Consequently, different techniques should be used for the identification and quantification of MNPs in complex matrices to facilitate nanoplastics research and (eco)toxicological assessment. Examples are methods based on stoichiometry (e.g. Electron Microscopy (EM) coupled with Energy Dispersive X-ray Analysis (EDX)), refractive index (e.g. light scattering), density or chemical resistance. However, being less specific, these methods should be used in combination to enable full physicochemical characterisation of MNPs in foods while decreasing the uncertainties associated to the measurand.

Other important aspects highlighted were the importance of a clear and harmonised terminology for MNPs, particularly in terms of size range definition. This aspect is key in clarifying the problem formulation in relation to potential human exposure and possible toxicity implications. When plastics are released into the environment, their environmental fate may lead to their degradation, potentially ending in the formation of small particulates, most likely at the nanoscale. The nanometer size range may have the greatest relevance and hazard potential, but also the greatest analytical challenges.

The importance of a collaborative effort and international partnerships to address the current knowledge gaps was highlighted. National and international organisations worldwide are investing in research to fill gaps within the assessment of MNPs. In the EU for instance, a large research cluster called CUSP was funded by the European Commission and includes 75 organisations from 21 countries working within five large-scale research projects to understand the health impacts of micro- and nanoplastics. Areas of work include the development of analytical methods and representative materials, data sharing, inter-laboratory comparisons, exposure assessment, risk assessment, communication and dissemination [8]. US FDA is coordinating a Nanoplastics Interest Group, composed of 20 agencies, more than 100 members across the US government. Also in this case the goal is to ensure collaboration between laboratories and research groups to address the analytical challenges and other knowledge gaps, to organise interlaboratory comparisons and content exchange between researchers and risk assessors. It was emphasized that the importance of this collaboration is also to ensure that data developed from research activities meet current standards and regulatory needs, leading to the generation of meaningful data for regulatory use.

In parallel to try to solve gaps related to the analytical characterisation, it is important to invest resources in the identification of potential MNPs hazard. Definition of exposure levels is key to reflect on the physiological conditions and actual concentrations relevant for toxicity testing. For example, the use of suitable concentrations in *in vitro* and *in vivo* systems is essential to produce meaningful results. On the other hand, the investigation of critical aspects for risk assessment such as cellular internalisation are also considered important to inform risk assessors.

Lastly, the importance of innovative solutions for effective plastic circularity was raised. While working on international policies to decrease the amount of plastic, additionally efforts should be made to explore solutions on how to handle it. Given the complex and multidisciplinary nature of this issue that requires considerations on lifecycle assessment, socioeconomic components, as well as knowledge on polymer chemistry and material science, there is a need to gather all diverse stakeholders to discuss the priorities that are needed to move forward.

[8] https://cusp-research.eu/

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