METHODOLOGY AND SCIENTIFIC SUPPORT UNIT

Workshop on the EFSA Project NAMS4NANO

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WEB-conference

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Participants

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1. Welcome and apologies for absence

The Co-chairs welcomed the participants.

2. Adoption of agenda

The agenda was adopted without changes.

3. Background, EFSA NAMs Roadmap and workshop objectives

The EFSA NAMS4NANO project is part of the projects implementing the EFSA Roadmap on NAMs, that aim at promoting the implementation of New Approach Methodologies (NAMs) to perform comprehensive chemical risk assessments, minimising the need for additional animal testing, in the context of the EFSA remit (food and feed area). NAMs include *in vitro* and *in silico* studies, connected to modern technologies and big data focusing on relevant models for human health assessment. NAMs represent innovative tools as they improve the quality, efficiency and speed of chemical hazard and risk assessments while reducing dependency on animal testing (Chemicals Strategy for Sustainability[1]). Despite the well-defined European legal framework for the incorporation of non-animal approaches in chemical risk assessment and the fact that NAM-based data are key to the toxicological research area, their incorporation in the regulatory context is still limited. Within the EFSA's remit, information from animal studies is frequently available, but often with deficiencies that create uncertainties and inconclusive risk assessments, requiring additional testing.

In the food safety area, there are two main fields in which NAMs are considered sufficiently ready for incorporation in the risk assessment: 1) mechanistic-based risk assessment for chemicals in food and feed (one of the EFSA priorities is to develop a guidance on how to use this information) 2) addressing data gaps in risk assessment through the design and validation of NAM-based Integrated Assessment and Testing Strategies (IATAs). The EFSA approach is not to use NAMs in isolation but to integrate them into already existing *in vivo* data, human epidemiological data, human biomonitoring data and toxicokinetic data, to perform a better risk characterisation. In the EFSA 2027 Strategy[2], NAMs are included as a priority to minimise animal testing, produce more informative risk assessments, and make use of wider, improved and new data streams.

Based on these key elements, EFSA has defined a set of actions:

- Development of a NAMs Roadmap
- Increase international cooperation (e.g., through Accelerating the Pace of Chemical Risk Assessment (APCRA) network and the NAMs Working Group under the International Liaison Group on Methods for Risk Assessment of Chemicals in Food (ILMERAC))
- Selection of case studies for filling data gaps and exploring the use of Artificial Intelligence (AI) to integrate NAMs data in EFSA's risk assessment.

These actions have the common intent to explore qualification and verification of non-guideline NAMs methods and promote the use of results from valid methods in the regulatory context before their formal validation. The ongoing EFSA case studies cover different areas under the EFSA's remit and different toxicological endpoints. In addition, the AI case study aims to explore the possibility of using the OECD OHT 201[3] to perform data extraction, pre-validation, and integration in AOP-like networks.

The EFSA Roadmap outlines activity proposals in seven distinct but interacting scientific areas (e.g., development of additional AOPs/AOP networks, advanced cell culture models including organ-on-a chip (OoC), toxicokinetic assessment with a focus on physiological-based kinetic modelling (PBK), exposome, human susceptibility, data integration and new concepts in human health risk assessment). One of the main outcomes of the Roadmap is the proposal of using the step "*in situ validation*" before moving to the official validation through ECVAM and OECD, using in the regulatory context studies

[1] Strategy.pdf (europa.eu)

[2] https://www.efsa.europa.eu/sites/default/files/2021-07/efsa-strategy-2027.pdf

[3] https://www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm

that are already scientifically valid to be included in the Weight of Evidence for clarifying mechanistic information or other elements. The Roadmap is mainly focused on conventional materials: nanomaterials are mentioned but are not really covered. It should be noted that the promising capacity of NAMs for assessing nano-scale considerations has been already highlighted in the Nano Guidance documents published in 2021 and is under investigation in one of the ongoing EFSA case studies (NANOCELLUP)[4].

Since one of the priorities of the Roadmap implementation is to explore data integration, EFSA has selected for the NAMS4NANO project nanoscale considerations as high impact topic that could extend the use of NAMs in other areas through a cascade effect. Results from this project will be integrated with results from all other EFSA projects, mostly focused on conventional (non-nano) substances.

The objective of the present Workshop is to have an open discussion and collect experts' feedback on the EFSA proposal, supporting EFSA in the following steps, that include drafting the technical specification for the launch of a call for tender to EU Member States (MSs) for case studies on nanoscale assessment and the development of a qualification system for NAMs.

After the Workshop, EFSA will draft technical specification and launch an open call for a grant agreement with Member State organisations. The interested organisations should consider the call, organise consortia if needed, and prepare specific proposals, noting that the main organisation in the consortia can only be those included in the Art. 36 Organisations list, while other organisations can be included as sub-contractors.

4. Introduction to the EFSA NAMS4NANO project

The NAMS4NANO project presents three main pillars: 1) focus on nano scale considerations and establishment of case studies through the launch of a call for proposals followed by an update of the EFSA Nano Guidances 2) development of a qualification system as part of the call 3) NAMs data integration with already existing animal or human data for the incorporation in the risk assessment with a focus on recommendations on data reporting in dossiers; this third pillar is not specific for nanoscale issues, and will integrate case studies and activities from other EFSA projects.
Chemicals for which nano specific considerations are needed include both materials that meet the definition of engineered nanomaterials, as set out in the Novel Food Regulation (EU) 2015/2283[5], and conventional materials which do not meet the definition but contain small particles including particles at the nano scale according to the EFSA "Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles"[6] (Guidance on Particle-TR). EFSA considers 'nano' as a cross-cutting topic since applications requiring nano scale considerations can be related to different areas relevant to EFSA's chemical risk assessment (e.g., novel foods, food additives and flavourings, feed additives, food contact materials); in order to take into account all EFSA's areas, the project will include also pesticides and contaminants.
The nano example, which combines the integration of NAMs and non-NAMs methods and nano and non-nano considerations, has been identified as a very promising case to demonstrate how NAMs can be used avoiding additional animal testing. Through a cascade effect, results of this project will be then propagated to other areas.
The EFSA "Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health" (Guidance on Nano-RA) drives the risk assessment for nanomaterials and materials containing a nano fraction integrating nano considerations with the relevant sectoral guidances for conventional materials. It should be noted that for most of the chemicals assessed by EFSA, toxicity information about the chemistry of the material is already available, but specific nano considerations of toxicokinetic and toxicodynamic issues depending on the physical form (e.g., specific interactions, high surface/volume ratio, considerations on possible uptake by the cells and reactivity after the uptake of the particles) must also be addressed. The Guidance on Particle-TR already includes an option for complementing studies with NAMs to address nano scale considerations; the Guidance

[4] https://www.efsa.europa.eu/sites/default/files/2022-04/Presentation_Day2_NANOCELLUP.pdf

[5] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001

[6] https://www.efsa.europa.eu/en/efsajournal/pub/6769

on Nano-RA includes an option for integrating NAMs existing information into IATAs. The NAMS4NANO project will develop ”proof of concept” case studies covering all areas of EFSA’s interest using different NAMs that will be integrated in the Nano Guidances as annexes, serving as real examples for the applicants on how an IATA case on NAMs complementing the existing information could be performed. This could also facilitate the internal design of studies and provide more generic recommendations on how to cover data gaps with supplementing existing studies on NAMs or developing IATA.
The second specific element of the project is the establishment of a qualification system for NAMs developers, covering all kind of NAMs not only those related to the nano area.
The third main point of the project is data integration of results from this and other projects moving forward a clear recommendation on how to report data and information using harmonised data formats (using information from OECD and in cooperation with ECHA).

5. NAMs for addressing nano-scale considerations in food and feed safety assessment

The first proposal of the project is to develop proof of concept case studies to be published as annexes to the EFSA Nano Guidances to demonstrate that, if an applicant produces an IATA using existing knowledge (e.g., animal data, read-across data from other materials, human data) combining it with NAMs for addressing mechanistically nano specific issues, this information could result in a better safety assessment than the generation of new *in vivo* studies. Moreover, a key element to the nano scale considerations is being able to follow the fate of the nano particles through their biological interactions: while with *in vivo* studies this could be very difficult, well-designed NAMs studies can more easily provide this information.

The project aims to involve all the relevant EFSA areas, taking into consideration also pesticides (not only the active substance but also nano formulated pesticides) and areas in which applications are not expected, as food contaminants with a focus on nanoplastics, providing support and confidence to applicants in using NAMs instead of conducting new animal studies. The large majority of expected case studies involve oral exposure but, especially in the case of feed additives or pesticides, the EFSA risk assessments need to include also occupational exposure and environmental exposure for residents (mostly inhalation and dermal exposure).

5.1. Discussion

The group was asked to provide feedback on the feasibility of the project, suggestion on priorities for case studies and which elements should be included in each case study proposal. The main discussion points raised are listed below.

- The case study may cover a full risk assessment for a material/substance or a group requiring nanoscale considerations or be targeted to the assessment for specific toxicological endpoints or toxicokinetic issues pre-identified as those leading the safety assessment. The newly designed studies should be based on NAMs, while the assessment should also consider existing animal or human data. EFSA is open for the discussion on the selection of toxicological endpoints as long as they are relevant for nano specific considerations. The Guidance on Particle-TR already clarifies for each endpoint which studies can cover nano-specific considerations.
- Data integration will include the integration of already existing *in vivo* and *in vitro* evidence of the selected chemical with *de novo* produced data by NAMs approaches, to provide better mechanistic understanding. EFSA’s proposal is to use as initial format the IATA template from OECD to clarify which information are available and how they can be complemented using NAMs (also integrating nano specific considerations with non-nano specific considerations). Data integration will also include recommendations to applicants on how to report NAMs data in the dossiers using harmonised data formats (e.g., OECD Harmonised Templates)[7].

[7] https://www.oecd.org/ehs/templates/

- EFSA cannot provide networking suggestions or force any consortia formation, but has informed the cross-cutting Working Group on Nanotechnologies (ccWG Nano), the NAMs Working Group on ILMERAC and the Nanonetwork on the launch of the call. Considering the long-term duration of the project, additional calls will be launched to cover different tasks with different submission timelines.
- In the food area for regulated products data poor substances are not considered a main concern due to the presence of sectoral information requirements that should provide basic information for every substance under EFSA's remit. In practice, the available studies may have shortcomings, and this is particularly the case for nanoscale considerations as even most OECD test guidelines have not been updated for covering nano-specific issues. The key element for the case studies is to provide clear indications also to applicants on how to avoid additional *in vivo* studies.
- Regarding the risk assessment of nanoplastics, there are two complementary situations. In some cases, e.g. linked to food contact materials, existing information on chemical composition is expected to be available, and should be complemented with the toxicokinetic and toxicodynamic specificities of nano forms using NAMs, generating *de novo* studies to fulfil data gaps. For nanoplastics as contaminants in food, the proposal is to contribute the nanoplastics evaluation to be done under a group of international regulatory agencies. In general, international efforts in the field should be followed[8].

6. Qualification system for NAMs methods and tools

The second main proposal of the project is to develop a qualification system that would cover all NAMs, and not only those relevant for the nano scale assessment, facilitating the regulatory use of NAMs tools and methods before the standardisation process. EFSA's objective is to complement and not substitute the validation step with an option that can speed up the regulatory acceptance of methods that scientifically are sufficiently good but not validated yet.

6.1. Discussion

The group was asked to provide feedback on the availability of other validation experiences, suggestions on methods, SOPs, technologies, and critical elements to be included in the proposal for a qualification system. EFSA is aware of relevant systems developed by EMA and US FDA, related experience and lesson learnt will be considered for the development of an ad hoc approach in line with EFSA regulatory frameworks.

7. Collecting and integrating NAMs-based data into IUCLID

In 2019 the European Commission published the European Green Deal[9], which includes in its action plan the Chemicals Strategy for Sustainability[10] (CSS). Filling knowledge gaps, promoting innovation and alternative testing methods are some of the main objectives of the Strategy. As a contribution to the European Green Deal, EFSA and the European Chemicals Agency (ECHA) drafted a joint position paper around the idea of "One Substance - One Assessment" for chemicals, with the aim of simplifying and consolidating the current legal framework across Europe. One of the main identified objectives of the CSS is focusing on data. Data should be easily findable and accessible, and data, tools and platforms should be developed according to the current format (e.g., IUCLID and IPCHEM for exposure data) in order to contribute to the overall development of a Common Data Platform on Chemicals. In EFSA there are different activities, with the support of Sister Agencies (e.g., ECHA, EMA) and JRC, for the establishment of tools for making academic data easily available and accessible, and for the establishment of an EU repository of health-based guidance values (e.g., ADI for pesticides).

The Common Open Data Platform on Chemicals aims to ensure a single access point to data and information chemicals in the EU, with the view that all authorities can be aware of each other's

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

[9] https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6691

[10] https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf

regulatory actions and have an easy access to the same data. Moreover, Stakeholders together with Authorities, could have access to the best available tools for safety assessments and, together with citizens, can be publicly and timely informed about the regulatory processes and their outcomes, in line with the new Transparency Regulation[11]. IUCLID has been selected as basis for collecting and disseminating hazard data.

Some key elements of the Open Data Platform of Chemicals are the presence of a common ontology, a controlled vocabulary to report chemical information, and the establishment of a new repository of health-based values: all Authorities and Stakeholders should have a unique access point to all EU and international guidance values, legislations that require setting such limits and also limit values derived by regulators during risk assessment process. The EFSA OpenFoodTox database has been identified by the European Commission as a starting point for the repository of health-based limit values and the OpenFoodTox 3.0 project will be integrated in the Open Data Platform of Chemicals.

The NAMS4NANO project includes a specific Work Package on data integration. The objectives of this work package on data integration, derived from the EFSA NAMs Roadmap, and are:

1) Developments of templates for reporting NAMs-based data into IUCLID: the focus should be on the applicants' side, a technical guidance for using IUCLID should be provided and an analysis on the current IUCLID formats (i.e. OHTs, endpoint summary, flexible records) on how these can be adjusted and modified to capture NAMs based data should be performed.
2) Collecting and integrating nanomaterials NAMs-based data into IUCLID resulting from the "Nano proof of concept case studies".
3) Collecting and integrating data resulting from the NAMs Case Studies and other EFSA projects into IUCLID using available formats and tools ongoing in EFSA. EFSA recently launched a project on Multi-OMICs and Inter-species Workflow to derive Human Reference Points and Health-based Guidance Values from quantitative *in vitro* data.

IUCLID is the essential tool managed by ECHA for any organisation or individual that needs to record, store, submit and exchange data on chemical substances in the format of the OECD Harmonised Templates (OHTs). It ensures a harmonised way of collecting and share data, but also a harmonised terminology. Within the OHTs, is important to mention OHT 201 (achieved by OECD, JRC and ECHA) on intermediate effects with the aim of collecting, exchanging and (re)using harmonised mechanistic data derived from multiple NAMs-based approaches (e.g., *in vitro*, *in silico*, OMICs). The OHT 201 has been designed to accommodate and allow users to report data but also to follow the same ontology as defined in the different AOP Frameworks and could be the starting point not only for nano materials in IUCLID.

Among IUCLID tools, the IUCLID Uploader is a KNIME based tool that allows migrating data from existing databases into IUCLID and allows data validation against the IUCLID format and the generation of IUCLID files that can be then shared within IUCLID and across Authorities and Agencies. A second tool is the Data Extractor, which allows users to extract ad hoc information from IUCLID in accordance with a set of user-defined rules. The proposal for integrating data into IUCLID is to allow experts and EFSA staff to report data directly in IUCLID through IUCLUD tools developing new templates starting from the existing ones (i.e. OHTs for Nanomaterials 101-113 and OHT 201). This approach could lead towards more interoperability among current databases in Europe, having a machine-readable format to be shared with Open Data Platform, but also to be published on the EFSA portal and knowledge junction database. Regarding the link of IUCLID with other databases and tools, data from IUCLID can be retrieved while using the OECD QSAR Toolbox only for REACH related dossiers and data in IUCLID can be migrated into the eChemPortal.

[11] Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC

7.1. Discussion

Participants were asked to provide their view on the use of IUCLID to store NAMs data and suggestions on other methods, templates, guidance documents to be used. The main discussion points raised are listed below.

- The eNanoMapper[12] database is fully compatible and could be migrated into IUCLID, that should be seen as a repository to collect, store and disseminate data. EFSA is currently migrating other databases (e.g., OpenFoodTox) and the goal should be to have a harmonised way to store information in Europe, with an aligned terminology (e.g., OHT 201). Data stored in the eNanoMapper database come from research projects and if agreed with the owner, can be made publicly available.
- IUCLID and OHTs are flexible tools, but if a group involved in the project would identify the need to develop a new OHT or extend an already existing one it would be possible to liaise with OECD to implement the modification in IUCLID. EFSA is already in contact with ECHA who expressed interest in supporting this project.
- Reporting NAMs based studies results in a relevant way for regulatory risk assessment is a key element. Reporting templates can also be helpful in the study designing process. A conventional chemical substance can be identified through the specification of the IUPAC name, but to define nano materials all the characteristics that could determine specific effects (e.g., morphology, size distribution or surface properties) are necessary. IUCLID is user friendly, but the chemical identification of nano materials is indeed considered challenging. In IUCLID it is possible to link documents concerning different effects through cross reference functions, building a database on a reference substance and connecting database with datasets directly through the reference substance.
- Follow-up activities with ECHA on the issue of materials that contain a nanoscale fraction, very relevant under REACH as well, have been encouraged.

8. Final discussion

The participants from the workshop provided general support to the feeling that in the nano area the current state of the art is considered ready to produce safety assessment using existing information combined with NAMs. The main discussion points raised are listed below.

- EFSA should provide flexibility regarding the case study design, the number of endpoints, NAMs approaches to be used and the possibility for extrapolation of elements proposed in other areas. EFSA will follow a tiered approach, launching subsequent calls depending on the number of cases received initially. Proposals for case studies can be relevant to several areas. Each case study proposal will have a specific contract and will require collaboration with all the other cases. Interlaboratory comparison will be welcomed but not mandatory. However, case studies should be able to demonstrate that is possible to use of *ad hoc* methods, so replicability of results should be guaranteed including a full description of the methods and access to raw data (in some cases EFSA may work in parallel will JRC for checking reproducibility) and data quality is considered essential as well as reporting quality for allowing regulatory verification.
- For conventional materials the link with other EU projects is extensively presented in the review described in the EFSA Roadmap; a review of the EU projects and NAMs specifically relevant for the assessment of nanotechnology could be additionally requested. Full alignment with PARC, and deliverables from projects such as PATROLS, GRACIOUS, and Sunshine projects will be ensured.
- Ensuring a link with eNanoMapper has been considered a key element that will be further investigated. The consortium is also aiming to report NAMs data in OHT 201.

[12] http://www.enanomapper.net/data

- To ensure physiological relevance, investigating the gastrointestinal digestion process of the pristine material for the oral route of exposure to predict or model what happens during digestion is among the identified priorities.

9. Next steps

In accordance with the results of this workshop, EFSA will finalise the technical specifications for launching the procurement call before Summer 2022. The experts were informed that their participation in this workshop is not considered a conflict of interest and does not affect the participation of their organisations in the call.