SCIENTIFIC COMMITTEE AND EMERGING RISKS UNIT

Network on Nanotechnologies in Food and Feed

Minutes of the 8th meeting

Held on 15-16 November 2018, Rome

(Agreed on 13 December 2018)[1]

Participants

- **Network Representatives of Member States (including EFTA Countries):**

Country	**Name[2]**
Austria	*Apologies*
Belgium	Jan Mast
Bulgaria	Aksinia Antonova
Cyprus	*Apologies*
Croatia	Darko Mikec
Czech Republic	Vladimir Ostry
Denmark	Katrin Loeschner
Estonia	Kaja Kasemets
Finland	Pertti Koivisto
France	Bruno Teste
Germany	Alfonso Lampen
Greece	Aristotelis Xenakis
Hungary	Andrea Zentai
Ireland	Karl McDonald
Italy	Francesco Cubadda
Lithuania	*Apologies*
Luxembourg	Micheline Rosch
Netherlands	Jacqueline Castenmiller
Norway	Gro Mathisen
Poland	Wojciech Wasowicz
Portugal	Maria de Lourdes Bastos
Romania	Gina Popovici
Slovakia	Peter Simon
Slovenia	Viviana Golja

1 Minutes should be published within 15 working days of the final day of the relevant meeting.

2 Indicate first full name and them surname (John Smith) throughout the document

Spain	José Manuel Barat Baviera
Sweden	Lena Hellmer
United Kingdom	David Gott

- **Members of WG on Nanotechnology**

Alicja Mortensen (WG Chair), Qasim Chaudhry (WG vice Chair), Agnes Oomen, Stefan Weigel

- **Hearing Experts**

Roland Franz

- **Observers**

ECHA, EMA and OECD: apologies

- **European Commission:**

Takis Daskaleros (DG SANTE), Hubert Rauscher (DG JRC)

- **EFSA:**

SCER Unit: Reinhilde Schoonjans (meeting Chair), Berrak Eryasa

NUTRI Unit: Reinhard Ackerl, Andrea Germini

FIP Unit: Ana Rincon

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Daniela Hofstaedter (Austria), Vaclovas Jurgelevicius (Lithuania) and Popi Kanari (Cyprus).

2. Adoption of agenda

The agenda was adopted with a few editorial changes.

3. Agreement of the minutes of the 7th meeting of the Network on Nanotechnologies in Food and Feed held on 28 November and 29 November 2017, Parma

The minutes were agreed by written procedure on 20 March 2018 and published on the EFSA website[3].

4. Declarations of interest and confidentiality statements

Network members duly addressed declarations of interest and confidentiality statement according to the EFSA policy.

[3] https://www.efsa.europa.eu/en/events/event/181115-1

Declarations of Interest of Working Groups members: In accordance with EFSA's Policy on Independence[4] and the Decision of the Executive Director on Competing Interest Management[5] EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process. Certain interests were declared orally by the members before the beginning of the meeting. For further details on the outcome of the screening of the Oral Declaration(s) of Interest made at the beginning of the meeting, please refer topic nr 9.

5. Tour the Table

1.1. EFSA: Presentation pilot phase of the guidance and EFSA cross-cutting working group for nanomaterial dossiers support

The Chair, Reinhilde Schoonjans, introduced the pilot phase of the EFSA Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health, that was published in July 2018[6]. The timelines and steps on its implementation were presented. The uniform implementation of the guidance across EFSA will be supported by a new cross-cutting WG that is being established and that will provide experts advice upon request.

1.2. Members states brief overview of what are the experiences and expected implementation rate of the EFSA Guidance.

The following MS gave their feedback during the tour the table:

Czech Republic: There are plans to prepare a questionnaire for food business operators on the subject of 'Nanotechnology in Food' by the end of 2018 and the outcomes will be presented the next Annual Network meeting, together with other relevant information from the Czech Republic on health risk assessment of nanotechnology products.

Hungary: The EFSA Guidance was distributed to the interested parties but there was no submission on possible case studies, nor other type of feedback on the implementation of the EFSA Guidance. The Nano Network will be informed in the case that any information becomes available at the next meeting.

Croatia: It was noted that so far no cases were reported for risk assessment on nanoparticles in food/feed or FCMs to the Croatian Food Agency which is the national body for risk assessment on chemicals contaminants and microbial agents in food and feed.

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[4] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf

[5] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf

[6] Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health: https://www.efsa.europa.eu/en/press/news/180704

Italy: The 3rd National conferences on nano safety assessment in the agri-food sector is scheduled with the stakeholders and the scientific community and will focus on the EFSA Guidance. It was reported that a new project is on-going with a focus on the in-vitro acellular dissolution studies (GI digestion and lysosomal fate) on real world particulate materials as detailed in the EFSA guidance.

Greece: The Greek Authority of Safety (EFET) reported that there were no cases submitted to the national authority at the moment.

Ireland: Currently, there are no case studies to be presented as the laboratories contacted have reported that they have not yet done enough work in this area for implementing the Guidance. The laboratory did indicate that it took part in a Rikilt Proficiency Test (PT) for gold nano in water and would be hoping a PT scheme will be coming for food in 2019.

Germany: The EFSA Guidance was forwarded to the food industry, but no nanomaterial is claimed to be used. Also, the food control agencies are notified about the EFSA Guidance and, although there no concrete cases, these agencies are able to follow a semi quantitative assessment.

Spain, Denmark, Estonia, Romania, Finland, Sweden, Greece, Slovakia, Croatia, Bulgaria, Poland, UK, Slovenia, France reported that there are no specific cases of implementing the guidance, but relevant research and risk assessment initiatives are ongoing.

Belgium, Slovenia, Portugal, The Netherlands, Norway, Finland, France and Germany will present a more detailed topic later in the agenda.

The Nano network was asked to continue to identify the users of the EFSA Guidance (in the private and public sectors). The Chair suggested to get in contact with the national nominated experts[7] to the ECHA Nanomaterials Expert Group[8]. This might facilitate to follow up national activities ongoing under the REACH framework which are complementary to EFSA's work on food safety.

6. EC legislation overview

Takis Daskaleros (EC), presented an overview of the legal provisions regarding nanomaterials in the EU Food Regulations. The current EC definition of nanomaterials is under revision and this will serve as the basis for the revision of the Novel Food (NF) definition of 'engineered nanomaterials' that also applies to the other food related legislations. A key feature of the NF definition of nanomaterials is functional intentionality i.e. the condition that the nanomaterial must be intentionally manufactured to enable a nano related function in the food product which in turn trigger its pre-market assessment under the various food safety legislations, and its labelling under the Food Information to Consumers legislation. In real life, implementation of the food nanodefinition poses significant

[7] https://echa.europa.eu/regulations/nanomaterials/nanomaterials-expert-group/members

[8] https://echa.europa.eu/regulations/nanomaterials/nanomaterials-expert-group

challenges as when a nanomaterial is identified in a particular product, it is very difficult to 'prove' whether it was intentionally engineered and added to perform a nano related function or whether the conventional material was added and ended up in nano form due to changes in its physical state during its processing and addition to the product or due to the analytical manipulations. Nevertheless, regardless of the regulatory status of a nanomaterial identified in a food, it needs to be assessed for its safety as set out in the EFSA guidance.

Although safety-testing methodologies have improved over recent times, there is still a need for analytical tools (validated and standardised analytical methodologies, standards of nanomaterials alone and in food matrices, laboratory capacity building, etc.) for Risk Assessment and Enforcement. The Commission (JRC and SANTE) and the MS are working together to develop, validate and apply such analytical measurements/techniques. In addition, in the context of novel foods, economic operators are responsible for providing an appropriate analytical method together with their novel food application of an engineered nanomaterial novel food.

7. The EC Definition and JRC reports supporting its implementation. Part 1: concepts and terms

Hubert Rauscher (JRC) presented the EC recommended definition of nanomaterial (2011/696/EU), its revision and related Guidance. The report explains general terms, concepts and key parameters and raises the uniform interpretation and measurement approaches.

8. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Case studies and possible adjustments of the guidance

Belgium

Jan Mast (BE) presented results on the validation and implementation of an analytical methodology to characterize the fraction of nano-sized particles in E171 (titanium dioxide) and E174 (silver). This work was partly sponsored by EFSA and in line with the recently published EFSA Guidance. The study characterized the pristine food additives and the marketed products containing these food additives. For silver, the results showed different levels of particles in the two and suggested that the processing of E174 in products induced formation of silver NP. The data obtained for E171 demonstrated that there were several types of TiO2 nano-sized particles in pristine E171 and in food products added E171. No transformation was observed between pristine materials and products. It was discussed that the product stability needs to be part of the assessment and the safety implication is the responsibility under that assessment.

For implementing the legislation and the EFSA guidance, the challenge is to build up specialized expertise and analytical capacity. Other sectors than the food sector have this capacity with top technologies in place and collaborations are recommended.

EFSA

Ana Rincon (EFSA) showed examples from the re-evaluation of food additives and new evaluations, on how the EFSA guidance is being interpreted. The question that arises is if the characterisation of the particle size - including the nano-range –is always needed for any substance (as a powder) to be evaluated and which methodology has to be used. In the EFSA Guidance, it is recommended that particle size distribution should be determined by more than one independent technique (one of which being electron microscopy).

The WG members commented that particle size distribution should be provided as a part of the characterisation of any powdered material and the traditional analytical sieving is not a sufficient technique for that. Data on particle size distribution generated using EM should be requested in case of a micronized material. The following reflections were raised by individual participants in the discussion that took place afterwards:

- Solubility: If the dissolution rate is very well characterised, data generated with EM is not needed for powders that do not form a dispersion (particles suspended in the medium) and show a high dissolution rate in relevant media (= negative confirmation of the presence of nanoparticles and therefore an exit point of the guidance). As discussed in the guidance section 4.3.3, it is also important in this regard that a difference is made between 'solubility' and 'dispersion' of nanoparticles (e.g. silica is sometimes claimed to be soluble in water, but it is not and EM will show that particles float around as a dispersion).
- Other techniques: The NanoDefine decision scheme offers screening method options to approach the physical chemical characterisation of particulate materials. For instance, DLS can be used but only if material is not polydisperse, which rarely happen. If the material is polydisperse, DLS will only show larger particles and not smaller ones and therefore not an appropriate method for many cases. It should be noted that other techniques than EM give different type of size information, for example, for DLS also the static water layer around the particle is included. Furthermore, when using DLS method, it is assumed that the particles are spherical, which may not always be the case. This should be taken into consideration when EM is not provided. In practice, the discussion tended towards the conclusion that, except for NMs with high dissolution rate (see bullet point above), EM information is needed.
- Uptake: There is a different uptake by enterocytes for the nanomaterials around 250nm, though the scientific insights on this issue needs further evolvement. Hence for physiological reasons, it is important to have proper size measurements. The size and shape of particles can only be seen through EM. This substantiate the need for EM as the standard approach for characterising size distributions of particulate materials.

Overall, the experts reiterated the importance of providing adequate data on particle size distribution as part of the physical characterisation of the substances to be evaluated in the application dossiers submitted to EFSA and in view of the

safety for the consumers. As a rule, currently EM is the technique that can adequately scan the nanorange and demonstrate if nanoparticles are present in a powdered material. For all materials in the remit of the EFSA Guidance, it is required that the size parameter should always be measured by at least two independent techniques, one being electron microscopy. If electron microscopy is not applicable (e.g. for some organic nanomaterials), it is recommended to use another imaging technique instead of electron microscopy.

Portugal

Maria L. Bastos (PT) presented findings on the reliability of external and internal doses of metallic nanoparticles: number of nanoparticles versus concentration of the metal. The results are under publication. The network discussed the expression of internalization and cytotoxicity both by metal concentration and NPs concentration, which seems to be recommendable. The study however indicated that internal dose quantification in numbers concentration by EM is not a feasible method to be requested consistently in safety assessments.

Slovenia

Viviana Golja presented a case study of safety assessment of food contact material containing TiO_2 nanoparticles using the new EFSA guidance document. A case study showed that the release of nano TiO_2 particles from quasi-ceramic non-stick pan coatings into 3% acetic acid as an acidic food simulant and by mechanical wear into food may be possible. Based on the available information from migration and mechanical tests, the potential exposure of consumers to released particles was assessed. Further developments of risk assessment for NP release from FCM is needed, also taking into account the many associated uncertainties (in physicochemical characterisation, in hazard characterisation and in exposure assessment).

Viviana Golja also shared recommendations for follow-up research, including the checking of persistence of the released particles in foods and GI fluids during digestion, checking the impact of interaction of nanoparticles with real food components (similar to protein corona in blood serum), testing into real foods and developments of more appropriate test conditions, and the generation of adequate toxicity data for TiO_2 nanoparticles released from such coatings.

In the discussion it was noted that indirect measurement method for the potential release of TiO_2 nanoparticles was applied, size distribution of the intact material was determined only manually (from the high resolution SEM images) and indirectly (by dynamic light scattering measurements of simulant after migration test; polydispersity index indicated polydisperse particle size distribution). For that reason, the nature of the released TiO_2 containing material could not be adequately assessed. Therefore, comparison of the so derived exposure estimates with DNEL values obtained with well-defined and very small TiO_2 nanoparticles for risk assessment considerations is likely to be not appropriate. At the moment more appropriate DNEL values (taking into account larger TiO_2 particles with the appropriate crystallinity) are not available.

Finland

Pertti Koivisto presented Nanofiber production at the Technical Research Centre of Finland (VTT). These nanotechnology-based food products are mainly cellulose (E460 in additives) micro- and nanofibrils with various properties. The analysis and detection of nanocellulose or similar nanomaterials from various matrixes should be done in order to generate data for safety assessment. Nanocellulose, microcellulose as novel food, as any other regulated product, to be used in food products and in feed, but also in food packing materials, has to be previously assessed and in this case according to the EFSA Guidance.
Enough sensitive methods need to be developed, for example for migration studies.

The safety assessments of the nanocellulose products particularly at the in-vitro digestion testing phase, should take into account variation in its digestibility in the in-vitro digestion tests. Nanocellulose is not digestible in human GI system whereas it undergoes microbial fermentation in rumen of ruminant animals or in caecum of laboratory rats.

Germany/France

Alfonso Lampen presented the project SolNanoTox, which is a bilateral cooperation project between German and French institutions to study factors that determine intestine and liver toxicity of nanoparticles. Similar nanoparticles, that are used in food and packaging, but having different solubility characteristics, were selected for in vitro and in vivo investigation on uptake and mechanisms involved. The cellular uptake occurs predominantly in nanoparticle form, which may possibly lead to a 'Trojan horse' effect. However, the effects are stronger from ionic species and liver cells showed more effects than the intestinal cells. The toxic effects are assumed to be derived from oxidative stress, mitochondrial dysfunction and disturbed metal ion homeostasis.

Importantly, it was also found that the effects of Aluminium were dependent on the NP/ion species ratio, and that the Aluminium can convert into one another in biological fluids (e.g. during in vitro digestion).

The project also tried to find out the fate of Al particles in the body by analysing its corona composition, which should reflect the biological interactions.

The network commented that Omics approach in general may be useful in the Adverse Outcome Pathways (AOPs) assessments as further developments for toxicity (in general) are needed for Mode of action (MoA) and Key Events (KEs). It is expected that nanomaterials will follow the same pathways of harm. Nevertheless, Omics data currently available, especially metabolomics, do not allow quantitative assessment (e.g. for the level of oxidative stress). Through metabolomics, more understanding will be gained for what is happening in the cell in relation to nanoparticles. There are data available from the in-vitro analyses and models are being developed to see what are the effects on the target tissue and its relation to AOPs.

The Netherlands

Jacqueline Castenmiller presented a retrospective assessment by which the stepwise framework of the EFSA Guidance (GD) was used on two cases: synthetic amorphous silica (SAS, SiO2, E551) and titanium dioxide (TiO2, E171). This retrospective assessment allowed to identify (and value) the information available (or not). The guidance is found to be well-structured and clear to follow. However, the guidance can lead to iterative processes and following of the guidance would be an expensive process. The physicochemical characterisation is recognised as a highly important initial step for evaluating a nanomaterial. Then, the EFSA Guidance recommends a tiered approach for toxicity testing, similar to that in other EFSA guidance documents for regulatory products. This means that not all steps are mandatory, and if the test results of the initial tier(s) provide sufficient data to reach conclusions on the safety of the compound at the proposed uses, further testing would not be necessary. This not only leads to less animal testing and cost reduction, but also without compromising a risk assessment of a nanomaterial. Assessment of relevancy for humans of some adverse effects recorded in toxicity studies in laboratory animals may require additional studies to elucidate Mode of Action (MoA).

The Netherlands

Nanotechnology is a dynamic field with new and innovative products, developments and nanomaterials being developed. Producers are responsible for safety of their products. For the regulatory authorities, the question is how to keep overview on type of product and their safety when a health risk may not yet be adequately characterised. Agnes Oomen presented a new tool for "signal identification and prioritisation" which products/materials should attract attention of risk assessors. The assessors gather information from open literature for "nano and food". Information is further categorised and severity scores are assigned following a systematic methodology. The scoring is based on nano-specific questions related to risk and it is performed by three experts. The tool is able to differentiate between the products, developments and materials. The signalling tool is based on a conservative approach that includes an alerting system, such as when an adverse reaction is detected. The tool will be available for public access in the following year.

Norway

The Norwegian Scientific Committee for Food and Environment, the national counterpart of the EFSA Scientific Committee, has not received questions with regard to nanomaterials and has not assessed any nanomaterial. It was discussed if the network can identify some substances that are found important to be assessed using the EFSA Guidance in the implementation phase. The idea is to suggest a self-initiation of an assessment to the Norwegian Scientific Committee for Food and Environment by identifying cases for safety testing. The chair proposed to use the signals selected through the screening activities in the Netherlands (see presentation of Agnes Oomen (RIVM).

9. Request for specific advice

A short meeting with the members of the working group took place to plan the implementation of the EFSA Guidance on an issue raised by the NDA Panel. With regard to this topic, Dr David Gott and Dr Alicja Mortensen declared the following interest: they were chair and members of the ANS Panel WG on new applications respectively. This WG of the ANS Panel requested to the applicant additional data on the particle size distribution of the substance/complex under evaluation. Currently, these data are being assessed by the NDA Panel as nutrient sources have been transferred under the revised task division of EFSA panel as from July 2018[9]. The new data submitted by the applicant have been discussed in this meeting. In accordance with EFSA's Policy on Independence[10] and the Decision of the Executive Director on Competing Interest Management[11], and taking into account the specific matters discussed at the meeting in question, the interest above was not deemed to represent a Conflict of Interest for the expert concerned.

10. Any Other Business

Nano-emulsions

EFSA's Emerging Risks network, operating under the EFSA Scientific Committee and Emerging Risks (SCER) Unit, had received the results from an internal Literature search of nano-emulsion use in food. From the open literature it can be observed that the main applications proposed are as preservatives and vitamin/supplements.

Article Presentation: Elespru et al., 2018: Genotoxicity Assessment of Nanomaterials: Recommendations on Best Practices, Assays, and Methods

No novelties were presented in the article and the approach in the EFSA guidance document is in line with the findings of the authors.

Presentation on the European Union Observatory for Nanomaterials (EUON)

The presentation was delivered by the Chair on behalf of the European Chemicals Agency (ECHA) colleagues. Under EUON, a study on the information for risk assessment for nano-pigments was conducted and published in 2018. Background

[9] https://www.efsa.europa.eu/en/panels/nda

[10] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf

[11] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf

information as well as the full report is available on the European Chemicals Agency (ECHA)'s website.

11. Date for next meetings

The following dates were discussed:

- TBC, 2019, Ispra, ITALY
- Nano Stakeholders Engagement Event – TBC- Availabilities of the WG were discussed.

12. Conclusions

The members of the nano network made efforts to distribute the EFSA guidance for risk assessment to the users in their countries. This has led to an increased understanding of the EFSA Guidance. The correct implementation of this guidance is pivotal to ensure appropriate risk assessments of products used in the food and feed chain. At this network meeting, some issues concerning applicability of the guidance were presented and discussed among the participants.

The members of the network agreed amendments of the current guidance, especially on the physical chemical characterisation, which might be useful to the basis on the outcome of the piloting phase. The applicants are responsible for submission of the relevant data using the adequate methodology.

13. Closure of the meeting

The Chair thanked the members of the network for their contributions and time to attend the meeting.