European Food Safety Authority

Scientific Committee Unit

Network for

Risk Assessment of Nanotechnologies in Food and Feed

Minutes of the 2nd meeting

Held on 03-04 04 2012, Parma

(Agreed on 15 September 2012)

Participants

- **Network Representatives:**

Angelika Keckeis (AT), Jan Mast (BE), Popi Kanari (CY), Gabriela Salejová (CZ), Alfonso Lampen (DE), Josep Samitier (ES), Liv Kukkonen (FI), Gilles Rivière (FR), Darko Mikec (HR), Andrea Zentai (HU), Patrick O'Mahony (IE), Francesco Cubadda (IT), Mindaugas Morkunas (LT), Zorica Dekic (ME), Suzana Popovska (MK), Anton Rietveld (NL), Wojciech Wąsowicz (PL), Maja Remškar (SI), Zehra Karagoz-Emiroglu (TR), David Gott (UK).

- **Hearing Experts:**

Qasim Chaudhry, Rolf Hertel, Vittorio Silano, Stefan Weigel.

- **European Commission:**

Georgios Katalagarianakis, Hermann Stamm.

- **EFSA:**

SCOM Unit: Djien Liem (Network Chair), Reinhilde Schoonjans (Co-chair), Daniela Maurici, Theresa McFadden, Francesca Piombini.

1. Welcome and apologies for absence

Djien Liem, Head of the Scientific Committee Unit (SCOM) and Chair of the Network welcomed the participants to the 2nd meeting of the EFSA Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed. The Network was set up to exchange information on nanotechnology in the food/feed area, to identify priorities or knowledge gaps in this sector, and to promote scientific co-operation for risk assessment. The priorities as identified at the first meeting of the Network will be further discussed during this second meeting of the Network.
During a tour de table the participants of the Network presented themselves, their background and affiliation. Apologies were received from the Bulgarian, Danish, Greek, Norwegian, Portuguese and Slovak representatives.

2. Adoption of agenda

The agenda was adopted without changes.

3. Declarations of interest

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes regarding Declarations of Interests (DoIs)[1] and the Decision of the Executive Director implementing this Policy[2], EFSA screened the Annual Declaration of interest (ADoI) filled in by the experts invited for the present meeting. No conflicts of interest related to the issues discussed in this meeting have been identified during the screening process or in the Oral Declaration of interest (ODoI) at the beginning of this meeting.

4. Agreement of the minutes of the 1st meeting of the Network on Risk Assessment of Nanotechnologies in Food and Feed held on 11 February 2011, Parma.

The minutes were agreed by written procedure on 12 May 2011 and published on the EFSA website http://www.efsa.europa.eu/en/events/event/scaf110222-m.pdf.

5. Topics for discussion

5.1 Definition of nanomaterial

Prior to the meeting, the Network was provided with the Recommendation of the European Commission on a definition of nanomaterial as published on 18 October 2011[3] and the opinion of the European Commission's Scientific Committee of Emerging and Newly Identified Health Risks (SCENIHR) of 6 July 2010 that provided the scientific basis thereto[4].
The Network discussed the potential consequences for EFSA's work when such definition would be applied to food and feed:

1) The inclusion of agglomerates and aggregates into the definition of nanomaterial was considered useful to cover top-down produced nanoparticles, but would on the other hand have an impact on reproducible risk assessment of nanomaterials for example due to different (and often lower) bioavailability and toxicity as compared with original nanoparticles. Italy underlined that agglomeration and aggregation of a nanomaterial should be always determined at the dose levels used in the actual testing media for toxicological testing. Germany supported the tiered approach proposed by SCENIHR for different size categories of nanomaterials, but noted that the threshold of 0.15 % suggested by SCENIHR is not consistent with the threshold of 50 % as recommended by the EC.

2) The inclusion of agglomerates and aggregates would also imply that risk assessment should be carried out on a mixture of different-size nanoparticles with distinct properties rather than with a homogeneous preparation of nanoparticles. Slovenia, Germany, Poland and Czech Republic proposed to carry out both: firstly on the nanoparticles of homogeneous size, and then on mixtures. Italy is of the opinion that experimental data on the relation between average particle size and toxicity are needed. Belgium raised the point that the polydispersity in size of the nanomaterial used for risk analyses should be in line with the polydispersity of the nanomaterial used in practice when it is in its most disperse state, taken into account and maintaining the structure of the material.

[1] http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf
[2] http://www.efsa.europa.eu/en/keydocs/docs/independencerules.pdf
[3] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF; 275/40 Official Journal
[4] http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_030.pdf

3) Network members acknowledged that inclusion of agglomerates and/or aggregates of nanoparticles in the test doses may result in a reduction of the effects detected rather than in their increase (or vice versa). Slovenia mentioned that the degree of agglomeration in some bio-media similar to body fluids in animals can be tested *in vitro*, which is useful when trying to estimate the effect of agglomeration on safety. On the other hand, some processes inside the body can cause de-agglomeration and dissolution of nanoparticles; therefore the *in vitro* results should be considered carefully. Italy underlined that a suitable dispersion protocol should be used during the preparation of nanomaterial suspensions, so that aggregation is avoided. Furthermore, thorough toxicological testing at low doses should always be carried out and attention should be paid to repeated dose toxicity with a variety of endpoints. Belgium raised the point that size distribution measurements are desirable on the different doses used for testing. Germany suggested the determination of size distribution of free nanomaterials, aggregates and agglomerates in the target cells/tissues in order to facilitate the interpretation of the test results.

4) Follow-up actions for the proposed EC definition were reported from Belgium where ongoing research looks into quantitative analysis of the physical characteristics of primary particles and aggregates of manufactured nanomaterials by transmission electron microscopy. This research focuses on the estimation of the uncertainty of the measured percentage of particles < 100 nm. Germany reported on projects under consideration to elucidate possible consequences of the application of SCENIHR recommendations for future risk assessments, i.e. to check if the available techniques are appropriate to assess if > 0.15 % of the number size distribution is < 100 nm.

5) The Recommendation on a definition will be reviewed by the EC before December 2014. Network members (Poland, Portugal, and Czech Republic) commented that the outcome of ongoing research activities will be important for such revision (see for example item 5.2). Slovenia suggested that the term 'internal structure' as now proposed in the definition should be replaced by 'internal fragmentation with a possibility to be released out of the material', in order to exclude every bulk material composed of nanograins, for example steel or polymers.

The representative from the European Commission's Joint Research Centre (JRC), Dr Herman Stamm, explained the technical elements of the definition in detail. The Network ratified that EFSA should further inform the EC when refining the definition on nanomaterial and its applicability to food and feed. EFSA shall also indicate which risk assessment elements might be made difficult by some components of the definition. Issues to be considered include for instance that aggregates can release smaller particles when going through changing pH conditions and changing ionic strengths as occurring during their passage through the human or animal gastrointestinal tract. Another example is the accumulation of nanosilver in certain cells near the digestive tract and aggregation in certain body parts. For the longer term planning, the Network ratified that the EFSA Guidance document of 2011 is to be revised to make it consistent, as much as possible, with the definition once adopted.

5.2 Presentation of the FP7 project NanoLyse

Dr Stefan Weigel of the RIKILT Institute of Food Safety presented the major achievements of the NanoLyse project[5], aimed at developing and validating analytical tools for detecting nanoparticles in foods. Different instruments, methods and parameters to detect and characterise nanomaterial in complex matrices were explained in detail and their strengths and weaknesses were reviewed. One particular criterion under investigation is the capacity of the method to count particles in the size-

[5] http://www.nanolyse.eu/default.aspx

range of 1-20 nm. Problematic issues and future challenges for testing food and feed for the presence of nanomaterial were identified. The overall aim is to propose one common, validated detection method to be uniformly applied in the EU.
The Member States agreed to share with the NanoLyse group the contact details of national laboratories that have experience with detecting and measuring nanomaterials in complex matrices. The NanoLyse group will contact these labs when distributing protocols for testing/validation and for technology transfer. It was concluded that the outcomes of the NanoLyse project are awaited and are important for the adaptation of the Recommendation on a Definition by the EC in 2014 (see Item 5.1.5).

5.3 Update on EU Research projects

In his presentation, Dr Georgios Katalagarianakis from DG Research & Innovation (RTD), highlighted the strong commitment of the EU for technology progress in the area of nanotechnology, for its safe implementation and for monitoring the relevant societal needs with respect to nanotechnology now and in the future. The compendium of projects in the European NanoSafety Cluster, which is the safety pillar of the NanoFUTURES[6] hub, and in particular the projects therein which are relevant for food were explained following the overview matrix of the compendium 2012[7]. A snapshot of the progress in the most important areas of nanosafety was given: Material characterisation is well advanced for most common nanomaterials but has encountered difficulties with variations, stability, changing environment, ageing etc.; Hazards for nano are mostly understood with difficulties noticed regarding quantification, combination, long-term and special cases; Eco-toxicity has taken some delay; Exposure monitoring advances fast to cover for such delay but the challenges in this area are unclear metrics (number, mass, surface), release from the matrix, and the fate of the nanomaterial. Life-Cycle Analysis advanced well and some progress was marked for risk reduction. Faster progress is needed for risk evaluation and risk communication.
International co-operation for safety assessment - for instance for information technology used, or for the formulation of risk assessment conclusions, or for determining regulatory test requirements - is a key element and remains high priority for the EU. A stronger co-ordination between the EC and European Member States would be helpful in order to develop joint priorities. One of the most important aspects for such co-operation is the need for an effective system for sharing early information relevant for risk assessment of nanomaterials in food production processes and in food, on their effects on organisms, and their fate in the environment. This EFSA Network is a helpful system for such information sharing.

5.4 Activities at the Joint Research Centre concerning Nanomaterials in EU Regulations

The JRC representative, Dr Herman Stamm, announced the launch in May 2012 of an inter-laboratory comparison study on particle size distribution measurements in the range of 1-200 nm in simple matrices. National control laboratories in Member States were to be invited for participation and the EFSA Network agreed to provide Dr Stamm with the contact persons of relevant national control laboratories. A number of technical tools were under consideration for testing but no best practice has yet been identified. The outcome of this ring-test will allow the assessment of the state of readiness of EU analytical laboratories for application of the proposed definition of nanomaterial in a legal framework. In the discussion following his presentation, Dr Stamm mentioned that this work was being implemented for nanomaterial chemicals under the REACH legislative framework.

[6] http://www.nanofutures.eu/
[7] http://www.nanosafetycluster.eu/uploads/files/pdf/Compendium2012_u2_web.pdf

5.5 Feedback from the Network on the priorities identified at the 1st meeting of the Network for further co-operation and development

The priority issues regarding nanotechnology in food[8] were discussed and updated by the Network as follows:

1. The EC Recommendation on a Definition of nanomaterial is to be developed and clarified further, ideally before making an inventory list of nanomaterial in food/feed on the market. Member States that have embarked on inventories of general uses of nanomaterial are FR, IT, BE, NL, DE, SE, DK as a consortium together with the JRC, and the UK and CY to a certain extend.

2. The analysis and monitoring of products under development and on the market for the presence of nanomaterial is considered important and information is needed on the set-up of a system thereto, a legal framework and/or control mechanisms. For these purposes, detection methods need to be available and validated. The technology from NanoLyse is therefore awaited for transfer to the Member States and dissemination in 2013.

3. Validated *in vitro* and *in vivo* tests following oral exposure are needed. The Network members asked to be alerted on toxicity testing approaches and data when such would become available (even when from the field of cosmetics). During this annual meeting Network representatives reported that the following toxicological studies are performed:

    - Inhalatory studies with nanosilver conducted in which only the highest dose, but not the lower dose, caused toxic effects;
    - Studies on oral uptake of nanosilver;
    - Oral 5 days repeated dose studies on rats will be concluded soon for TiO_2, wherein the lowest dose gave results while no bioaccumulation was observed. A 90-day oral feeding study is also being planned.
    - The EU Nanogenotox project was also mentioned and more info is given in 5.7 under the item research needs.

    Throughout the year, Network members can alert each other on available results and toxicity data trough a dedicated online exchange platform Extranet.

5.6 Feedback from the Network on its tasks of the Terms of Reference.

The Network provided feedback on each of the tasks under the Terms of Reference[9] as follows.

- To **share best practices**, Network members reported on national and international meetings and workshops that were organised in 2011 for informing regulators, scientific community and the stakeholders about risk assessment of nanomaterial in food/feed. Slovenia also reported a scientific experiment wherein the spread of nanoparticles from fireworks was measured by scanning the mobility and particle sizes. The same instrument can be used in other activities: spraying, defence against hail, forest fire, volcano eruption, dispersing of soil under strong wind, etc. The velocity of spread of nanoparticles in relation to their size and the absolute amounts of nanoparticles dispersed through fireworks were reported in this study[10].

[8] Identified at the 1st meeting of this Network in 2011: minutes http://www.efsa.europa.eu/en/events/event/scaf110222-m.pdf
[9] http://www.efsa.europa.eu/en/scnetworks/docs/scnanotechnologiesnetworktor.pdf
[10] http://cobiss.izum.si/scripts/cobiss?command=DISPLAY&lani=en&base=COBIB&RID=24897063#

- In order to **streamline common research needs**, members of the Network discussed ongoing research projects at their national levels or their participation in EU projects:

    - The NanoLyse project (see item 5.2);
    - The Nanorisk project in Belgium (CODA)[11] aims to adapt methods for assessing the exposure to nanoparticles and to provide complementary detection methods for the detection of nanoparticles in complex matrices such as food and feed. The proposed approach is based on a step-by-step, size-dependent fractionation of the matrix, combined with different detection methods such as TEM, NTA and ICP-MS. Existing *in vivo* methods are being assessed and adapted for the assessment of oral toxicity of nanoparticles. Particular attention will be paid to toxicokinetic and subchronic studies, in order to identify potential target organs for the nanoparticles, to detect their presence therein and/or potential toxic effects caused. *In vitro* toxicity studies will contribute to the understanding of the specific toxicological effects of nanoparticles such as absorption and transport through barriers, oxidative stress and inflammation.
    - The Nanogenotox project[12] aims to develop a robust and consolidated methodology to assess the genotoxic potential of nanomaterials and to establish a correlation between *in vitro* and *in vivo* results. 15 manufactured nanomaterials (4 TiO_2, 4 SiO_2 and 7 carbon nanotubes), commercially available or soon to be on the market, are being investigated. They were chosen based on their potential use in common consumer products including food and accordingly the oral exposure route has been investigated. Silica nanomaterials are considered the most relevant for use in food and feed applications by the Italian Network member and the toxicokinetic and general toxicity of these silica have been studied by his institute which is the Italian consortium partner of the EU project.
    - Slovenia reported on the Micro-proton induced X-ray emission (micro-PIXE) localisation study of Ag in digestive glands of a nano-Ag fed terrestrial arthropod (*Porcellio scaber*)[13]. The results obtained documented high amounts of Ag inside S-cells of the digestive gland epithelium. However, TEM investigation did not show particle aggregates inside digestive gland cells. Also no adverse effect on feeding behaviour was recorded which is a measure of toxic effects. The presence of Ag inside the cells is interpreted as a result of the assimilation of dissoluted Ag ions from ingested nano-Ag particles. Assimilation of excessive amounts of ingested metal ions in S-cells is a well known metal detoxification mechanism in isopods.

    ❖ During the discussions, the Network identified a need to know the national laboratories that are competent to analyse food samples upon request and that are able to verify presence of and characterise nanoparticles. Therefore, the Network decided on creating an effective template for listing the laboratories in EU MS that are competent in testing nanomaterial, the physicochemical/analytical methodologies used in those labs, and the nature of the nanomaterials tested.

    ❖ As to the need for certified reference materials and standard material to work with in research, safety testing or detection, the JRC representative referred to homogeneous Silica for size measurements. The Network underlined the existing need for also soluble nano reference material.

[11] http://www.coda-cerva.be/index.php?option=com_content&view=article&id=366%3Ananorisk&catid=181%3Alatest-news&Itemid=264&lang=en

[12] http://www.nanogenotox.eu/

[13] http://www.bionanoteam.com/publications/micro-pixe-study-of-ag-in-digestive-glands-of-a-nano-ag-fed-arthropod/

- Regarding the **mapping of new expertise** needed in the remit of the Network and the work on nanosafety, Austria mentioned two calls for researchers: one regarding safety assessment of nanomaterials in consumer products and safety precautions for workers; and one for environmental monitoring and exposure[14]. Slovenia reported on a public dialogue through telephone surveys[15], showing that only 12% of the respondents understood what nanotechnology is and therefore more (risk) communication expertise regarding nanotechnology in food is required. Interestingly, 75% of the respondents said to decline the use of nanotechnology in food.

- Regarding opportunities for **mutual co-operations** between Member States, references were made to international conferences or workshops, and the EU community Research projects, wherein such co-operations can be integrated.

6. Any Other Business

The Network is committed to modulate its working procedures as new needs for sharing information arise. All presentations given at the meeting were made available to the Network and immediately after the meeting the summaries and conclusions of each discussion were shared.

7. Next meetings

The next yearly meeting for this Network will be scheduled in 2013.

[14] http://www.ffg.at/nano-ehs
[15] http://www.arhiv.mkgp.gov.si/fileadmin/mkgp.gov.si/pageuploads/EFSA/nov11/javnomnenjska_anketa_SI.pdf