Network on Risk Assessment of Nanotechnologies in Food and Feed

Minutes of the 5th meeting

Held on 7-8 July 2015, Parma

(Agreed on 10 December 2015)

Participants

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

Country	Name
Austria	Melanie Kuffner
Belgium	Jan Mast
Bulgaria	Angel Angelov
Cyprus	Apologies
Croatia	Darko Mikec
Czech Republic	Vladimir Ostrý
Denmark	Alicja Mortensen
Estonia	No nominations received
Finland	Pertti Koivisto
France	Apologies
Germany	Alfonso Lampen
Greece	Aristotelis Xenakis
Hungary	Krisztian Varga
Ireland	Patrick O'Mahony
Italy	Francesco Cubadda
Latvia	No nominations received
Lithuania	Apologies
Luxembourg	No nominations received
Malta	No nominations received
Netherlands	Jaqueline Castenmiller
Poland	Wojciech Wąsowicz
Portugal	Helena Carmo
Romania	No nominations received
Slovakia	Peter Simon
Slovenia	Viviana Golja
Spain	José Manuel Barat Baviera
Sweden	No nominations received
United Kingdom	David Michael Gott
Iceland	No nominations received

Liechtenstein	No nominations received
Norway	Ragna Bogen Hetland
Switzerland	No nominations received

- **Hearing Experts:**

Qasim Chaudhry (FERA), Claus Svendsen (NERC-CEH) and Stefan Weigel (RIKILT).

- **European Commission:**

Siret Surva (DG SANTE); Hubert Rauscher (JRC);

Dolores Henan, Ruben Pita and Falk Ehmann (EMA); Cecilia Tanarro (ECHA) participated via teleconference.

- **EFSA:**

SCER Unit: Reinhilde Schoonjans (Chair), Tilemachos Goumperis

FIP Unit: Paolo Colombo, Ana Rincon, Federica Lodi, Anne Theobald

FEED Unit: Maria Vittoria Vettori

PRAS Unit: Andrea Terron, Maria Arena

NDA Unit: Wolfgang Gelbmann

ApDESK Unit: Tom Meyvis

1. Welcome and apologies for absence

The Chair, Reinhilde Schoonjans, welcomed the participants. Apologies were received from network members as indicated in the above table.

2. Adoption of agenda

The agenda was adopted without changes.

3. Agreement of the minutes of the 4th meeting of the Network on 21-22 October 2014, Parma.

The minutes were agreed by written procedure on 26 January 2015 and published on the EFSA website.

4. Topics for discussion

4.1 Nano Definition and its technical aspects

Hubert Rauscher (JRC) gave a presentation to the network on the Science for Policy report Part 3 'Towards a review of the EC Recommendation for a definition

of the term "nanomaterial"[1]. Scientific-technical evaluations were considered when reporting the options to clarify the definition (EC Recommendation 2011/696/EU) and to facilitate its implementation. This recommended definition has the purpose of being a general source of reference for many application fields, including the regulatory purpose of food labelling.

The JRC evaluation supports the recommendation that the scope of the definition should remain the same and address natural, incidental as well as manufactured nanomaterials. There is little evidence to support deviating from size as the sole defining property of a nanoparticle or from the 1 nm to 100 nm size range as a definition of the nanoscale.

The report mentioned that there is a need to clarify the following issues in the definition and/or provide additional implementing guidance: particle, particle size and external dimension, minimal external dimension, constituent particle. There is a conceptual difference between a threshold which refers to the number fraction of particles with external dimensions between 1 nm and 100 nm in a material (currently 50 %), and a content threshold for such materials in a product. Using the phrase 'mainly consisting of particles' in the definition (rather than the currently used 'containing particles …') could prevent the misunderstanding that products containing nanoparticles become nanomaterials themselves. A flexible threshold of nanoparticle (1-100 nm) fraction (1-50%), would allow regulators to address specific concerns.

Also the role of the volume specific surface area (VSSA) deserves clarification (see next agenda item) and a method to prove that a material is not a nanomaterial would be helpful. A strategy on how to avoid unintended inclusion of materials and the list of explicitly included materials also deserves attention. A discussion is on-going about including or excluding, for example, single molecules, micelles and non-solid materials. It was concluded that additional guidance for the implementation of the definition would be useful, particularly on good measurement/sampling practice with a list of methods and emphasising the responsibility of the analyst to judge the reliability of the method.

The EFSA Nano Network strongly supports this suggestion to provide a detailed implementing guidance.

4.2 Progress report NanoDefine

Stefan Weigel (RIKILT) presented the planning and progress of the NanoDefine project (EU FP7). The twenty-eight NanoDefine project consortium members from all over Europe are developing an integrated analytical approach to implement the EC Recommended Definition of Nanomaterial (see previous agenda item).

This project addresses the analytical challenges inherent to the recommendation: (1) the number/size distribution is preferred, but the conversion from intensities, volumes or mass to numbers is prone to errors; (2) measuring below 30 nm can only be done with few techniques and measuring

[1] See http://publications.jrc.ec.europa.eu/repository/bitstream/JRC95675/towards%20review%20ec%20rec%20def%20nanomaterial%20-%20part%203_report_online%20id.pdf

simultaneously 1-100 + above 100 nm needs to be addressed; (3) agglomerates and aggregates require de-agglomeration or finding particles in aggregates.

The concept to implement the definition is to use a tiered approach (from widely available methods to more sophisticated ones for complex samples) and a NanoDefiner e-tool for method selection and NM classification. Existing methods and algorithms will undergo a theoretical and an experimental evaluation. For volume specific surface area (VSSA) there will be collaboration with the NanoReg project. New instruments and software will be developed for enhancing data quality. Reference materials, validation of methods and standardisation (in liaison with CEN/ISO) are foreseen. Furthermore, there will be recommendations to revise the definition based on analytical possibilities for e.g. external dimensions of individual particles, particle size distribution, aggregates, role of VSSA, colloids, and mixtures.

NanoDefine is expected to deliver the test schemes and tools for possible consideration by concerned industries and regulatory agencies by October 2017.

4.3 Solubility – dissolution – ions and translocation/toxicity

Qasim Chaudhry (FERA) moderated this discussion session.

4.3.1 Solubility

A reflection on the issue of solubility is needed since this is often invoked to imply lower risk, due to loss of the particulate nature. Few materials, however, are readily soluble and while dissolving, smaller – potentially more toxic – particles are generated.

The Italian delegate acknowledged that intracellular presence and toxicity increase in a size-dependent manner. This makes the issue of solubility and adequate toxicity testing important, especially when NM becomes smaller and smaller. The Portuguese delegate added that in addition, the individual metabolism differs among different people.

The Spanish delegate said that in addition to the issue of solubility in water, the interactions with acids also need to be considered. He expressed the concern that mimicking such interactions by testing compounds in vitro may not be realistic enough.

Alfonso Lampen (DE) presented an introduction to the SolNanTox project. This is an on-going DE-FR research project that is expected to unravel the influence on uptake and toxicity of solubility (Al -NP) versus non-solubility (TiO2). Different work packages of the project will focus on mechanisms of uptake and toxic effects and will run until August 2017. This comparative study should provide insight into the critical aspect of solubility for risk assessment.

The Nano Network was informed that this subject is also included in the NanoReg (FP7) project.

4.3.2 Soft Nanomaterial

The term ‘soft’ nanomaterials is generally used for liposomes, nano-emulsions, and (bio)polymer based encapsulates. The Irish delegate reported to the Network a discussion between industries and scientists on the fact of whether or

not soft nanomaterials require risk assessment. However, if a product is a novel food, for example, then it will require a safety assessment before being allowed on the market. Similarly, this is the case for new supplements, food contact materials etc. From these discussions it appeared that some details of the legislation (e.g. on novel food, supplements) and its implementation in the EU, might not be sufficiently clear to all stakeholders. Therefore, it is proposed to include in the agenda of next year's meeting, a topic on the implementation of the Novel Food Regulation, and its revised version.

The EFSA Nano Network, in its meeting of July 2015, recognised the further need for consideration of 'soft' formulations designed to deliver nutrients/supplements in food and health-food products at the nano-scale. This is because some of the nano-scale delivery systems have been described to significantly enhance the uptake and bioavailability of the encapsulated substances.

4.3.3 Translocation

An EFSA colleague raised the point that coatings are authorised on a wider scale for products beyond the remit of EFSA (e.g. to make pills). These coatings can protect the material at one site and facilitate the take-up by cells at a given target site. There is a link between the issues regarding formulations and the assessment of NMs: examples are vitamin C and vitamin A that are usually water dispersible, but can now be designed to have changing basic chemical properties. The UK delegate confirmed that, while there are regulations on core materials, there are regulatory issues for delivery systems such as for nutrient sources and for mixtures.

In 2011 the Scientific Committee of EFSA published its 'Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain'[2]. The Danish delegate remarked that EFSA has to take forward the issues that have been raised in the meantime. Adsorption, which is to be assessed case-by-case for nutrient sources, food additives or novel food, has to be compared with the non-nano form. Regarding systemic absorption of nanomaterial after oral exposure, standards to measure the ADME for nanomaterial are to be developed.

Alfonso Lampen (DE) presented an on-going NL-DE project that is expected to unravel the influence/protection of food matrix components and digestion on the cytotoxicity of NPs. Bioavailability of NPs from the gastro-intestinal tract is studied with the aid of Ag NPs. The results demonstrated a coating-related effect and negative Zeta Potential mediated enhanced uptake. Transport mechanisms of NPs through the gastrointestinal barrier are studied with the aid of Fe NPs. The results demonstrated coating-related effects and enhanced uptake via M-cells.

Helena Campo (PT) presented the influence of shape in the biokinetics of Au NPs. The results demonstrated that there was less toxicity observed with the star-shaped particles than with the spheres. In vitro, there is also a clear

[2] See http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2140.pdf

increased cytotoxicity for larger Au NPs. In vivo, there was a poor recovery of administered Au and EtOH in vehicle does not seem to influence the biokinetic profile after oral administration. Larger particles appear in blood after 24h, are excreted in urine and are present in the kidneys. It was concluded from this research in Portugal that Au NPs are only very poorly absorbed after oral administration.

4.4 Updates from Member states

Germany and Portugal gave feedback on relevant research in the previous agenda item.

4.4.1 BE: release of silver nanoparticles from confectionary

Jan Mast (BE) presented an analysis of the release of silver nanoparticles from decoration of pastry. Silver in food is authorised for specific uses and is indicated on the label as E174 or as 'silver'. The techniques used in the analysis were TEM, SP-ICP-MS and ICP-MS. It was demonstrated that silver nanoparticles are released from the silver-coloured coating of confectionary: more than 95% of these particles are smaller than 100 nm, which corresponds to 20% of the mass of silver.

4.4.2 NL: Update on on-going research with TiO2

Jacqueline Castenmiller (NL) presented an on-going research with the aim of obtaining insight into the potential health risk of titanium dioxide nanoparticles present in food. Titanium dioxide (TiO2) is a food additive used to enhance the white colour and brightness. The step-wise risk assessment approach includes the following steps. First the dietary intake is investigated by analysing the presence of nano TiO2 in 24 foods and 3 personal care products. In a second step the toxicokinetic data are investigated and a kinetic model is deduced. This kinetic model is subsequently applied for estimating human organ concentrations after 20, 40 and 80 years of daily exposure via food. The kinetic model is also applied to estimate animal organ concentrations in toxicity studies according to the applied dosing regime. At step five, the external doses from dietary intake in humans and doses in hazard assessment, are used for risk assessment and the determination of margins of exposure (MoE). Also at step five, the internal modelled concentrations in human organs and organs from rats and mice, are used for risk assessment and determination of margins of safety (MoS). This risk assessment will be finalised in the forthcoming months.

4.4.3 ES: Update on on-going research

Jose M Barat (ES) presented the activities of the Spanish network for nanomaterials in food. This network consists of industrial partners, Spanish administration, research institutions and consumers organisations. At the fifth meeting on 1 June 2015, the focus was to identify groups that work on food and feed safety of nanomaterials and to stimulate cooperation. Various improvements in food applications by using nanomaterials are being discussed, mainly new ways to improve food packaging, the use of nanoparticles of essential oils as antimicrobial agents and controlled release through encapsulation in mesoporous silica particles. On-going safety research in Spain is looking at release of nanoparticles from packaging, toxicology for the

environment, toxicology of nanoclays and nanosilica, and the use of molluscs, zebrafish and *C. elegans* as model systems for safety studies.

4.4.4 IT: analytical methods

Francesco Cubadda (IT) presented the development of analytical methods for quantitative characterisation of particles (size distribution and mass concentration) in food matrices. Accurate size characterization of particles separated by FFF was achieved by ICP-MS/MS (with size calibrants) and by MALS. Quantification of the silicon present in the size fractions separated by FFF, was achieved by element-specific detection with ICP-MS/MS using all the three silicon isotopes, pre-channel mass-calibration with silica nanoparticles and post-channel mass-calibration with elemental standards. The FFF-MALS-ICP-MS/MS method enabled dimensional and mass determination of silica particles over the size range of approximately 20–200 nm with satisfactory recoveries of the analyte material. The method enabled characterisation of two test samples, i.e. the reference material ERM-FD100 and a silica suspension having nominal diameters of 20 and 140 nm, respectively. The method is now being successfully applied to the analytical determination of synthetic amorphous silica (SAS) in food.

4.4.5 IT: oral toxicity study with SAS

Francesco Cubadda (IT) presented the progress of work package of the FP7 project NANoREG: Subchronic oral toxicity study on synthetic amorphous silica (SAS) to be performed on the basis of the OECD TG 408 with the aim of identifying hazards and obtaining dose-response data. The global market volume of SAS is 1.5 million tons a year (i.e. not limited to food applications) and dietary exposure of consumers to nanosized SiO2 occurs. He presented the details of the study design and management for general toxicity: the NM characterisation and biodistribution, reproductive toxicity, genotoxicity and male fertility, and immunotoxicity. The final results will be available before the SAS re-evaluation by EFSA.

4.5 Risk Assessment of nanomaterial at ECHA

Celia Tanarro (ECHA) reported on the activities of the European Chemicals Agency (ECHA) on nanomaterials within the registration and evaluation of nanomaterials under REACH. This is an extensive legislation covering most chemicals and where the burden of proof is placed with industries that must ensure the safe use of chemicals. This legal framework also aims to reduce animal testing by promoting alternative methods (e.g. QSARs). ECHA and the Member States evaluate the information submitted in the registration dossiers and may ask for further information. This framework allows for authorisations and risk management restrictions upon risk analysis and socio-economic analysis.

For nanomaterials, there is no explicit reference in REACH so far, but they are treated as substances in their own right. Few NMs (13 out of a total of 12,439 unique substances) have been registered by using the voluntary field in the registration dossier.

ECHA is taking initiatives to support registrants with guidance and advice on nano specific issues as follows: ECHA provides support to the Competent

Authority Subgroup on Nanomaterials (CASG-Nano), the ECHA Nanomaterial working group (NMWG), the OECD activities, relevant FP7 projects and CEN/ISO activities. ECHA's Group Assessing Already Registered Nanomaterials (GAARN) is composed of ECHA staff, Commission staff, Member States Competent Authorities and representative registrants. The challenges for registering substances with nanoforms under REACH and on the information requirements are (1) substance identification, physical chemical properties, and (2) hazard information (toxicology and ecotoxicology). GAARN therefore issued three best practice reports (informal and based on consensus) for assessing and managing the safety of nanomaterials under the REACH regulation. Now ECHA is preparing guidance on nanomaterials to assist stakeholders in implementing their obligations, but it is not legally binding. ECHA guidance usually undergoes extensive consultations to make it acceptable to those using it (and those enforcing the legislation).

In 2012, ECHA prepared updates to the guidance on Information Requirements and Chemical Safety Assessment (IR&CSA[3]) in the form of Appendices to the guidance on 'Recommendations for nanomaterials'. These Appendices explained the issues that are different for nanomaterials, for instance: Appendix to R7a on specific considerations regarding the IR on granulometry and Appendix to R14 including specific consideration regarding exposure assessment for nanomaterials (available devices, measurement strategy etc.).

The new guidance projects on NM entail efforts to (1) Define the borderline between different nanoforms and different substances to be considered in their own right); (2) Clarify concept and/or principles on how nanoforms can be 'clustered' into different groups; and (3) Clarify what is required in terms of tonnage calculation and related hazard information. ECHA will also make efforts for read-across between nanoforms.

It is currently under consideration to update several guidance documents [currently available] on nanomaterials: Appendices to Chapters R7a, R7b and R7c (Information requirements for physico-chemical properties, human health and the environment) and Appendix to Chapter R14 (Occupational exposure estimation).

The EFSA Nano Network welcomed the update by ECHA with the view to explore synergies between ECHA and EFSA, e.g. to give guidance on how coating and shape of particles influence their physico-chemical measurements. The Nano Network remarked that more complex studies, such as long term Environmental Risk Assessments, are bringing forward new ideas and new methods that should also be considered. The Nano Network encourages taking these considerations on board during guidance development and not only for human health impact assessments.

4.6 Risk Assessment of nanomaterial at EMA

Dolores Hernan, Ruben Pita and Falk Ehmann (EMA), presented activities on Nanomedicines at the European Medicines Agency (EMA). Under the EMA legal framework the marketing authorisation shall be refused if it is clear that the risk-

[3] See http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

benefit ratio is not considered to be favourable, if the therapeutic efficacy is insufficiently substantiated by the applicant, or if the qualitative and quantitative composition is not as declared.

A Nanomedicine is a system purposely designed for clinical applications and having at least one component in the nano-scale size. The design and size result in having definable specific properties and characteristics related to the intended use (route of administration, dose) and associated with the expected clinical advantages (e.g. preferential organ/tissue distribution, integration of efficacious molecules that could otherwise not be used because of their high toxicity, reduction of dose and toxicity). EMA has about 20 applications pending and has given over 50 reports on scientific advices and protocol assistance on nanomedicines.

EMA issued reflection papers on nanomedicines: for Block Co-Polymer Micelles, for Liposomal formulations, for Iron-oxide nanoparticles, and one on surface coating[4].

The International Pharmaceutical Regulators Forum (IPRF) has a Nano working group with the objective of cooperating, data sharing and promotion of potential consensus finding on standards. The workplan details the objectives and deliverables, including posting of updates on the IPRF public website (https://www.i-p-r-f.org/en/).

The EFSA Nano Network welcomed this overview of EMA activities and encouraged exploring synergies between EMA and EFSA, especially in the area of soft nanomaterials. Potential for cooperation between EMA and EFSA in this area arise from the fact that soft materials can be designed to carry a drug/conjugate or fortified food/nutraceutical further in the body and/or release it in a controlled manner e.g. at a targeted rate and/or targeted body part. In this context the Nano Network reminded to check the sensitivity of the current toxicity tests. It also questioned if a certain chemical with unique CAS ID will need a new CAS ID when bound to a monomer. In this area, risk assessment should not try to put emphasis on the size, but on the specific properties of the soft nanomaterials that determine their property-based functionality.

4.7 Risk Assessments of nanomaterial at EFSA

Reinhilde Schoonjans (EFSA) and EFSA colleagues presented an overview of all the EFSA activities regarding the assessment of nanomaterials used in agri/food/feed. In 2011, the Scientific Committee of EFSA published its 'Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain'. The approaches described therein concern mainly the oral route of exposure and are to be implemented by applicants and Panel risk assessors. This is a leading guidance. Since 2011, the Scientific Committee monitors new developments in the legislation, instrumentation, methodology for RA and study results through its Nano Network of Member State delegates.

[4] See http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2013/08/news_detail_001875.jsp&mid=WC0b01ac058004d5c1

At EFSA, applications are being assessed for substances to be used as food contact materials, food additives, as novel food or as feed additives. It is to be noted that each of these uses has to be assessed under a different legal framework. In order to prepare for future applications, in 2014 EFSA procured an inventory of nanomaterials/nano-applications on the market or reasonably foreseen to be placed on the market[5]. A distinction needs to be made between engineered NM and bulk material coincidently comprising a nanofraction.

Food contact materials: EFSA has received and evaluated applications explicitly covering engineered nanomaterial to be used in food contact material with plastics. The risk assessments were based on zero exposure scenarios as there was no demonstrated migration into the food. Other applications are currently under evaluation. No applications have been received for nanoforms as flavourings.

Food additives: past and on-going applications are all on bulk materials. The ANS Panel is carrying out a re-evaluation programme for the majority of food additives and few of them may comprise a nanofraction (e.g. Silver (E174) and titanium dioxide (E171)). For the re-evaluation of already authorised food additives, there are neither applicants nor dossiers. EFSA launches calls for data so that data are gathered from the public domain or interested parties should provide and/or generate appropriate data. The EFSA calls for data on the bulk material may include questions about content of nanosized particles. TiO2 is being evaluated in line with a parallel evaluation as a feed additive, which is based on a different legal framework. Regulation (EU) No 231/2012 does not specify a limit for nanofraction in food additives. According to the information provided by interested parties, food additives may comprise a small nanoscale fraction, which can be considered as unintentionally present or formed. Moreover, the nanoform might even counteract the function of the food additive, e.g. nano-TiO2 is not whitening. Issues for special attention are the sufficient characterisation of nanofractions in biological studies (e.g. % of substance in nanoform; methodology used) and the amount of nanofraction potentially present in the food additive on the market.

Feed additives: the assessment of bulk TiO2 as a feed additive is under re-evaluation in parallel with its re-evaluation as a food additive. Size distribution data are part of the standard risk assessment dossier as submitted by the applicant. This material is grinded and coincidently comprises a nanofraction.

Novel foods: EFSA has not yet received an application for a novel food explicitly comprising a nanoform. The new Novel Food legislation explicitly includes 'engineered nanomaterials' and will soon be adopted.

Pesticides: EFSA assesses only the active ingredient. There is no nano-specific risk assessment yet of the active ingredients. In some of the formulations, however, which fall under the responsibility of the Member States, there are nanoforms. The pesticides network and the EFSA PPR Panel are in favour of developing regulatory guidance (including environmental risk assessment), most ideally by the EFSA Scientific Committee to ensure harmonisation in risk assessment[6].

[5] See http://www.efsa.europa.eu/de/supporting/doc/621e.pdf
[6] See http://www.efsa.europa.eu/sites/default/files/event/141111a-m.pdf

Contaminants: EFSA recently received a request from BfR for a scientific opinion on the presence of plastic microparticles and nanoparticles in food, with particular focus on seafood (EFSA-Q-2015-00159). The work is on-going.

4.8 Update on the regulatory framework

Siret Surva (DG SANTE, Unit E6 Innovation and sustainability) updated the Nano Network on 'Nanomaterials in food' and the current situation in the food legislation. A modification of the current definition of engineered NPs in Regulation (EU) No 2011/1169 on food information to consumers (FIC) is in progress, based on the Recommendation 2011/696/EU. A labelling requirement is provided in Article 18 of the FIC Regulation, saying that 'All ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word "nano" in brackets'.

The ENVI committee of the European Parliament has recently voted in favour of the compromise proposal that revises the novel food regulation. The text has been adopted by the plenary session of the EP in November 2015. The definition of engineered NP will be the same as currently in the FIC. This will be aligned later to the Commission Recommendation. Regarding the methods to be used for nanomaterials, EFSA has to verify that the most up-to-date methods will be used, that reference will be made to the conclusions of OECD Council recommendations, and that the appropriateness of the methods used are substantiated by the applicants.

It was confirmed that up to now there is no concrete application for a feed additive whose function is based on a particle size in the nano-scale, such as binding toxins to manufactured clays. For the plastics Regulation, the following substances have authorisations relating to nano-form: Titanium nitride nanoparticles, Silicon Dioxide, Carbon Black, (butadiene, ethyl acrylate, methyl methacrylate, styrene) copolymer in nanoform crosslinked with divinylbenzene, crosslinked with 1,3-butanediol dimethacrylate or not cross-linked; Kaolin.

The Nano Network noted that many different activities are running in parallel on the topic of nanomaterial and should be well coordinated between risk management and risk assessment. The concern is that the scientific work is being done on a definition which is mainly a risk management element of the regulatory framework.

4.9 Nanoparticles in the environment

Claus Svendsen (NERC-CEH) presented findings resulting from the FP7project NanoFATE, the TINE project and the Guide nano project. Methods for exposure and hazard characterisation in environmental risk assessment (ERA) of nanoparticles were presented. The approach is to first perform basic ERA by calculating the PEC/PNEC ratio for water and soil, and then to use more realistic exposure scenarios including real world exposure forms, real world media and bioavailability effects. The commercial, but pristine, NMs used were ZnO, Ag and CeO2. Systems and organisms were chosen to be inclusive and realistic, but also as standard as possible. The chosen environmental media and systems were the standard ecotox test media, fresh and marine waters, soil pore waters, soils and

sewage sludges. Both soil and water test species from less than 1 mm to more than 5 cm were used as organisms.

The questions to be addressed were: (1) Are the fate and behaviour of NM metals the same as of ionic metals? (2) Is the availability and uptake the same for NM and ionic metals? (3) Is the effect of internalised NM-derived metal the same as of ionic metal?

(1) It was demonstrated that in soil NP coating is important for exposure, fate and toxicity (e.g. higher toxicity for ZnO NP when coated). This is possibly relevant for nanopesticides and fertilisers. Also aging of NPs is important for exposure and toxicity (e.g. increased AgNP toxicity upon aging). This is relevant e.g. in relation to animal manures as long-term aging affects the relevance of conclusions from standard tests. It was noted, however, that even for NPs like ZnO and Ag, both expected to act through ion release, it is not possible to generalise these conclusions.

(2) It is probably not the case that dissolution/uptake by earthworms is only pore water based and only ion based. Therefore, further hypotheses of different uptake for NP (different from ionic) were explored. At high doses the uptake of Zn NP was higher than Zn ionic, but this could be a high dose artefact. In another experiment with sealed and unsealed earthworms, it was shown that Ion and NP exposures produce comparable uptake. Dietary intake is the main exposure route and is 10 times higher than dermal exposure. Thus, care should be taken when extrapolating from NP behaviour observed in the unrealistically high doses used for hazard testing, to realistic NP behaviour at environmental concentrations. The bioavailability studies showed that OM increase and/or pH increase result in diminished toxicity. This triggered the questions whether at low and environmentally relevant concentrations it might be all ions; and what are the possible long-term effects of accumulated NPs.

(3) Regarding the effects and the time scale, the point of debate is if the exposure media should be aged prior to the test, in order to achieve more realistic test outcomes. It was found that the fate time is longer than the testing time and that the aging of ZnO differs from the aging of Ag. From the TINE project, it was concluded that the effects of NPs are not only ion driven but that nano metal derived 'manure/sludge' has very different behaviour, uptake and effects to ionic equivalents.

5. Any Other Business

- Date for next meeting: The 2016 Nano Network meeting is planned on 30 June (13.00h) to 1 July (13.00h) in Madrid. The network was informed about the workshop of the Global Summit on Regulatory Science (GSRS15) on Nanomaterial Characterisation and Standards for Regulatory Consideration that will be hosted by EFSA on 11 October 2015 in Parma.
- Annual reports: The network was informed that the annual reports of this network have been covered by external media and are regularly viewed from EFSA's website e.g. the 2013 Advisory Forum network report was viewed 1,022 times during 2014, and the report was downloaded 370 times.

- Feedback from the Member States: Portugal, Slovenia and Norway reported in writing about new activities at national level. The files are kept on the common drive for Nano Network members.
- Training courses: The Nano Network members were invited to participate in the following trainings organised by EFSA: Benchmark Dose modelling course, Computational toxicology and modelling tools course, Systematic Review, and Expert knowledge elicitation. EFSA welcomed four participants from the Nano Network to these trainings.
- Possible topics for next year:
  - Supported by IE and BE delegates, it is proposed to discuss the new version and implementation of the Novel Food Regulation. It is considered urgent that EFSA prepares itself to be ready for full assessment of applications in that domain and to update the current guidance document of the Scientific Committee.
  - As research is now largely based on the materials of the NM repository of the JRC, it is supported by BE that more materials should be made available for research. This can be through co-operations from industries and/or through reference material development while expanding the repository.
  - It was also suggested to discuss the idea of pooling (on-going) safety research in order to extract the data in a harmonized way. This could facilitate the comparison of similarities and discrepancies with the aim to inform best practice protocols. There is a need for harmonised protocols and interlab studies to perform the toxicity studies.
  - The Nano Network wishes to receive an update on the implementation of the risk assessment approach as proposed by NL and debate its wider application.
  - Supported by EMA, it is considered important to mention the availability of validated test methods and standards when ensuring appropriate reporting of uncertainties in forthcoming risk assessments. Reference can be made to the on-going EFSA work in this area that will be in a pilot phase with the EFSA Panels in 2016 and some national risk assessment bodies at the time of the next Nano Network meeting.

- aSASp statement on amorphous silica: on 20 October 2014 the Association of Synthetic Amorphous Silica producers (aSASp) published its 'Statement for Synthetic Amorphous Silica regarding the definition of "engineered nanomaterials" for use in food in the European Union'[7]. It states that 'The recent EFSA Scientific Opinion No. 3712 of June 12, 2014[8]

[7] See http://www.asasp.eu/docs/ASASP_nanoinfood_20141020.pdf
[8] EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Statement on the safety assessment of the substance silicon dioxide, silanated, FCM Substance No 87 for use in food contact materials. EFSA Journal 2014;12(6):3712, 7 pp. doi:10.2903/j.efsa.2014.3712

has also concluded that silicon dioxide (i.e., SAS) does not include any isolated nanoparticles since only aggregates larger than 100 nm along with larger agglomerates were observed.'

- EFSA wishes to clarify that regarding the EFSA opinion referred to in the aSASp statement, the conclusion of the CEF Panel is that the substance silicon dioxide, silanated, is safe for use in the production of plastic food contact materials. This conclusion is based on the information provided by the applicant showing that no primary particles were detected in the starting silicon dioxide or the silanated silicon dioxide used in the production of the plastic film, only aggregates and agglomerates. Furthermore, no migration of the substance was detected in the simulants used. The conclusion of the EFSA opinion is not that silicon dioxide, as primary particles (E551), does not include any isolated nanoparticles as such. The information of size distribution/aggregation/agglomeration on the silicon dioxide, silanated, provided and referred to in the CEF opinion was used for the evaluation of the current use of silicon dioxide, silanated, in FCM.

Abbreviations

ANS Panel	Panel on Food Additives and Nutrient Sources Added to Food
CEF Panel	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEN/ISO	European Committee for Standardization/Organization for Standardization
EFSA	European Food Safety Authority
ELC	The Federation of European Specialty Food Ingredients Industries
ECHA	European Chemicals Agency
EMA	European Medicines Agency
ENVI	Environment, Public Health and Food Safety committee of the European Parliament
ERA	Environmental Risk Assessment
FIC	Regulation (EU) No 2011/1169 on Food Information to Consumers
FFF	Field-Flow Fractionation
FP7	Framework Program 7
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
JRC	European Commission's Joint Research Centre
MALS	Multi-Angle Light Scattering
MS	Mass Spectrometry
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
PPR Panel	Panel on Plant Protection Products and their Residues
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation and Authorisation of CHemicals
SAS	Synthetic Amorphous Silica
SP-ICP-MS	Single Particle Inductively Coupled Plasma Mass Spectrometry
TEM	Transmission Electron Microscopy