Network on Risk Assessment of Nanotechnologies in Food and Feed

Minutes of the 3rd meeting

Held on 06-07 June 2013, Parma

(Agreed on 15 September 2013)

Participants

- **Network Representatives of Member States:**

Country*	Name
Austria	Daniela Hofstaedter
Belgium	Jan Mast
Czech Republic	Vladimir Ostrý
Finland	Pertti Koivisto
France	Gilles Rivière
Germany	Alfonso Lampen
Hungary	Andrea Zentai
Ireland	Patrick O'Mahony
Italy	Francesco Cubadda
Netherlands	Anton Rietveld
Poland	Wojciech Wąsowicz
Portugal	Maria de Lourdes Bastos
Slovenia	Maja Remškar
Spain	Juan Frank Sanguesa
United Kingdom	Manisha Upadhyay

*****No nominations received from Estonia, Latvia, Malta, Romania and Sweden

- **Scientific Committee Panel Members:**
  - Qasim Chaudhry, Alicja Mortensen
- **European Commission and/or Member States representatives:**
  - Karin Aschberger (JRC)
- **EFSA:**
  - SCER Unit (Reinhilde Schoonjans (Chair), Miriam Jacobs (Co-Chair), Andrea Germini)
  - AFSCO (Jeff Moon)
  - FEED Unit (Maria Vittoria Vettori)
  - PRAS Unit (Manuela Tiramani, Andrea Terron)
- **Others:**
  - Norway (Ragna Bogen Hetland)

European Food Safety Authority – Via Carlo Magno 1/a, 43126 Parma, ITALY
Tel: (+39) 0521 036 200 • Fax: (+39) 0521 036 0200 • www.efsa.europa.eu

1. Welcome and apologies for absence

The Chair welcomed the participants, who introduced themselves during a tour the table.

Apologies were received from 6 Network Representatives of Member States (Nadezhda Sertova (BG), Kanari Popi (CY), Binderup Mona-Lise (DK), Falaras Polycarpos (GR), Jurgelevicius Vaclovas (LT) and Sajbidor Jan (SK)). Apologies from 4 observers of other countries (Mikec Darco (HR), Popovska Suzana (MK), Karagöz Emiroğlu Zehra (TR) and Dekic Zorica (ME)).

2. Adoption of agenda

The agenda was adopted with a proposal for one additional presentation from Alfonso Lampen (DE) not originally foreseen in the agenda.

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], members of networks, peer review meetings, networking meetings and their alternates shall be invited to complete and submit an Annual Declaration of Interest (ADoI).

EFSA screened the ADoI filled in by the experts invited for the present meeting. No conflicts of interests related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest (ODoI) at the beginning of this meeting.

4. Agreement of the minutes of the 2nd meeting of the Network on Risk Assessment of Nanotechnologies in Food and Feed held on 03-04 April 2012, Parma

The minutes were agreed by written procedure on 15 September 2012 and published on the EFSA website on 21 September 2012.

5. Topics for discussion

5.1 Feedback from the Network on the tasks of the Terms of Reference and mandate renewal

Jeff Moon presented the main points from a review performed last year on EFSA's networks, the recommendations and next steps. In general the EFSA networks were found useful and essential for cooperation between EFSA and national organisations for specific domains. The review highlighted some challenges for the future, especially the support needed for experts from national authorities to actively participate.

It was noted that different EFSA networks have different types of activities (e.g. discuss risk assessment, collection of data, etc.), but they all operate under the same framework. Since it is the intention of the EFSA SCER Unit to propose the renewal of the nanonetwork after its first 3-years mandate, the current Terms of Reference will be updated and submitted to EFSA and its Advisory Forum before the end of 2013. During this process, the nanonetwork members will be consulted for specifying the concrete activities to be performed by the

[1] http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf
[2] http://www.efsa.europa.eu/en/keydocs/docs/independencerules.pdf

network. Members of the Network shall commit to liaise as appropriate at national level before and after each Network meeting. This is crucial as the required expertise for the Network is often shared between different experts who are employed by different organisations at national level. Such commitment will be formalised by inclusion in the Terms of Reference for the next 3-years mandate. Sciencenet is proposed as ideal platform to share information between annual meetings to keep the Network operational. Renewal of the Network will imply new nominations of participating national experts through the EFSA Advisory forum.

5.2 EFSA Procurement Nanomaterials inventory

EFSA launched in autumn 2012 the call for tenders CFT/EFSA/FEED/2012/01[3] to make inventory lists of nanomaterial in food/feed already on the market or reasonably envisaged to be used on the market. The procurement contract was awarded on 1 March 2013. Its technical specification and task deliverables expected by the March 2014 were presented by Maria Vittoria Vettori. The core tasks are to perform a scientific literature review, to enter into direct contacts with food/feed producers and to make inventory lists according to the use of the nanomaterial e.g. as feed additive or food contact material.

The Network offered to provide the contractor with relevant contacts of food/feed producers. Some Network members (Gilles Ivière, Manisha Upadhyay, and Jan Mast), shared their experience from creating national registers, that producers do not always share information unless legally obliged to do so. Even more, food/feed producers might not be aware they are using nanomaterials. Sampling of products on the market was considered by Maja Remškar as an alternative and deterministic approach, and Alfonso Lampen suggested that such monitoring programs could be launched via food control bodies. Compiling inventory lists will also be facilitated once the new regulations, particularly the one for labelling, will enter into force.

5.3 Update from EU Member States: IT and BE presenting EU project 'Nanogenotox' and specific research results

The EU project towards a method for detecting the potential genotoxicity of nanomaterials "Nanogenotox" published its final report in March 2013[4] and the main results were presented to the Network by Jan Mast and Francesco Cubadda. 16 manufactured nanomaterials from the JRC repository and NRCWE were selected for the project (6 forms of TiO2, 4 forms of synthetic amorphous silica (SAS) and 6 carbon nanotubes (CNTs). Detailed physico-chemical characterisation of the test material, including the primary particle and aggregate/agglomerate size and size-distributions, are essential for proper interpretation of experimental results and a SOP for producing a suitable stock dispersion of NM to prepare exposure media was developed for application in *in vitro* and *in vivo* toxicity testing. It was emphasised that tests should be performed with a well characterised dispersion of the nanomaterial.

One major objective of the project was to establish robust methodology to screen *in vitro* genotoxicity of MNs in pulmonary, intestinal and dermal cell systems. While the *in vitro* mouse lymphoma assay was uniformly negative, the outcome of the comet assay and the micronucleus assay varied greatly among the different cell systems and their predictive value in identifying MNs that are genotoxic *in vivo* that could be carcinogenic is presently unclear. More information on the mechanisms of the detected *in vitro* genotoxicity on one

[3] see the technical specification http://www.efsa.europa.eu/en/tendersawarded/tender/cftefsafeed201201.htm
[4] http://www.nanogenotox.eu/

hand and of the MNs that are genotoxic *in vivo* or carcinogenic on the other hand, is needed before this question can be answered.

The project also looked at toxicokinetics and tissue distribution of MNs and identification of organs at risk for genotoxicity testing. The oral route of exposure was chosen as this is the common route of exposure for consumers. The absorption of NMs varies greatly after oral exposure, therefore, intravenous route (IV) was also explored in order to circumvent the biological barriers present and results in a direct system availability of the NM in the blood and internal organs. For all TiO2 and SAS nanomaterials, oral administration resulted in a rather low uptake via the GI-tract after repeated oral administration, whereas for multiple wall carbon nanotubes (MWCNT) no uptake from the GI tract was demonstrated. Results from the IV route show that liver, spleen and lung and to a limited extend the kidney are targeted and that MNs can persist in organs for a prolonged period of time until at least 90 days.

For *in vivo* genotoxicity tests, male rats were exposed to three doses of nanomaterial dispersions through instillation and gavage. With the comet assay on collected tissue samples, the responses were largely negative for most of the MNs tested and the organs considered. In most cases, when positive results were obtained, no dose response relationship could be established which makes it difficult to conclude on the *in vivo* genotoxicity of the NMs tested. No mutation damage was observed in bone marrow after gavage with either of four SAS, which may be explained by the low bioavailability of SAS after gavage (as observed in the toxicokinetics studies) or by SAS dissolution. None of the tested TiO2 and SAS nanomaterials induced micronuclei formation in bone marrow after gavage while two SAS (NM-202 and -203) induced an increase of micronuclei in colon samples but only at the lowest dose.

The overall conclusion was that the NMs investigated in Nanogenotox do not so far show strong genotoxicity *in vivo* or *in vitro*. However, in several cases, even at the lowest tested doses, some genotoxic effects were detectable *in vitro* and *in vivo*.

During the following discussion, it was clarified that the characterisation of the NM in the comet assay is key and needs checking, but it is important to demonstrate the actual uptake of the NM in the cell. It remained unclear if the chosen positive control (ZnO) is the most appropriate one and how to define the best positive control for each test.

During the meeting, further general conclusions from the Nanogenotox project were endorsed (see item nr 9).

Also the recent EU project NanoReg[5] was introduced, aiming at providing legislators with a set of tools for risk assessment and decision making instruments for the short to medium term, by gathering data and performing pilot risk assessment, including exposure monitoring and control, for a selected number of nanomaterials used in products. Within this project, Italy will follow-up the Nanogenotox SAS study by performing a repeated-dose 90-day oral toxicity study in rat, which is extremely relevant to food safety due to the widespread presence of nano-SiO2 in the food additive E551.

Italy then presented the results of a repeated dose oral study on nano-TiO2 (Nanotoxicology 2013, DOI: 10.3109/17435390.2013.822114). Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat increased total Ti tissue levels in spleen and, especially, in ovaries. In the spleen of treated animals TiO2 aggregates could be detected by single-particle ICP-MS, even though Ti tissue levels remained low reflecting the low doses (1, 2 mg/kg body weight per day) and the short exposure time (5 days). Sensitive Ti determination in tissues was possible due to the use of an interference-free ICP-MS method with a high detection power. TiO2 nanoparticles elicited sex-related effects in endocrine-active tissues such as thyroid (both sexes), adrenal cortex (females only), adrenal medulla

[5] http://nanoreg.eu/

(both sexes) and ovarian granulosa; changes in the serum levels of testosterone (males and females) and T3 (males only) were concurrently present, in the absence of general toxicity signs. Overall, the findings prompt to comprehensively assess endocrine and reproductive effects in the safety evaluation of nanomaterials.

5.4 Update from EU Member States: PT research results

Maria de Lourdes Bastos from the University of Porto introduced research results with Gold nano particles with different coating and sizes, magnetite (Fe3O4)IONPS, and with magnetite superparamagnetic iron oxide nanoparticles (coated or uncoated).

In summary, the *in vitro* tests with various cell lines, showed that all the tested AuNPs were efficiently taken up by both types of cells (Caco-2 and HepG2) in a concentration-dependent manner. Monodispersed or agglomerates of AuNPs were observed either inside endosomes or in the intercellular spaces. No evidence of cytotoxicity was found for AuNPs under the test conditions, and it was not possible to evaluate *in vitro* the passage of the Au-NPs using the transwell approach with Caco-2 (due to membrane filter-NPs interactions). Exposure for 24 h to low concentrations of Cit-AuNPs produced DNA damage in HepG2 cells while MUA-coated AuNPs did not. Decreased neuronal cell viability was observed both for bulk iron material and SPIONS, but through different mechanisms (ROS-dependent for bulk iron and ROS-independent for SPIONS). In the Blood Brain Barrier Model the magnetite NPs (IONPs) differentially increased cellular permeability according to the surface coating.

Using *in vivo* tests with rats exposed to a single intraveneous (iv) administration, it was found that after iv administration, AuNPs mainly accumulate in the liver, then spleen, although to a much lesser extent at different time points (30 min, 24 h or 28 days). This distribution profile is very similar for the different tested coatings (citrate, MUA and pentapeptides). There is a long residence time of the AuNPs specially in the liver (about 30% of injected dose 28 days after a single injection).

Biomedicine was the focus of the research, but the Network acknowledged that for food/feed oral administration should be evaluated in parallel with intravenous administration to evaluate biokinetics. Since this would increase the experimental test numbers considerably, it was suggested to only conduct such larger tests for exploratory research in relation to potential hazards. It is not suggested for regulatory testing, especially for food/feed safety where the relevant administration route would be the oral route.

5.5 Update from EU Member States: UK research results

Results from a recent UK research project that investigated the toxicokinetics of nano-sized and larger particles of titanium dioxide (TiO_2) were presented by Qasim Chaudhry. TiO_2 was chosen because it is an insoluble and persistent material that is an approved food additive (E171). Humans are exposed to it from a variety of non-food sources (paints, cosmetics, personal care products) as well as from food, and reported estimates (for a US adult) suggest a daily intake of ~1mg Ti/kg bw. The tests were performed *in vitro* using human cell model of gut epithelium, and *in vivo* with rat model. A separate project investigated blood and urine samples of human volunteers. The results of the studies showed that sample preparation of the test material, to avoid formation of agglomerates that are no longer nanosized, proved to be an important hurdle to overcome. The results of the study will be submitted for publication soon.

5.6 Activities at the Joint Research Centre concerning Nanomaterials in EU Regulation

Karin Aschberger from the Nanobiosciences Unit introduced the JRC units that are involved in various activities on nanomaterials: research, safety assessment, policy support and international harmonisation.

She presented results from tests (Nanobiotechnology group) investigating the toxicity and biokinetics (translocation across cellular barrier) of radiolablelled amorphous SiO_2 NPs (a widely used food additive) of three different sizes (20, 40, 100 nm) in an *in vitro* human intestinal model (Caco-2 cells). All three sizes did not induce toxicity in Caco-2 cells, neither after acute nor repeated-dose exposure and the Caco-2 barrier integrity was not compromised after exposure up to 22 days. Staining with Ru(bipy)3 showed that SiO_2 85 nm particles were efficiently taken up by Caco-2 cells and localized mainly in the lysosomes. No particles were found in nuclei or mitochondria. These results show that SiO_2 NPs were translocated across the *in vitro* intestinal barrier in small amounts and the degree of passage was size dependent.

The NANO SUPPORT project (finalised in February 2013) was based on an administrative arrangement between DG ENV and JRC in close collaboration with ECHA. From a total of >26.000 dossiers on 4700 substances registered in the REACH database (March 2011), 25 dossiers on 19 substances were found likely to cover nanomaterials. Only 3 dossiers were clearly identified to cover nanomaterials. All 25 dossiers were analysed in detail and assessed for physicochemical properties, manufacture and use, human health, fate, ecotoxicity, PBT, C&L, CSR. It was observed that the identification of substances was mainly based on chemical composition and there was insufficient information to describe different forms of a substance. In general that there was no clear link between the data presented (e.g. in the endpoint sections) and how they relate to the scope of the registration. Since its establishment in January 2012 by DG ENV and chaired by ECHA, the Group for Assessing Already Registered Nanomaterials (GAARN) is convening regularly experts from the EC, from ECHA and Member States. The purpose of GAARN is to build a consensus in an informal setting on best practices for assessing and managing the safety of nanomaterials under the REACH Regulation and thereby increase confidence and mutual understanding among stakeholders so that nanomaterials can be sustainably developed. Conclusions and best practices from GAARN are shared with stakeholders.

Foods and feeding stuffs including additives and flavourings, are exempt[6] from registration and authorisation under REACH on the grounds that there is other legislation which adequately regulates them. This applies to both human and/or animal nutrition. However, substances assessed by EFSA may have other uses and therefore may also have to be registered under REACH (e.g. TiO_2).

Initiatives that will increase consumer awareness include the updates of the labelling requirements for cosmetics and foods, and the production of inventory lists/product register on types and uses of nanomaterials on the EU market.

One further important initiative is the 'Research prioritisation to deliver an Intelligent Testing Strategy for engineered Nanomaterials' (ITS Nano) in which JRC was project partner. The aim of this 15 month FP7 project (completed in May 2013) is to advise on and organise the requirements for physical-chemical identification of nanomaterial, with respect to exposure, hazard identification, modelling, grouping and ranking, in order to facilitate risk assessment.

[6] see exemptions from REACH http://www.reach-serv.com/index.php?option=com_content&task=view&id=37&Itemid=64#food and http://echa.europa.eu/contact/helpdesk-contact-form/enquiry-on-reach-from-non-eu-countries

The ambition is to provide models and test-strategies to predict risks of nanomaterials. Reports and free webinars that detail this project's objectives are available online[7].

5.7 Update from EU Member States: SI Research, studies of effects of nano-TiO2 and nano-Ag on digestive glands of isopoda Porcellio scaber

Various results of *in vivo* feeding experiments with nanoparticles were introduced by Maja Remškar. The hepatopancreas of *Porcellio scaber* (otherwise known as the common rough woodlouse or rough woodlouse) fed with nanoparticles were isolated to check the digestive gland membrane stability. For TiO_2 particles, it was found that digestive gland cell membranes were destabilized in some animals and cellular internalisation of Ti was found when exposure concentrations were high and when the cell membrane was destabilised. For Ag particles also cell membrane destabilisation was observed by particles from exterior and Ag ions entered cells where they were stored and detoxified in metal storing granules. Elemental analyses showed the presence of Ag in the digestive gland epithelium of animals fed on nano-Ag dosed food and not in control animals.

Additionally, ongoing research in Slovenia is examining the pollution resulting from nanoparticles massively released during firework displays, particularly NP composed of Magnesium, Aluminium, Lead, Iron, Potassium and Calcium. Their dispersal, travel distance and deposition are raising potential safety questions to be further addressed.

5.8 Update from EU Member States: DK research

Nanosilver as a food supplement (a source of Silver) and in food contact materials (protecting agent) was investigated in Danish research projects, focussing on detecting toxic effects. The results were presented by Alicja Mortensen. Observations from a 28-day oral repeated dose toxicity study, raised a suspicion for immunotoxicity based on the lower relative thymus weight. However, there was no correlation found with circulating leucocyte subset numbers and no determining conclusions were drawn. Also the tissue distribution of silver NP versus silver acetate (ionic form) were investigated during a 28-day oral repeated dose toxicity study in rats. Similarly to the UK results with TiO_2, it was seen that excretion of Ag in urine was low but high in faeces. Through light microscopy and autometalographic staining, Ag were seen in the lamina propria of the ileum, mainly in the tips of the villi but not in the epithelial cells. Using transmission electron microscopy, lysosomes containing nano particles were observed also particles in the basal lamina. It was confirmed that the observed granules in the lysosomes of macrophages consisted of Ag and the presence of also selenium and sulphur were confirmed in the silver granules. This may indicated the presence of a metal binding vehicle protein transporting the silver inside the cell. To determine whether these were AgNP or Ag ions the Network suggested to also check the carbon source. If it is transported as Ag ion, there are no particular NP considerations during the risk assessment. It was agreed that the gut should indeed be the target organ after oral exposure, and that our interest should concentrate here to understand transport mechanism and toxicity.

5.9 Update from EU Member States: DE research

Alfonso Lampen presented results from ongoing artificial digestion experiments with coated Ag nanoparticles. The physico-chemical characterisation prior to digestions entailed a

[7] ITS Nano webinar can be viewed at http://www.its-nano.eu/webinar

cooperation of different technologies to measure the hydrodynamic radius and core-dimensions. The ascertained nanosized spheres were subsequently exposed to a series of digestive fluids, in conditions that mimic human digestion. After such artificial digestion, the physico-chemical properties of the nanoparticles were altered and their size was significantly increased due to di- and trimerisation (paper in preparation). These findings of the influence of digestion, informed further risk assessment safety testing of this research group. Research is still ongoing and the full results will reported at a later stage.

5.10 Discussion on nanotoxicology

To focus the discussion on nanotoxicology and potential areas of participant interest in relation to food and feed safety, the meeting participants received beforehand background information on nano materials in food and feed. This included updates on various international activities, recent relevant legislation, and *in vitro* and *in vivo* test developments of relevance for oral exposure (see Appendix, updated following comments received during the Network meeting).

The participants were asked to share their views on four priority questions relevant for nanotoxicology in the food and feed area in relation to: (1) the adequacy of genotoxicity tests, (2) the relevance of developing GI tract tests, particularly digestion models, (3) the adequacy of other *in vivo* tests, and (4) the adequacy of test methods for environmental risk assessment. The last topic was not addressed due to time constraints.

5.10.1 Agreements following discussions regarding genotoxicity and based on the results from the nanogenotox studies

In general:

- According to the behaviour of the nanomaterial and their specificities, any genotoxic test guideline should be amended to include some toxicokinetic testing as there is a critical need to always investigate whether the tested nanomaterials reach the target cells and not just rely on genotoxicity methods commonly used.

  See also the considerations noted in the EFSA SC genotoxicity opinion[8] and the EFSA SC nano opinion[9].

For *in vitro*:

- Any genotoxicity assessment *in vitro* should specify the dispersion protocol used to prepare the nanomaterials and characterise the dispersed particles, and provide information on the availability of the nanomaterials to reach the cells/tissues and their uptake.

[8] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. [69 pp.] http://www.efsa.europa.eu/en/efsajournal/doc/2379.pdf

[9] EFSA Scientific Committee; Scientific Opinion on Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011;9(5):2140 [36 pp.] http://www.efsa.europa.eu/en/efsajournal/doc/2140.pdf

- The most sensitive and relevant cell type according to the relevant exposure route should be used, and appropriate positive and negative controls should be included.

For *in vivo*:

- For hazard identification of substance-related genotoxicity, the OECD test guideline 487 can be used but with target cells corresponding to the route of exposure. However, particle uptake into the cells of the chosen test system should be demonstrated, otherwise negative results might occur due to lack of exposure.
- The *in vivo* mammalian erythrocyte micronucleus OECD test guideline (TG 474) can also be used, however, similarly, it must be demonstrated that the test item reaches the target cells *in vivo*.
- The results of the Nanogenotox project also suggest that other *in vivo* tests might be applicable for genotoxicity investigation of nanomaterials, for example the *in vivo* micronucleus assay on intestine cells or colon as some genotoxic effects were observed *in vivo* on those organs.

In conclusion, the Network summarised that:

- There is a common agreement that the Ames test and the chromosome aberration test are not reliable tests for nanomaterials.
- The availability of validated positive controls for genotoxicity testing still remains an issue: *it is important to develop reference positive controls for the validated test methods to ensure that positive and negative results obtained are reliable. ZnO may be used as positive control only in some specific test system (e.g micronucleus); it is not universally applicable.*
- Specificity and sensitivity of *in vitro* and *in vivo* tests for the evaluation of the genotoxic potential of MN, and therefore their predictivity, is still unclear.
- A testing strategy for the assessment of the genotoxicity endpoints should be defined:
- A prerequisite is an adequate characterisation of the nanomaterial and representative test materials (physico-chemical properties including coatings and biokinetics).
- Greater sensitivity was observed with the *in vivo* (oral exposure) Comet assay but, the following considerations should be made:
    - Specificity data are lacking: *It was remarked that from the literature it is obvious that Ames test normally doesn't work with nanoparticles and that the Comet assay seems to be more important in this regard. It is more used because it is easier to perform. But, the test is very sensitive and may yield a large number of positive results not always in a very robust way. Moreover, methodologically for the gastrointestinal system it is not ideal due to the background.*
    - To be focussed on GI tract as a first site of contact: *It is important that is should focus on the gut.*
    - Considered as indicator tests (for DNA damage) and seen as a follow up study in case of equivocal, inconclusive or positive *in vitro* results: *Some* Network *participants were concerned that undue weight might be given to the Comet assay, and that it should not be used as the sole test. It does not measure genotoxicity directly, furthermore point mutations or chromosomal aberration are not considered by the comet*

assay. It is a sensitive but indicative test of DNA damage and it does not indicate what is the cause of the damage and if the damage is permanent or not, or if it is at the repair stage. The comet assay is rather for hazard identification not for hazard characterization. Test strategies are extensively discussed in the EFSA Scientific Committee opinion and recommendations for genotoxicity test strategies (2011)[10]. No OECD TG protocol at present, but internationally agreed protocols are available.

5.10.2 Agreements following discussions regarding the adequacy of *in vitro* test methods for food and feed (oral exposure) - The relevance of developing GI tract tests

Digestion:

- Existing models (such as low throughput TNO models) are found useful and more recent (faster, high throughput) models for digestion are soon to be published. These would facilitate tests for regulatory purposes.
- Results show morphological modification:
  - In case of agglomeration: no general statements can be made. *Is was observed however, that in the whole scheme of testing oral exposure, in vitro systems that check the morphological changes in a simulated GI tract are very relevant because we need to make sure that what reaches the GI tract is the actual nanoparticle and not a ionized or physically or chemically altered form that could be assessed as a normal chemical (see next point). On the other hand, the formation of aggregates or agglomerates may occur, but these can still qualify as NM not only formally based on their primary particle size, but also with respect to their nano-specific properties resulting from e.g. their high VSSA.*
- Can be a first tier to stop the RA since loosing nanostructure means becoming a 'normal chemical' (see EFSA GD). *It was underlined that nanoparticles are normal chemicals that have specific physical properties and the nano specific considerations in RA only apply if the NM retains the physical structure.*
- While one participant considers that there are validation difficulties for these tests with respect to absorption and uptake, and that a more valid priority would be running 28 and 90 day studies with a range of well characterised nanomaterials including different sizes of particles to establish baseline pathology and identify any additional tissues required for risk assessment, the majority were in support of further development of digestion models as a component of nanotoxicity hazard assessment.
- The Network recommended that such models be explored for possible test guideline development, however the current lack of positive controls would need to be addressed before embarking on (pre) validation activities.
- The coatings of the nanomaterials tested can greatly affect the result in digestion models.

With cell lines as uptake and absorption models:

[10] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. [69 pp.] http://www.efsa.europa.eu/en/efsajournal/doc/2379.pdf

- Role of Caco-2 cells: more basic research is needed regarding uptake and absorption.

The Network was informed that a COST action[11] is being set up to work on *in silico* modelling of nanomaterial toxicity.

5.10.3 Agreements following discussions regarding the adequacy of other *in vivo* tests

- Other tests as described in the EFSA GD: if absorption takes place, general toxicology and reproductive endpoints need to be explored.
- In addition, this Network advises to give special attention on endocrine endpoints.
- Additional consideration should include the use of EM in general toxicity studies.
- Selection of target organs to be examined should come from the ADME study and from the histopathology evaluation.
- This will help to characterise the test material in the tissue and further characterization histopathological changes by increasing the threshold of sensitivity of the investigation.
- There is a need to differentiate between ionic deposits and nanomaterials, as the deposition of ions may not be as nano form.

5.11 Updates on the EU project 'NanoLyse'

A brief overview of the NanoLyse project[12] on analytical methods for the detection and characterisation of nanoparticles in food was given by Qasim Chaudhry. A variety of methods was assessed/developed for inorganic and organic nanomaterials in different food matrices. The final report is in preparation and will describe for each case (NP/matrix) the methods tested. An analytical scheme for each NP/matrix needs: (1) extraction, concentration, and/or fractionation (2) particle detection and (3) chemical characterisation. This involved the use of more than one analytical method.

One of the NanoLyse researchers is currently performing a EU wide market survey for food products containing silica as food additive (E551). The Network was invited to forward samples or knowledge about types of food products to which silica is added.

6. Any Other Business

6.1 Inventory List of laboratories

The members of the Network updated the list of national laboratories that are competent to analyse food samples upon request and that are able to verify presence of and characterise nanoparticles. The list, available to the Network through Sciencenet, provides details on the physicochemical/analytical methodologies used in those labs, and the nature of the nanomaterials tested.

[11] http://www.modena-cost.eu/
[12] www.nanolyse.eu

With the approval of the Network, this list was shared with the NanoLyse consortium for information dissemination purposes.

6.2 Scientific Publications

Summaries from MS national activities in the area of food and feed as well as relevant scientific papers to share with the Network were uploaded to the online platform for the Network (Sciencenet) under the documents of the present meeting. Network members were reminded to use this forum also for accessing meeting documents.

6.3 Publication of the 2012 annual report of the Network

The Network was informed that at the end of 2012, the EFSA staff produced and published the annual report for the EFSA advisory forum. This technical report is searchable in the EFSA register of questions and the EFSA journal: http://www.efsa.europa.eu/en/efsajournal/doc/362e.pdf

7. Next meeting

In case this Network will be renewed, its next yearly meeting will be scheduled in 2014.

Appendix 1: Background information on nanomaterials safety in food and feed

The EFSA staff of the Scientific Committee and Emerging Risks Unit compiled the following non-exhaustive information (mainly from public sources) and provided it as background documentation prior to the meeting on the EFSA Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed on 6-7 June 2013. It is meant as a reflections paper to focus the discussions on what is needed next for food and feed regulatory purposes.

This background information has been subjected to comments from the nanonetwork participants only. This background information is not and cannot be regarded as representing the position, the views or the policy of the European Food Safety Authority or of any national or EU Institution, agency or body.

1. Introduction

This background information on the risk assessment of nanotechnologies in food and feed for the EFSA Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed (EFSA Nano Network), established in accordance with EFSA strategy for co-operation and networking with Member States, provides updates on legislative and test developments since the Nano Network meeting held in April 2012. It also provides brief updates on the priorities discussed in the annual report of the Nano Network from 2012 (EFSA 2012).
The updates are specifically regarding (1) The EC
recommended definition of nanomaterial; (2) The analysis and monitoring of nanomaterials and EC legal framework/safety control mechanisms, particularly the Commission's 'Second Regulatory Review on Nanomaterials' held in October 2012; and (3) The current status and availability of relevant and validated *in vitro* and *in vivo* tests following oral exposure.
On the basis of key examples and the current status of standardised test methods applicable for nanotoxicity testing, the EFSA Nano Network participants are invited to reflect on particular areas that would be highly relevant for EFSA, in relation to food and feed safety testing.

2. Definition of Nanomaterials

In the 'Second Regulatory Review on Nanomaterials'[13], the 2011 Recommendation on the definition of nanomaterials (Commission Recommendation 2011/696/EU, OJ L 275, 20.10.2011) was confirmed, defining 'nanomaterial' as "*a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %... "* This is the definition to be used in EU legislation and instruments of implementation where appropriate and will be reviewed in 2014.
(http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2012:0572:FIN:en:PDF).

2.1 Relevant safety aspects under European discussion

In 2009, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) stated that: *"As there is not yet a generally applicable paradigm for nanomaterial hazard identification, a case-by case approach for the risk assessment of nanomaterials is still warranted*". This was confirmed by EFSA (2011) and also adopted by the European Medicines Agency (EMA) for medicinal products.

[13] 'The Second Regulatory Review on Nanomaterials', a communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee in October 2012

The Second Regulatory Review reports that several risk assessments and risk/benefit assessments have been completed and various products in different sectors have been authorised (such as 20 medicines and three food contact materials: silicon dioxide, carbon black and titanium nitride. Silicon dioxide has also been authorised as food additive. However the evaluation of silicon dioxide predates nanomaterials regulatory activities. The material as produced and tested would be predominantly in the nano-scale but there was no characterisation of the material and the approval predates the recognition of nanomaterials). The Scientific Committee on Consumer Safety (SCCS) has assessed and approved the safety of one nanomaterial used as a UV filter and is completing the assessment of three other nanomaterials. Other substances will be assessed as the case arises (e.g. UV filters, food and feed ingredients) (Commission Recommendation 2011/696/EU, OJ L 275, 20.10.2011).
The Second Regulatory Review was published together with a Commission Staff Working Paper (SWP) on 'Nanomaterial Types and Uses, including Safety Aspects', in response to the European Parliament's concern that the Commission's approach to nanomaterials is jeopardised by the lack of information on the use and safety of nanomaterials already on the market.
The SWP therefore provides some more detailed information on the definition of nanomaterials, nanomaterial markets, uses, benefits, health and safety aspects, risk assessment, and information and databases on nanomaterials.
Further background information on this and relevant EU and collaborative international OECD regulatory activities are provided in the sections below and references therein.

With respect to such information collection, and particularly addressing the priority needs identified by the EFSA Nano Network, EFSA has just embarked upon a procurement with an external contractor to prepare an overview report on the current knowledge in the field of nanotechnology, and to produce inventory lists of nanomaterials used and foreseen to be used in food and feed. This includes food additives, food contact materials and feed additive applications. It also includes a review of the relevant existing legislation in non EU countries, and whether guidelines for the assessment of effects on human health and the environment exist. The project started in March 2013, and will run for a year. Following the project completion, the report will then be available to be used as a background document by EFSA's Panels, the Scientific Committee and the Nano Network, for consideration and potential update of specific guidance documents.

3. International test methods and supporting research

3.1 Overview

The harmonization and standardization of measurement and test methods in support of risk assessment of nanomaterials continues to be promoted through the OECD and by a Commission Mandate to the European Standards Organisations[14].
In addition to cooperation with the OECD, the Commission has a regular dialogue with the United States, in the context of the Transatlantic Economic Council (TEC), for example, to harmonisation where possible, and avoid unnecessary divergences.

[14] European Standards Organizations (ESOs) are officially recognised by the European Commission and act as a European platform through which European Standards are developed. In the European Union, only standards developed by the European Committee for Standardization (CEN), the European Committee for Electrotechnical Standardization (CENELEC) and the European Telecommunications Standards Institute (ETSI) are recognised as 'European Standards'. They work jointly in the interest of European harmonization, creating both standards requested by the market and harmonized standards in support of European legislation. They are the regional mirror bodies to their international counterparts, i.e. ISO (the International Organization for Standardization), IEC (the International Electrotechnical Commission) and ITU-T (the International Telecommunication Union, telecommunication standardization sector) respectively.

Research concerning safety and the development of reliable test methods for nanomaterial detection and safety continue to be a key priority under the EU Framework Programmes and for the Commission's Joint Research Centre (e.g. Requirements on measurements for the implementation of the EC definition of the term 'nanomaterial'. Linsinger et al., 2012).
In 2013 and 2014, the OECD is holding a series of workshops addressing test guideline needs, as initiated by member countries and data availability. The 'Nanotoxicology considerations for environmental fate and ecotoxicology workshop' was held in January 2013, and the report will be available following joint meeting declassification in June 2013. Germany and the US are the lead member countries. A related physico-chemical properties workshop was held in March 2013, and the report is currently being drafted. Three more workshops are planned: one concerning genotoxicity aspects in Autumn 2013, led by France and Canada (this is being developed hand-in-hand with the EU Framework 7 funded programme 'Nanogenotox' (www.nanogenotox.eu)), another concerning toxicokinetics and mechanistic issues, in early 2014, led by Germany, and in March 2014 there will be a follow–up workshop regarding the categorisation of nanomaterials, led by the US and Netherlands, with strong interest from all member delegations.
A recent international COST (European Cooperation in Science and Technology) workshop on the use of QSAR methods to model biological effects of nanomaterials (www.cost.esf.org/events/qntr) identified roadblocks to achieving useful models for assessing nanoparticle risks, and methods for overcoming them. A number of tasks that need to be completed in order to create models useful for nanoparticle regulation within the ten-year time frame requested by regulators, were divided into a road map of three time horizons (2, 5 and 10 years) that the consensus of COST workshop participants identified as being realistically achievable. See also Appendix E, regarding future OECD QSAR Toolbox plans.
In order to have an idea of current research and review activity in the nanotoxicology in food and feed area, a quick summary Pubmed literature scan for the years 2012-2013, was conducted on 22 April 2013 using Nanotoxicol* as a search term. This produced 88 citations, twenty of which were specifically related to inhalation, two were specifically related to dermal exposure, and so therefore more than 60 papers were potentially of food and feed safety relevance. Relevant review papers published in this short time period include genotoxicity test methods and computational tools.

3.2 Test methods applicability

3.2.1 Test guidelines in use up to 2011

Current EFSA Scientific Committee guidance 'Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain' (EFSA 2011a) addresses the tests and recommendations available up until 2011.
It is generally recognised that for nanomaterials, *in vivo* methods are needed to first assess the toxicity. However a recent transatlantic workshop report also suggests that the more low order, whole organisms such as the developmental models including *C. Elegans* and Zebra fish embryo tests would be a useful prior test to conduct, to assist in prioritisation testing in the higher level mammalian tests for example (Silbergeld et al., 2011). Assessment of the behaviour of nanomaterials in the test system is of particular importance in *in vitro* test systems, as the physico-chemical properties affect the experimental conditions.

Genotoxicity testing

Genotoxicity testing for nanomaterials is one of the important areas for nanotoxicity testing. In the comments received for the EFSA nano guidance opinion in 2011, the UK pointed out that as '*there is limited information on nanoparticles (NP), a larger test baseline would be perhaps advisable. A problem of course, may be the lack of a sufficient spread of reference NPs, known to be*

genotoxic/carcinogenic and we are not sure how well validated the assays for genotoxicity/carcinogenicity/mutagenicity are against nanoparticles.' (EFSA 2011b).

The EFSA 2011 opinion noted that:

'*A bacterial reverse mutation assay is usually recommended for the detection of gene mutations, as also included in the work of the Scientific Committee on genotoxicity testing strategies. However, since engineered nanomaterials (ENM) may not be able to penetrate the bacterial cell wall (Landsiedel et al., 2009) and because bacterial cells, unlike mammalian cells, do not have the ability to phagocytose particles, the use of a bacterial reverse mutation test for detection of genotoxicity of ENM is not considered to be appropriate.*

The following in vitro tests are required for ENM added to, or migrating into food:

1. *A test for induction of gene mutations in mammalian cells (preferably the mouse lymphoma tk assay with colony sizing) (OECD test guideline 476)*
2. *An in vitro micronucleus assay (OECD test guideline 487)*

There may be circumstances under which it may be justified to deviate from the above-mentioned core set (e.g. when there is a need to test the ENM in a matrix that cannot be added in vitro). In such cases a scientific justification should be provided and additional types of considerations or in vivo studies may be needed. In certain instances (e.g. with induction of reactive oxygen species, soluble ENM, very small ENM) a bacterial reverse mutation test might still be informative.'

- More recently there has been an FP7 'Nanotest' funded genotoxicity review of *in vitro* and *in vivo* studies with engineered nanoparticles (www.nanotest-fp7eu; Magdolenova et al., 2013). It is noted that physico-chemical properties and experimental conditions, affect the genotoxic response. From 4346 articles on NP toxicity, 94 *in vitro* genotoxicity studies and 22 *in vivo* genotoxicity studies are described. The review identifies that the most used assays are the comet assay (58 *in vitro*, 9 *in vivo*), the micronucleus assay (31 *in vitro*, 14 *in vivo*), the chromosome aberrations test (10 *in vitro*, 1 *in vivo*) and the bacterial reverse mutation assay (13 studies). A need for appropriate methodologies to be used for investigation of genotoxic effects of nanomaterials, *in vitro* and *in vivo* is identified in the review.
- Concerns are expressed regarding the suitability of the Ames test for NP testing, '*as larger NPs are unable to cross the cell wall. If they do enter the cell, NPs could possibly interfere with histidine synthesis and induce false-negative (down-regulation) or positive (up-regulation) results. The Ames test has nevertheless been used to assess genotoxicity of a variety of NPs, and has so far given largely negative results'*. Reviewed in Magdolenova et al., 2013.

In the same vein, the bacterial reverse mutation test is therefore not suitable for assessing human-related nanoparticle genotoxicity due to the bacterial cell wall barrier. This same review (Magdolenova et al., 2013) considers the comet assay to be the most sensitive method to detect nanoparticle genotoxic potential, as low level DNA damage and specific DNA lesions can be identified , however it needs to be used as a component of a comprehensive integrated testing strategy (ITS), as it is an indicator test, it detects DNA damage which is a sign of structural aberration, but does not specify what type of aberration has occurred, thus it does not measure genotoxicity directly. How to use the Comet assay as part of test strategies is discussed in the EFSA Genotoxicity testing Strategies Opinion 2011 (EFSA 2011).

In vitro digestion models

There are also tools, such as *in vitro* digestion models, recommended in the 2011 guidance (EFSA 2011 and references therein), that could provide a highly relevant test method tool with respect to understanding and testing digestion and absorption of nanomaterials consumed orally. Digestion models can be better understood in three parts; one concerned with the upper digestive tract, the

second for small intestinal tract absorption, and the third incorporating metabolism. The large intestinal tract is generally not taken into account, as the small intestine is the primary site for food digestion and absorption. The first part of the digestive tract, i.e. mouth, stomach and duodenum (first part of the small intestine) can be represented by an *in vitro* digestion model. The absorption of digested matter would still require an appropriate and validated *in vitro* test to be part of the test battery.

'In vitro digestion can be used to demonstrate dissolution/degradation of the engineered nanomaterial (ENM). In these cases only limited or no further testing might be needed. Various models are available, most have been designed to assess the release or dissolution of non-nanomaterials (Oomen et al., 2002; Dressman et al., 1998; Krul et al., 2000; Brandon et al., 2006). With an in vitro digestion model, the conditions of the human gastrointestinal tract can be simulated, i.e. temperature, mixing, transit time, composition of salt, enzymes and other constituents such as bile. In vitro digestion models have been applied to determine the release of various orally ingested compounds e.g. contaminants from soil (Oomen et al., 2003; Van de Wiele et al., 2007), food contaminants (Dall'Asta et al., 2010; Versantvoort et al., 2005), food mutagens (Krul et al., 2000), food components (Blanquet-Diot et al., 2009; Tydeman et al., 2010), contaminants in toys (Brandon et al., 2006) and drugs (Dressman et al., 1998; Kostewicz et al., 2002; Blanquet et al., 2004). These models vary in the degree in which they simulate human gastro intestinal tract conditions from very simple to rather sophisticated. To which extent the different in vitro models lead to different conclusions regarding dissolution and degradation of nanomaterials has not yet been studied.' (EFSA 2011).

The model described by Oomen et al. in 2003 includes a standard meal to which a chemical of interest is added. Digestion is simulated by adding in sequential order: saliva; gastric juice; duodenal juice; bile and a bicarbonate solution. The TNO gastro-Intestinal tract Model (TIM), a dynamic computer-controlled *in vitro* system that mimics the human physiological conditions in the stomach and small intestine, is more complex. It contains four compartments and has been used to study the absorption of heterocyclic aromatic amines and the degradation of gluten (Krul et al., 2000; Mitea et al., 2008).

As yet, no validated models are available.

These two examples, from genotoxicity and digestion, might be priority areas that the Nano Network could discuss. Any conclusions could then be minuted, so that they can be taken forward, for instance to the Commission services PARERE group[15], which might be one of the appropriate Commission forums in which to express the need for the development and validation of such test methods for nanotoxicity testing relevant to food and feed.

3.2.2. Test methods and modelling for environmental risk assessment

The EFSA 2011 opinion does not focus on environmental risk assessment for nanomaterials, and internationally, there does not appear to be a strong focus on this area.

[15] As a member of the Commission services Preliminary Assessment of Regulatory Relevance network (PARERE), EFSA may be consulted on alternative risk assessment methods. This network was created by the Commission to expedite the process of regulatory acceptance of alternative methods. It was considered that regulators should be involved as early as possible in providing a preliminary view on the potential regulatory relevance of methods submitted to ECVAM for validation. PARERE's tasks are as follows: To assess the regulatory relevance, across different regulatory frameworks, of new alternative methods proposed for (pre)validation; To facilitate timely information flow from/to regulators in relation to alternative methods under development and taking account of the respective regulatory needs during validation; To allow early awareness and buy-in for alternative methods in the pipeline.

Recently Holden et al. (2012) have published measurements of the effects of nanomaterials on a subcellular or population level and related those effects to mechanisms within dynamic energy budget (DEB) models of growth and reproduction, by using high throughput/content screening (HTS/HCS) with cells or environmentally-relevant organisms.
DEB model predictions are compared with experimental data on organism and population responses, and microcosm studies to measure the potential for community- or ecosystem-level effects by nanomaterials that are likely to be produced in large quantities and for which either HTS/HCS or DEB modelling suggest their potential to harm populations and ecosystems.
The authors suggest that to keep pace with nanomaterial development, rapid assessment of the mechanisms of nanomaterial effects and modelling are needed. DEB models provide a method for mathematically representing effects such as the generation of reactive oxygen species and their associated damage. These models account for organism-level effects on metabolism and reproduction and can predict outcomes of nanomaterial-organism combinations on populations; those predictions can then suggest nanomaterial characteristics to be avoided.

3.2.3. Test guidelines published post 2011

There may be newly published test guidelines that should be examined more closely for addressing the needs of nanosafety testing. For example the extended one generation assay OECD Test Guideline 443 published in 2012 will have reproductive toxicity safety assessment applications for nanomaterials.

4. Relevant EC, EU Agency and OECD background information

4.1 Coverage of nanomaterials in REACH registrations and Classification Labelling Packaging (CLP) notifications

At an EC stakeholder workshop organised to present and discuss the Second Regulatory Review on Nanomaterials in January 2013 the main conclusions presented by DG ENV were that REACH registration and proof of safe use can be applied to nanomaterials, but that a case by case approach should be followed.
Each type of nanomaterial should be clearly described, and data generation and testing for nanomaterials are possible based on current risk assessment requirements assuming that data is provided for each nano "case" and there is a good description of test conditions and type of nanomaterial.

With respect to the REACH requirements for nanosafety, from February 2012 to January 2013, a voluntary tick box “nanomaterial” was ticked in 7 registrations, and 18 notifications, however many more registered substances are nanomaterials. Registration dossiers are generally unclear whether and how they cover nanomaterials. Therefore the EC has concluded that more specific requirements in REACH Annexes are necessary and an impact assessment is being conducted for registry nanomaterials below REACH tonnage, "to identify and develop the most adequate means to increase transparency and ensure regulatory oversight, including an in-depth analysis of the data gathering needs for such purpose. This analysis will include those Nanomaterials that fulfil the criteria for classification as hazardous under Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures must be classified and labelled. Many of the related provisions, including safety data sheets and classification and labelling already apply now, independently of the tonnage. Substances, including nanomaterials, meeting the classification criteria as hazardous and put on the market, must be notified to ECHA.

ECHA has published advice on how to enter nanomaterial information in IUCLID, and updated IUCLID 5.5 (2 April 2013) to include 13 new OECD agreed physical-chemical properties templates for nanomaterials. The guidance for nanomaterials has also been updated, on the basis of the final reports from REACH Implementation Projects on Nanomaterials (RIPoN2 Information requirements and RIPoN3 Chemicals

safety assessment) and the Commission Recommendation on the definition of Nanomaterial. ECHA and Member States have also included some substances with nanoforms in the Community Rolling Action Plan (CoRAP) list of substances to be evaluated in 2012-2014. In addition the Classification and Labelling Inventory and the web portal on 'Registered substances of ECHA' also contain information on substances with nanoforms.

The EC envisages modifications in some of the REACH Annexes and encourages ECHA to further develop guidance for registrations after 2013. There will be an impact assessment of relevant regulatory options, in particular possible amendments of REACH Annexes, to ensure further clarity on how nanomaterials are addressed and safety demonstrated in registration dossiers. If appropriate the EC will come forward with a draft implementing act by December 2013. http://ec.europa.eu/enterprise/sectors/chemicals/reach/nanomaterials/

4.2 ECHA working group on nanomaterials

Best practices for the assessment of nanomaterials are being collected from registrants that have already registered nanomaterials, and will be disseminated in the near future aimed at helping those registrants that need to register nanomaterials by 31 May 2013.
In June 2012 – '*ECHA organised a workshop concerning its first experiences with nanomaterials under REACH with an emphasis on the Evaluation process. In the two day event ECHA, Member State Competent Authorities (MSCAs), Accredited Stakeholders and the European Commission discussed how nanomaterials in general have been characterised in registration dossiers. Currently, the scope of the registration (i.e. whether and how many nano-forms are included) is often unclear and the level of nano-specific information provided (e.g. substance characterisation, hazards, exposure and risks) shows significant room for improvement. Over 50 expert participants from the MSCAs discussed the scientific and technical challenges as well as the regulatory processes that REACH offers to address safety aspects of nanomaterials.*
ECHA and MSCA representatives agreed upon a common approach to address the current information requirements in nanomaterial dossiers taking into account the scientific uncertainties and legislative framework provided by REACH. The workshop provided recommendations on how to proceed with nanomaterial substances under evaluation in the near future, with ECHA continuing further dossier evaluation activities. ECHA first aims to provide clarity on the physico-chemical characteristics of nanomaterials, and will use the available REACH instruments to obtain available data or request new data to be generated. A gradual approach is therefore being taken, combined with a collaborative and constructive interaction with registrants as the first steps towards future safety assessments of nanomaterials under REACH.'

ECHA intends to disseminate the best practices that are currently collected from relevant stakeholders that registered nanomaterials and which were discussed in the first Group Assessing Already Registered Nanomaterials meeting, prior to the workshop cited above. These best practices will be published on ECHA's website by the summer of 2013.

Nanomaterials report: http://ec.europa.eu/environment/chemicals/nanotech/index.htm
Guidance on information requirements and chemical safety assessment for nanomaterials: http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

4.3 DG SANCO's activities on consumer products including pharmaceuticals in nanoforms

There are no specific nanomaterials provisions in the General Product Safety Directive, but cosmetics, medical devices with nanomaterials in Class III (the highest risk), and the Commission proposal (September 2012) for a Regulation on medical devices, do contain specific rules for them. For pharmaceuticals, authorisation of medicines applies similarly to nano containing medicines, with 20 authorised to date, and further assessments are carried out as required.

For food and feed there are no validated methods/suitable reference standards available for detecting, identifying and quantifying nanoparticles in complex matrixes (e.g. food). As food structure is highly complex and varied, and many types of nanoparticle exist, no single, universally applicable method is expected in the near future. Tailored methods and reference standards (particles in relevant media) need to be developed and validated for specific applications and use in control laboratories, and this work in ongoing at the EC JRC as well as the EU FP7 project 'NanoLyse'.

Labelling:
With respect to nanomaterials definition in EC Regulation (EC) No 1169/2011 on food information, no nano labelling is proposed in the Commission proposal on Food Information. However the first reading European Parliament amendment has a labelling requirement although no definition is given. This has been accepted in principle by the EC, with a cross-reference to the definition of the "future novel food Regulation". Further readings are under negotiation, with the definition and labelling as agreed in the novel food. Nano provisions are included in EC Regulation (EC) No 1169/2011 as of 13/12/2014.
EC Nano definition (Article 2.(t)) is legally binding for labelling purposes. The labelling requirement (Article 18) states that: All ingredients present in the form of engineered nanomaterials must be clearly indicated in the list of ingredients, followed by the word 'nano' in brackets. There is a possibility to adapt the definition (Article 18(5)), and to incorporate technical and scientific progress or to definitions agreed at international level by delegated acts (subject to the control of the European Parliament and the Council).
The next steps are the adaptation of 'engineered nanomaterials' definition by December 2013 at the latest.

EC Food additives:
Regulation (EC) No 1333/2008 on food additives.
For all new food additives EFSA evaluations take into account nanotechnology. Nanomaterials are implicitly covered, and previously authorised food additives are considered as new additives if there is a significant change in production methods or in the starting materials used, or if there is a change in particle size, for example through nanotechnology, and therefore they need to be evaluated and authorised.

Flavourings and Enzymes:
Nano is implicitly covered: A new risk assessment is required for flavourings and enzymes where new production processes give rise to significant changes in the production process.

Novel foods:
Council Regulation (EC) No 258/97 on novel foods: As above, nano is implicitly covered: by a general provision: 'new production processes giving rise to significant changes in the composition or structure of foods or food ingredients': Novel food provisions apply only for foods which were not on the EU market before May 1997.

Food Contact materials:
Regulation (EC) No 10/2011 on measures for plastic materials and articles, as from May 2011, nanomaterials can only be used if listed in its Annex I. Art. 9(2) states: '*Substances in nanoform shall only be used if explicitly authorised and mentioned in the specifications in Annex I'.*
Regulation (EC) No 450/2009 on measures for active and intelligent materials and articles. Art. 6 states: '*Substances deliberately engineered to particle size which exhibit functional physical and chemical properties that significantly differ from these at a larger scale…'.*

Re-evaluation programme update.
Particle size is considered to be part of the re-evaluation programme. For example, with respect to calcium carbonate (E 170), EFSA concluded that "the available data are sufficient to conclude that the current levels of adventitious nanoscale material within macroscale calcium carbonate would not be an

additional toxicological concern". The re-evaluation of silicon dioxide (E 551) is to be completed by 2016 at the latest.

4.4 EMA activities

The legal Framework, Parliament and Council Directive 2001/83/EC establishes the Community code relating to medicinal products for human use (pharmaceutical legislation).
Of specific relevance are:
Article 8(3) -Particulars and documents, submitted in accordance with Annex I (a full dossier) to include the results of pharmaco-toxicological tests. The Directive 2003/63/EC or Annex I to Directive 2001/83/EC, as amended, contains detailed scientific and technical requirements. The evaluation of the potential environmental risks posed by the medicinal product is also required.
Recent activities include the Committee for Medicinal Products for Human Use (CHMP) Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products agreed by the Nanomedicines Drafting Group in October 2012. The adoption by the CHMP for release for consultation was on 17 January 2013, with the start of public consultation on 1 February 2013, and the deadline for comments is 1 July 2013 (1EMA/CHMP/13099/2013 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/02/WC500138390.pdf)

4.5 OECD activities

In 2006 the OECD launched a programme of work to analyse the potential safety concerns and to coordinate and harmonize internationally approaches for hazard, exposure and risk assessment for manufactured nanomaterials. In 2012, the OECD and its member countries have come to the same conclusion as the EC that the approaches for the testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials.
The OECD has concluded that in some cases, it might be necessary to adapt methods of sample preparation and dosimetry for safety testing. Similarly, adaptations may be needed for certain Test Guidelines. But the OECD do not consider it necessary to develop completely new approaches for nanomaterials, instead continuing to review all existing methodologies to identify and implement the necessary changes and or adaptations to the Test Guidelines that might be needed for their application to nanomaterials.

The OECD published guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials in December 2012 (OECD 2012).

The OECD QSAR Toolbox does not have any capacity to handle nanomaterials; however extension plans are under discussion in the next phase of development. The understanding of the hazards of the nanomaterials and their regulation poses increasing challenges to predictive methodologies, as it is not possible to test every variety of shape, size and composition of different nanomaterials from a given bulk substance for every endpoint. However it could be possible to read-across between the bulk form and nanoform of the same substance, read-across between different forms of the same substance, and assess options for QSAR models. This will all need further exploratory research.

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