European Food Safety Authority

Supporting Publications 2011:126

TECHNICAL REPORT OF EFSA

Outcome of the public consultation on the draft scientific opinion on Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed[1]

European Food Safety Authority[2]

European Food Safety Authority (EFSA), Parma, Italy

BACKGROUND

EFSA has undertaken a public consultation on the draft scientific opinion for Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed. The draft guidance was prepared by an EFSA Scientific Committee working group composed of external experts and members of the Scientific Committee. The draft guidance was endorsed for public consultation by the EFSA Scientific Committee on the 5th of January 2011. The public consultation started on the 14th of January and closed on the 25th of February (6 weeks). This report provides a summary of the comments and their consideration and includes a table with all the comments as provided.

CONSIDERATION OF COMMENTS RECEIVED

1. Comments received

At the end of the public consultation EFSA had received 256 comments from 35 interested parties including academia, industry, industry organisations, non-governmental organisations, national and international agencies and assessment bodies). Comments submitted formally on behalf of an organisation appear with the name of the organisation. The comments are tabulated in the appendix.

2. Screening and evaluation of comments received

All comments were subject to evaluation and assessment by the working group experts at a dedicated meeting. It was noted that many of the contributions reiterated arguments brought forward already by other organisations. Comments outside the risk assessment remit of EFSA were not addressed, but are included in the table of comments.

[1] On request from EFSA, Question No EFSA-Q-2009-009412, issued on 10 May 2011.

[2] Correspondence: scientific.committee@efsa.europa.eu

Suggested citation: European Food Safety Authority; Outcome of the public consultation on the draft scientific opinion on Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed. Supporting Publications 2011:126 [81 pp.]. Available online: www.efsa.europa.eu

2.1. Types of comments

In general, all different commenter providers were supportive of the outlined strategy and welcome the preparation of a practical guidance. Comments also indicated that it would be of value if more guidance could be provided to specify or describe qualitative endpoints. It is recognised that the guidance document acknowledges that there are several difficulties based on the current knowledge for detection and characterisation of engineered nanomaterials (ENM) in complex matrixes such as food and feed, that limitations in analytical methods also influences the exposure assessment. There is also limited experience with testing methods for ENM and that these difficulties give rise to several uncertainties. Comment providers recognised that there would be a need to update and refine the guidance as the science evolves, increase the use of *in vitro* methods and reduced reliance on *in vivo* methods. Several suggestions for clarifications and further explanations were provided.

3. Incorporation of the documents in the guidance document

A dedicated meeting with the Scientific Committee working group discussed all the comments and addressed how to incorporate them in the guidance. The comments received were appropriate and strongly contributed to enhance the scientific quality and clarity of the guidance document. The relevant comments were taken into account and the guidance document was revised accordingly.

The final guidance document was presented to the Scientific Committee at its April 5-6, 2011 plenary meeting and adopted on the 6th of April 2011.

APPENDIX

TABLE OF COMMENTS RECEIVED

No	Organisation	Section	Comment
1	Health Canada	General comments on the draft Guidance	Thank you for the opportunity to provide comments on Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed (Guidance). Given the evolving state of scientific knowledge, Health Canada recognizes the current challenge of developing more specific guidance documents. Health Canada would be happy to further collaborate with the European Food Safety Authority in sharing information and best practices to promote international regulatory cooperation in this area. Overall, we are pleased with the clarity of the Guidance and appreciate that issues related to the risk assessment of nanotechnology applications in the food and feed sectors have been addressed in a comprehensive fashion. We note that the Scientific Committee has developed this Guidance after a careful and thorough consideration of the current state of knowledge in 1) the available methodologies for ENM characterization in various food matrices that would be required to conduct exposure assessment as well as toxicity testing, and 2) limited information about the biological properties of ENM, their mechanisms of cellular interaction, and any resultant health effects. The Guidance highlights the areas of uncertainties that are associated with the health risk assessment of nanomaterials and thus stimulates discussion regarding the need for additional research to address known data gaps. Additional comments are provided for your consideration.
2	Federal Institute for Risk Assessment	General comments on the draft Guidance	Please clarify that various nanoforms of the same material may exist which differ in physicochemical properties (eg. size, surface coating, crystallinity) and thus, potentially, toxicity. EFSA may also whish to clarify that testing for an ENM should be performed with precisely the nanoform under assessment.
3	UK Food Standards Agency	General comments on the draft Guidance	These comments relate to genotoxicity, carcinogenicity and mutagenicity 1. The guidance is fine as it stands but may need revising after further developments with regard to genotoxicity testing, including the conclusions of EFSA's genotoxicity test strategy committee. 2. 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.

4	Max Rubner-Institut	General comments on the draft Guidance	The guidance draft overviews the difficulties in assessing safety of ENM quite good, but is lengthy and difficult to read. It does not prevent misinterpretations and is in parts inconsistent, as (exemplarily) shown below. Page 2, lines 38-40, page 9 (lines 273-276, fig.1): To use two general situations for the ENM risk assessment (non-nanoform material tested vs. non-nanoform material not tested) is at first glance a convincing idea. However, the strategy dealing with ENM without an approved non-nanoform is not presented consistently. In lines 273- 276 data to be submitted for an EFSA approval must include toxicity tests on the relevant non-nanoform as well as additional data on the chemical characterisation of the nanoform and "where appropriate" on the results of modified toxicity tests applied to the nanoform. In contrast, in figure 1 and all other paragraphs throughout the document it is recommended to perform toxicity test directly on the nanoform. Page2: Lines 41-46: We concluded from the text presented, that the same set of toxicity tests as regulated by law for the non-nanoform are recommended for "ENM with an approved non-nanoform and the same intended use in food/feed". In the case of differing results, additional data on the ENM are necessary. If no differences appear, both materials are of the same risk. However, throughout the document it is pointed out that the present toxicity tests might not be appropriate for ENM. Page 5: lines 143-144: We do not agree that a human exposure to nanomaterials is in general not given if "a delivery system for bulk materials is on the nanoscale". This might be true for most of the systems developed so far, but for some delivery systems it cannot be excluded. I.e. if complete nanocapsules are absorbed, this might in consequence result in a different body distribution of the encapsulated compound in comparison to its free form. Page 5: lines 152-154: It is said, that "the guidelines should indicate where necessary, the additional requirements in terms of endpoints, tests, and data that would have to be fulfilled to be able to perform conclusive risk assessment". It should be pointed out here, that this is not possible at present due to the limited knowledge on ENM and their behaviour in different environments. Page 8, lines 259-260: It is stated, that "in this ENM Guidance, the terms and definitions suggested by the SCENHIR are used" but only a link to the SCENIHR publications is given. To our opinion, the terms and definitions used throughout the guidance should be given highly visible within the guidance text. In contrast, alternative definition proposals (page 8, lines 212-258) might be given as an appendix. Page 10, line 320: Please give a precise definition of good solubility. What relative amount of a compound in which time, temperature, and environment needs to be solubilised? To our opinion the guidance draft illustrates that more information is needed to assess risk/safety of ENM, but due to the gaps in knowledge (are additional endpoints needed to address nano-specific risks, changes of properties of nanomaterials with respect to environment etc.) and the lack of analytical methods for complex matrices (such as food or feed), it is difficult to imagine how risk assessment should work in practice.

5	UK Food Standards Agency	General comments on the draft Guidance	1. The guidance seems basically sound. A reasonable and pragmatic approach which seems very appropriate and positive. 2. One criticism however is that the guidance may be too prescriptive for an area which is rapidly developing and more flexibility would avoid the guidance becoming outdated quickly. 3. An additional consideration is that at a time where efforts are being made to reduce animal testing, are all the studies listed for the risk assessment of engineered nanomaterials necessary and has EFSA considered an alternative approach to animal testing. For example, an alternative approach may be to distinguish where a new nano product may differ from the non-nanoform and devise an appropriate test. 4. The document does not mention how risk assessment for foods containing engineered nanomaterials is being approached in the rest of the world e.g. US, Japan although this is part of the terms of reference. 5. This guidance could usefully be set out in the context of naturally occurring nanostructures in food. For example thermal treatments, such as those often used to cook foods, may give rise to nanoscale protein structures and aggregates. 6. A surprising factor was that there was no reference in the opinion to allergenicity, as this is pertinent to consideration of nanoscale materials derived from proteins as there is evidence that the physical form of a protein may affect its digestibility and its allergenic potential.

6	Norwegian Scientific Committee for Food Safety (VKM)	General comments on the draft Guidance	Comments to "Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed" The Norwegian Scientific Committee for Food Safety (VKM) welcomes the initiative to prepare a guidance document for the safety assessment of applications related to nanotechnology in food and feed. We find the document well prepared and the recommendations, descriptions of tests and indication of endpoints that have to be known seem very useful. As new data in this field becomes available, updates of the guidelines would be welcomed. The Norwegian Scientific Committee for Food Safety highly support the statement that characterisation and knowledge of the properties of the ENM is of vital importance for the outcome of toxicological testing and the comparison of data. We therefore suggest that the importance of a detailed description of the conditions under which the ENM is characterised and used in test systems should be further emphasised. The different parameters for characterisation are described in Table 1, but we suggest that this also should be underlined in the text in chapter 3.1.3 (addition in line 430) and 5.3 (addition in line 587). Our suggestion is based on: Conditions that may influence the activity/properties of ENM and thus the outcome of a test: A. Storage: a. Temperature (room, refrigerator, freezer) b. As dry particles or dissolved c. If dissolved; in what type of solvent (e.g. PBS-buffer – with or without proteins, destilled water etc.) B. Treatment of ENM before given to cells/animals a. Type of solvent (e.g. buffer, culture medium – with or without serum etc.) b. Use of ultrasound (to avoid/reduce aggregation) C. Comparison with reference material: a. Similar treatment of test ENM and reference material/positive and negative control

7	Nanotechnology Industries Association	General comments on the draft Guidance	The NIA is grateful for the opportunity to provide comments on this draft scientific opinion. The NIA welcomes the European Commission's proactive lead in deciding to provide guidance on assessing the potential risks arising from the application of nanosciences and nanotechnologies to food and feed. We particularly appreciate the sound scientific approach that this draft guidance document is based on, and which led to the balanced, realistic recommendations provided in the draft guidance. In order to support and maintain the proactive, science-based effort provided in this guidance, however, we would like to encourage EFSA to provide a more interactive format for the submission and interactive review of the 'supplementary and specific information required on the potential additional hazards and risks that may arise from the nanoform' (quote lines 42-43), which this ENM Guidance is indicating. Such interactive process between those that submit data/information for approval and the relevant risk assessors responsible for approval would, in the first instance, help to identify those applications, for which no further data/information is required, as outlined in lines 51-54: 'Prior to commencing the detailed risk assessment [...].' An ongoing interactive review process would support specifically the approach described in lines 277 – 283: 'This ENM Guidance applies an approach, [...].' The NIA commends the EFSA Scientific Committee for highlighting the necessity to consider ongoing efforts in the research community, when noting that '[a]ppropriate in vitro and in vivo studies on the ENM should be undertaken [...].' (quote lines 56-59). The current work at the OECD Working Party on Manufactured Nanomaterials (WPMN) is of particular value in this context. The NIA that the term ''engineered nanomaterial (ENM)' is not defined in the guidance, but refers to the concept of a nanomaterial that is deliberately produced to be used in the food and feed area,', and that '[i]t is possible that the use of the term in this ENM Guidance will need to be revised once a legal definition [has] been agreed' (quotes lines 247-250), but we would like to caution that the implementability of the proposed risk assessment measures proposed in this ENM Guidance is directly dependent on the identity of the materials subject to these measures; both the scientific and economic impact of an application of the EHS Guidance under a given definition of the term 'nanomaterial' need to be assessed. In this context, we would like to remind the EFSA Scientific Committee 'that "nanomaterial" is a categorisation of a material by the size of its constituent parts. It neither implies a specific risk nor does it necessarily mean that this material actually has new hazard properties compared to its constituent parts.' In general, risk assessment needs to follow a defined, informed framework under full consideration of animal welfare; we encourage EFSA to regard any decision and guidance for potential additional data/information requirements the nanoform in this context.

8	UK Government Chemist	General comments on the draft Guidance	The remit of the UK Government Chemist includes providing advice to Her Majesty's Government and the wider community on dependencies between analytical science and regulatory requirements. Our input to this consultation is partially informed by our current work on the application of field flow fractionation (FFF) with ultraviolet–visible spectroscopy (UV-Vis) and inductively coupled plasma mass spectrometry (ICP-MS), to determine the size distribution and elemental composition of nanoparticles in food. We commend the Scientific Committee's continued efforts to highlight gaps in methodology for the characterisation, detection and measurement of engineered nanomaterials (ENM), particularly in complex matrices, and to develop practical advice that takes account of those barriers to effective risk assessment. Moreover, we agree that in this fast-moving area, the guidance will need to be reviewed and updated frequently. We believe the guidance to be generally informative and helpful - indeed, a number of the queries that we raised while reading the document were resolved satisfactorily by subsequent sections. There are a few remaining suggestions, which we will shortly submit against the individual chapters as requested.

9	ECPA-European Crop Protection Association	General comments on the draft Guidance	The European Crop Protection Association (ECPA) represents the crop protection industry interests at European level. Its members include all major companies and national associations across Europe. ECPA promotes modern agricultural technology in the context of sustainable development, one which protects the health of humans and the environment, and at the same time contributes towards an affordable healthy diet, competitive agriculture and high quality of life. ECPA members support fair, science-based regulation as a guarantee to the consumer and the user of high standards and safe products. ECPA welcomes the guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed and the opportunity to contribute to the consultation. This document provides overall a first good basis for the risk assessment of nanomaterials and identifies existing knowledge and gaps. ECPA has outlined the following arguments which are also detailed in the specific sections below: • ECPA welcomes that the draft guidance applies to engineered/manufactured nanomaterials (ENM) as deliberately produced to be used in the food and feed area. ECPA believes that naturally occurring or unintentionally produced nanomaterials should not be included per se in the scope of the risk assessment. Indeed, they relate in the overall majority of cases to particular substances already existing on the market for many years and not having been intentionally engineered specifically for their nano properties. This should be maintained in the final guidance. • ECPA believes that additional assessment should be undertaken on a case-by-case basis, depending on the risk profile of the substance and addressing concerns which are not currently accommodated by the existing regimes. As noted by the SCENIHR in its opinion dated December 2010, “a "nanomaterial" is a categorization of a material by the size of its constituent parts. (…) It neither implies a specific risk, nor does it necessarily mean that this material actually has new hazard properties compared to its constituent parts or larger sized counterparts”. • ECPA welcomes the follow-up by EFSA on the risk assessment paradigm of hazard identification and hazard characterisation and the cascade approach as developed in figure 2 line 509 and in section 5. Following this approach, an ENM “not present in food” would not require a risk assessment for the nanoform and would apply the risk assessment dedicated for the non-nano/conventional form. In practice, the risk assessment and guidances for PPP would apply. • However, the request for “genotoxicity studies, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM” as defined in lines 571-576 independent of the amount of migration is contradiction with the risk assessment paradigm of hazard identification and characterisation. ECPA does not support this approach and believes that this is counter-productive. Instead, additional testing requirements of ADME and 90-day studies should be determined on a case-by-case basis if the ENM is still “present” in its nano form in food/feed and only address concerns not accommodated by existing risk assessment requirements. • ECPA would also welcome guidance from EFSA and clarification on how this present guidance document will be implemented into the sector specific legislations. Particularly, for PPP, not only EFSA but as well Member States are conducting the assessments of PPP. Therefore it is important to clarify how this guidance will be taken into account in this process.

10	RIVM	General comments on the draft Guidance	First of all, we would like to express that we are quite pleased with this document, it is a useful, clear and comprehensible document. general comment: any additional test or endpoint for nanomaterials can only be justified if it can be integrated in risk analysis. The guidance should facilitate the regulatory process.
11	TNO	General comments on the draft Guidance	This guidance is intended as a practical guidance, however for a number of issues raised no (practical) solution has been provided. Terms as 'adequate' or 'sufficient' are used although no specific requirements have been proposed for these terms to be fulfilled.
12	AQUANOVA AG	General comments on the draft Guidance	Details of the three opinions - BfR (Bundesamt für Risikobetrachtung [Federal Office for Risk Assessment]: In its position paper "Selected Questions and Answers about Nanotechnology" of 9th September 2008 on the limitation of ENMs compared to conventional methods in food technology the BfR found: "What is the link between liposomes, micelles or vesicula and nanotechnology? Organic compounds like liposomes, micelles or vesicles are used in foods to encapsulate other substances like vitamins or flavourings, to transport them around the body and release them in a targeted manner. As the size of these "transport containers" is frequently in the nanometre range, they are also called nanocapsules. However, in contrast to inorganic, in-soluble nanoparticles, their nanoscalability does not lead to any new properties or, by extension, to any new biological effects. Hence, the use of nanoscale organic compounds is not classified as nanotechnology in the narrower sense by BfR. Organic substances like beta-cyclodextrin or polysorbates are frequently used for the capsule membrane. They are toxico-logically tested and assessed, and are approved as food additives (E 459 and E 432 up to E 436)." - Bund für Lebensmittelrecht e.V. (BLL, Association for Food Regulation), Bonn: The BLL also confirms this in its factual and position paper "Nanotechnology in the Food Sector" of March 2008. In this respect it says: "The usual technologies in food processing which are based on the production of extremely small particles must be dealt with separately from the new nanomaterials. There are also ingredients of foodstuffs which are present in nature in the nanoscale form. In such cases however the use of new types of nanoscale materials is not involved, but rather known foodstuff ingredients or substrates already known as foodstuffs (e.g. with starch and protein polymers) which are used with modified dimensions as required by the process. In this respect established technologies which have currently been used as safe methods in food production for decades, such as emulsification and homogenisation as well as methods based on colloidal properties with particle sizes in the nanoscale range are correctly not designated under the term nanotechnology." - American Chemistry Council: The American Chemistry Council uses in his statement "Consideration for a Defination of Engineered Nanomaterials" of 13. March, 2007 although as well the term "engineered nanomaterials" but makes there following exceptions: "Exclusions: … 4. Micelles and single polymer molecules."

13	AQUANOVA AG	General comments on the draft Guidance	Summary Consensus Discussion Scientific Experts Working Group Nanotechnology/Micelle Technology on 17th/18th April 2009 Participants: Prof. Dr. Biesalski (Univ. Stuttgart/Hohenheim), Prof. Dr. Elstner (TU / Munich), Dr. Reimann (Sworn Expert for Pharmaceuticals, Food and Food Supplements / Munich), Prof. Dr. Weber (Technische FH / Berlin), Dr. Weiser (AQUANOVA AG / Darmstadt), Prof. Dr. Weiss (Univ. Stuttgart/Hohenheim) The working group welcomes the scientific opinion of the EFSA on "The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety" of February 2009 which was published in the EFSA Journal (2009) 958 1-39. Based on pharmacological and toxicological aspects, questions arose which have to be considered during the processing of nanoscale particles in the food and feed sectors. Here, the EFSA mentions engineered Nanomaterials (ENMs). The conventional methods used in food technology, which have been established over decades, are not addressed in this paper. The working group is therefore in full agreement with the following supplementary and, above all, more precisely formulated opinions from (for details of the three opinions please see second comment): • BfR (Bundesamt für Risikobetrachtung [Federal Office for Risk Assessment]) • BLL (Bund für Lebensmittelrecht e.V. [Association for Food Regulation]), Bonn • American Chemistry Council and assumes that with the term "ENMs" mentioned by the EFSA solid and inorganic particles or substances are involved which cannot be metabolised and which come under the Novel Food Directive 258/97 Art. 1/2. This directive controls the question of the approval of such substances and particles, also independently of the question of geometry. Limitation of engineered nanomaterials / micelle technology: Micelle definition: Micelles are associated formations of ampliphilic or surface active agents which spontaneously combine in a dispersion medium (self-aggregation). Thus, micelle technology differs substantially from classical nanotechnology. Conclusion: Colloidal systems such as liposomes and micelles, which partly due to purely physical reasons are present through self-formation aided by approved additives (emulsifiers) during food production (technologically unavoidable with CMC = Critical Micelle Concentration), have been constituent parts of food for decades. Colloidal systems such as liposomes and micelles can and should therefore not be regarded nor understood as, and therefore not confused with ENMs from a scientific or a regulatory viewpoint for the purposes of the above mentioned EFSA paper.

14	Eurogroup for Animal Welfare / Animalfree Research	General comments on the draft Guidance	Eurogroup for Animals appreciates the opportunity to give our opinion on the EFSA's Draft Guidance Document on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed. We would like to invite EFSA to take the following comments, prepared by our Member Organisation Animalfree Research with support from Deutscher Tierschutzbund, into consideration in the further deliberations concerning the risk assessment of Engineered Nanomaterials (ENM) in food and feed. In all potential application areas of ENM, appropriate hazard assessment test methods are currently under investigation. Fundamental unresolved scientific questions, which are also depicted in the Draft Guidance Document itself, remain to be answered in order to obtain the level of knowledge necessary to determine hazard assessment strategies adequate in ensuring a scientifically sound human health protection. Considering that the application of ENM in food and feed is not driven by medical incentives, but mainly by economic and life-style driven interests, it does not appear ethically justifiable to expose humans or animals to ENM in food and feed – i.e. via the intentional eating of ENM - as long as their safety cannot be assessed on a scientifically sound basis. Any such attempt would not meet the mission of EFSA to contribute to a high level of protection of human life and health as laid down in EFSA's founding Regulation No. 178/2002. Furthermore, considering that for the safety testing of ENM validated test methods or testing strategies do not yet exist, it is not scientific state-of-the-art to develop new testing strategies based upon animal tests as it is currently foreseen in the Draft Guidance Document. Toxicological animal test methods have numerous scientific deficiencies and are not based upon modern scientific technologies. In acknowledging this, the US National Research Council has spelled out a paradigm change from in vivo to in vitro testing strategies as a vision and a goal for the 21st century (see comments to 5.1). International endeavours are currently striving for such a paradigm change for the toxicological testing of conventional chemicals. As regards the safety testing of nanomaterials, where new test methods and testing strategies are required in the first place, scientific and political efforts should set out to develop and validate scientifically sound non-animal testing strategies making use of modern toxicological test methods and technologies from the beginning. This would stand in line with EFSA's mission to ensure a high level of human health protection; and it would underline EFSA's commitment to playing a proactive role in animal welfare as confirmed by the EFSA Management Board on its meeting of 22 June 2004. Additionally, in the case of animal tests for the safety assessment of substances intended to be applied without medical motivation, such as ENM in food and feed, the harm-benefit-analysis called for by Article 38(2)(d) of Directive 2010/63/EC on the Protection of Laboratory Animals in combination with the Severity Classification for Animal Experiments laid down in Annex VIII of this Directive leads to the conclusion that the harms inflicted upon the animals would outweigh the scientific benefits. In consequence such animal testing should not be considered ethically acceptable (see comments to 5.4). In order to ensure a high level of human health protection and to play a proactive role in animal welfare, ENM in food and feed should only be permissible if their safety to human health can be ensured and if it can be assessed in scientifically validated non-animal testing strategies. In our comments to 5.2 and 5.3, we present an approach on how to fulfil this request.

15	CIAA	General comments on the draft Guidance	CIAA – the Confederation of food and drink industries of the EU – represents the food and drink manufacturing industry, the largest manufacturing sector, major employer and exporter in the EU. Our members are major food producers, federations and sector associations that represent small and medium sized businesses as well as large companies ahs carefully analysed the paper and comes to the following conclusions: It is a good, clearly structured paper reflecting the relevant aspects as regards guidance for risk assessment of nanomaterials and to be more specific ENM. We particularly welcome the recognition that the classical risk paradigm is applicable. We appreciate that there is recognition that food may contain components that have internal structures that individually could be present at the nanoscale, e.g. naturally occurring molecules, micelles or crystals and that "natural "components are considered within the context of this ENM Guidance only if they have been deliberately used or engineered to have nanoscale properties, or used e.g. to encapsulate bioactive compounds. The guidance addresses questions related to specific characteristics and properties of the nanomaterial. Of course also critical points are addressed such as the lack of testing methods for ENM. We appreciate the suggestions made for exposure assessment, namely that on the basis of the available consumption data, the anticipated average and high intakes in various population groups of the ENM food must be estimated, for which probabilistic methods may be useful.
16	Scientific Committee of the Belgian Food Safety Agency (FASFC)	General comments on the draft Guidance	The Scientific Committee of the Belgian Food Safety Agency greatly supports this initiative and is of the opinion that this guidance document is a useful document with clear recommendations. The Committee has following general comments: 1. "Terms used in the ENM Guidance", "Abbreviations" and "Glossary" should be harmonized (some examples are given below) 2. In L365-368 it is mentioned that physico-chemical parameters of ENM change in various environments and that the characterization of ENM has to be considered in various stages. This is an important remark as e.g. the charge and as such the adsorption behavior of the ENM can be different in the administration matrix (e.g. powder) compared to the digestive system (liquid, e.g. saliva). This should be indicated/repeated throughout the document (e.g. in Table 1 – different parameters should be measured at different pH values; see remarks below). 3. Toxicological testing and analysis of ENM in complex matrices remains a major concern. The guidance document should elaborate more on these points, although large research efforts are still needed.

17	BASF SE	General comments on the draft Guidance	BASF very much welcomes the EU-Commission's effort to provide guidance on risk assessments concerning potential risks arising from ENM in food and feed and we highly appreciate the opportunity to comment on the draft scientific opinion. BASF is active in the nutrition and crop protection businesses, thus we have a high level of expertise with regard to the risk assessment and toxicology in these areas. Moreover because we regard nanotechnology as a key enabling technology in our R&D, we actively contribute to the safety research of nanomaterials and publish our results in respected peer-reviewed scientific journals. We hope that our considerations find your support in the final recommendations of EFSA. Overall we find the report to be well balanced, comprehensive and based on sound science. It includes very pragmatic approaches and can also serve as a good example for other sectors outside of food and feed, in which questions about risk assessments are also relevant and under discussion. In the context of this comment, we will focus on the more general aspects of the risk assessment of nanomaterials, which are also relevant for food and feed. However we have also provided specific input with respect to food and feed as part of the contributions from the Federation of European Specialty Food Ingredients Industries (ELC) and the European Crop Protection Association.

18	ELC - Federation of European Specialty Food Ingredients Industries	General comments on the draft Guidance	General comments The ELC appreciates the opportunity to comment the draft scientific opinion on a "Guidance on risk assessments concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed". In our opinion the report is very balanced and takes up the relevant points regarding the risk assessment of this new technology. We appreciate that the well-established risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) was confirmed by EFSA in this context. This is what already has been done for new food additives or food ingredients in the past and there will be no fundamental change for food ingredients (incl. additives) obtained by nanotechnology in the future. We also appreciate EFSA's concept to use information available for an already approved bulk material in the assessment of a newly developed ENM. This is a reasonable approach and only if there are significant differences in the physical-chemical characteristics and behaviour, a comprehensive risk assessment of the new material might be necessary. We also support EFSA's view that a conventional risk assessment should be applied to all ENM's that lose their nano-specific properties by dissolution in the food matrix or in the body fluids or if they build up strong structures that are not released or reactive. This underlines that not size alone is the relevant criterion in the determination of a potential hazard of a material, it's about the novel properties not known for materials at larger scale. However, we would like to raise our concerns that this discussion on the risk assessment of nanotechnology in food and feed has always to be seen in the context of the developments of the legal framework. We understand EFSA's opinion as a scientific document. No conclusions should be drawn out of it with regard to the current discussions on a definition for engineered nanomaterials in the context of foods. The ELC would encourage preliminary discussions between the risk assessors and the applicants – e.g. before determining exposure, in order to avoid double costs on both sides. The ELC generally supports the comments made by our customers of the food industry (CIAA), in particular with regard to lines 572-576: it should be clearly established that if there is no exposure, and thus no risk, no additional tests should be required.

19	Cefic	General comments on the draft Guidance	Introduction In general, Cefic believes that the review is concise and well written. Cefic agrees with the notion that adequate characterization of ENM is essential for establishing its identify and physico-chemical forms in food/feed products. Moreover, it is true that the physico-chemical parameters are likely to change in various environments and that characterization of ENM has to be considered in various stages. Cefic agrees with the statement that the potential risk of an ENM will be determined by its chemical composition, physico-chemical properties, hazard characterization, and potential exposure. This conforms to the conventional risk assessment paradigm. However, the process for conducting an assessment, as described in this document, is unclear. ADME As a consequence of the importance of correct characterization, Cefic believes that the document minimizes the difficulty of conducting ADME studies. Indeed (page 22-line 719) “there may also be particular difficulties in measuring the amounts of ENM in blood, tissues, and excreta, and in establishing the form in which they are present in the body”… (Page 22- line 723) “For ADME studies it is essential that a measuring system is available either detecting the nanomaterial or its composition … “Fluorescence labeling or labeling with radio-labelled chemicals have the disadvantage that the label may be released from the ENM…” In addition to ascertaining whether the label remains with the nanoparticle in vivo– the authors should also consider the following issues/questions: • does the fluorescent labeling process change the biological activity/potential toxicity of the nanoparticle in vivo (e.g., particle-cell interactions) given that most of the surface composition of a nanoparticle is associated with the nanoparticle surface vs. the nanoparticle core constituent (i.e., shell vs. core – often times the shell comprises up to 80% of the entire ENM surface). The labeling of nano-particles in order to follow their way in the body could indeed lead to biased results because some surface treatments clearly modify the transfer kinetics and the elimination process via natural ways (urine, feces). • whether affixing a fluorescent label to the ENM might alter the formation of the particle corona in vivo, i.e., influencing or changing potential interactions of the ENM with gastrointestinal fluid contents, or blood contents following absorption. • In what physico-chemical form does the nanoparticle exist following passage through the stomach and subsequent absorption through the gastrointestinal tract ? (potential outcomes = agglomeration, soluble ions, monodispersed-intact nanoparticles) • how would a fluorescent label on a nanoparticle surface might serve to impact or hinder the efforts to provide relevant data or insights on the nanoparticle physical form after processing through the gastrointestinal tract? These are important considerations which transcend or move beyond simple ADME studies with bulk (non-nano) particle-types. Accordingly, it is unclear whether truly informative ADME studies can be conducted and conclusions derived using labeled nanoparticles; given the current technological limitations regarding fluorescent labelling of nanoparticles. An alternative method may be associated with the implementation of ADME/biokinetic studies with nanoparticulates that have been labeled with radioactive isotopes. However, these types of biokinetic studies may be relevant only for certain metal oxide nanoparticle-types (which labels the particle core component), and accordingly the methodologies should be validated in preliminary assays prior to the undertaking of isotope-based ADME studies.

20	Institute of Food Science and Technology	General comments on the draft Guidance	The Institute of Food Science & Technology (IFST) is the leading independent qualifying body for food professionals in Europe and the only professional body in the UK concerned with all aspects of food science and technology. As a registered charity, we are independent of government, industry, lobby or special interest groups. The IFST welcomes most aspects of the new EFSA guidelines but wishes to raise some concerns about certain aspects of the document, chiefly the definitions of ENMs used to define materials which undergo this new assessment process, and for which extensive physical chemical characterization of the ENMs is to be required. The IFST is also concerned that the ecological consequences of the disposal of ENMs, used as food contact materials, is not included in the guidelines for assessment.
21	Soil Association	General comments on the draft Guidance	The Soil Association is glad that practical guidance for the specific risk assessment of applications involving the use of nanoscience and nanotechnology in the area of food and feed has been produced, in as far as it acknowledges scientific concern that nanotechnologies may present new health risks as a result of their novel properties including their small size, solubility and persistence and reactivity. It is obviously beneficial that the Guidance aims to indicate the supplementary and specific information required on the potential hazards and risks that may arise from the nanoform of a particular material. However, the Soil Association remains concerned that there is currently not enough scientific understanding of how nanomaterials behave in the human body to predict with any certainty what kind of impact specific nanomaterials may have on human health, as acknowledged in the 2010 House of Lords report on Nanotechnologies and Food. This report concludes that persistent nanomaterials are of particular concern, since they do not break down in the stomach and may have the potential to leave the gut, travel through the body, and accumulate in the cells with long-term effects that cannot yet be determined. This report calls for more research to be done on the toxicological impact of nanomaterials to ensure that regulatory agencies can effectively assess the safety of products before they are allowed onto the market. We are glad that the Guidance acknowledges the current uncertainties related to the identification, characterisation and detection of engineered nanomaterials (ENM) because of a lack of suitable and validated test methods to cover all possible applications aspects, and properties of ENM. Similarly, it acknowledges that there are a number of uncertainties related to the applicability of current standard biological and toxicological testing methods of ENM. These uncertainties should be reflected in the conclusions of any risk assessment. However, in itself the Guidance is not enough to protect human health and the environment. In line with the precautionary principle, the Soil Association has signed up to a moratorium on the commercial release of food, food packaging, food contact materials and agrochemicals that contain manufactured nanomaterials until it is shown that they are safe (through the completion of publicly available, peer-reviewed safety studies) and until nanotechnology-specific regulation is introduced to protect the public, workers and the environment from their risks.

22	MRC Human Nutrition Research	General comments on the draft Guidance	The MRC Human Nutrition Research (HNR) was established in 1998 to advance knowledge of the relationships between human nutrition and health by providing a national centre of excellence for the measurement and interpretation of biochemical, functional and dietary indicators of nutritional status and health. HNR also acts as an independent, authoritative source of scientific advice and information on nutrition and health in order to foster evidence-based nutrition policy and practice. The Biomineral Research Group at HNR, led by Dr Jonathan Powell, has a long history of research interests in mineral based nano- and micro-particles in the gastrointestinal tract in terms of exposure, uptake and potential cellular effects. We study both endogenously-formed mineral particles (e.g. mineralised calcium) and exogenous mineral particles (e.g. dietary ferritin or food additives such as silicates and titanium dioxide) and we use a range of approaches from synthetic chemistry and basic cellular work through to whole-animal studies (human and murine). HNR welcomes the opportunity to comment on the EFSA guidance for the risk assessment of nanoscience and nanotechnology applications to food and feed. These comments were prepared by senior staff at HNR and do not necessarily reflect the view of the Medical Research Council. However, we hope they will make a useful contribution to this consultation and we would be pleased to have further discussions on specific issues if this would be helpful. Our general view is that the EFSA guidance document is very comprehensive and timely and should ensure that the nanotechnology-enabled food product applications received by EFSA are uniform and of high scientific standard. We would like to add that although there is a considerable history of risk assessment for particles in the respiratory tract this has not been the case in the gastrointestinal tract. Here, for ENMs intended to be incorporated into the diet the risk assessment could consider some of the gastrointestinal tract's unique characteristics. Unlike any other tissue the gut has specific mechanisms for the purposeful uptake of nanoparticles as well as the inevitable inadvertent pathways that nanoparticles are able to access [Powell JJ, Faria N, Thomas-McKay E, Pele LC: Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. Journal of autoimmunity 2010, 34(3):J226-233]. A further aspect of the unique gut environment is that it contains many luminal toxins and antigens and, due to entropic forces, particles will bind these in the lumen with relatively high affinity. This will change the overall properties of the particle surface and the cellular effects of the antigen or toxin. It should be noted that there are recent data showing that prion infectivity is greatly increased when prions are ingested with particulates [Johnson CJ, Pedersen JA, Chappell RJ, McKenzie D, Aiken JM. Oral transmissibility of prion disease is enhanced by binding to soil particles,PLoS Pathog. 2007 Jul;3(7):e93; Johnson CJ, McKenzie D, Pedersen JA, Aiken JM. Meat and bone meal and mineral feed additives may increase the risk of oral prion disease transmission.J Toxicol Environ Health A. 2011 Jan;74(2-4):161-6]. Immune cells from the gut will migrate to other organs and, therefore, there is a systemic route for distribution of particles from the gut as well as the obvious direct routing through venous and lymphatic channels. Additionally in the calculation of exposure during risk assessment it should be taken into consideration that some gut diseases may have an increased permeability with respect to nanoparticle uptake.

23	Humane Society International	General comments on the draft Guidance	The current uncertainties for risk assessment of nanotechnologies and their possible applications in the food and feed area, as well as in other areas of use, arise due to the presently limiting information in characterisation, detection and toxicology data. The lack of knowledge surrounding the current usage of engineered nanomaterials (ENM), and therefore exposure to such products, is an area requiring immediate attention. Whilst recognising that the currently used risk-assessment paradigm is applicable for ENM, our concerns centre around reliance upon conventional toxicity testing methods for the identification and characterisation of ENM hazards. HSI believes additional issues specific to ENM need to be addressed due to the different properties displayed by ENM when compared to the bulk-form material , and this will require testing method adaptation along with the treatment of ENM on a case-by-case basis. However, we do not agree that the current testing strategies are adequate for ENM. As is the case in the cosmetics sector, it will be extremely difficult in the food and feed industry to characterise ENM, and current guidelines do not address ENM. Until methods are in place to properly determine the behaviour of ENM in living organisms and make careful and informed risk assessments, it would not be defendable for regulators and industry to assert that ENM in food or feed products are 'safe'. We feel that it is more appropriate in the case of nanomaterials for companies to take a precautionary approach by avoiding exposing workers, consumers or the environment to these forms of substances. We do not believe that commercial and societal drives to produce and market the many new and exciting nano-containing applications should overtake the fundamental requisite to protect human and environmental health and safety. We wholly agree with the recommendations into furthering the currently limited knowledge and understanding of ENM behaviour and toxicokinetics. However, we do not support the assumption that the toxicological properties of substances, including ENM, ought to rely on in vivo studies. The level of uncertainty surrounding the hazard assessment of nanoparticles is of great concern. Uncertainties range from basic item preparation and characterisation to appropriate dosimetrics, target parameters such as skin irritation and corrosion, genotoxicity, inhalation effects, cyctotoxic effects, dermal absorption and lung retention, to name a few. If conventional uncertainty factors were to be applied to account for all areas of uncertainty in a ENM risk assessment, the resulting compounded UF could be too large to permit credible scientific conclusions to be drawn (e.g., US EPA risk assessment policy will not accept a compounded UF of more than 3000x—and it is quite conceivable that the compounded uncertainty associated with the hazard and risk assessment of for nanomaterials could exceed 3000x). There is also a level of clarity required regarding which form of nanomaterials to test i.e. manufactured, formulations and food matrix forms, where there is potential for overlap of results. In such cases it is possible that testing will balloon above a reasonable level, and duplicative testing will occur. It is therefore necessary at this stage that a battery of tests be compiled to ensure that an intelligent testing system is applied, and unnecessary (animal) testing does not ensue.
24	Food Safety Authority of Ireland	General comments on the draft Guidance	In learning lessons from the GMO scenario, I wonder if it would be prudent to mention long term effects, unintended effects and examination thereof somewhere in the guidance?

25	Food Safety Authority of Ireland	General comments on the draft Guidance	This is a significant document and forms a good basis on which to inform future risk assessment strategies. It remains to be seen how much detail can in fact be included in such guidance in light of the limited knowledge available on characterisation, detection and quantification of the various nanomaterials and eventual uses in food and feed. A case by case basis will be central to any strategy and as with other guidance documents, regular reviews will be required to incorporate or account for new developments in the technology.
26	FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)	General comments on the draft Guidance	FEFANA believes that the draft Guidance is acceptable at it stands. It provides relevant and pragmatic information which will help to further evaluate the nanotechnogically based products in the future. Therefore, FEFANA appreciates and support EFSA initiative.

27	University of Modena and ReggioEmilia	General comments on the draft Guidance	31 “A loss of nano-specific properties will move the risk assessment into a 32 conventional risk assessment and the nano-specific risk assessment procedure will no longer apply.” It is not true. Biopersistant microsized particles present in food cannot be digestible and there are no pieces of evidence of their elimination by stool. They remain on the bowel mucosa but can be entrapped in the wall and can determine a foreign body reaction. 1 M. Ballestri, A.Baraldi, A.M. Gatti, L.Furci, A.Bagni, P.Loria, R.Rapanà, N. Carulli, A.Albertazzi “ Liver and kidney foreign bodies granulomatosis in a patient with maloocclusion, bruxism, and worn dental prostheses” Gastroenterology , 2001, 121; 1234-38. 2 A.M. Gatti , F. Rivasi “ Biocompatibility of micro- and nanoparticles Part I in liver and kidney.” Biomaterials june 2002, vol 23 , issue 11 , 2381-2387. 3 AM Gatti Biocompatibility of micro- and nano-particles in the colon (part II) Biomaterials vol.25, 3, Feb 2004 385-392 4 AM. Gatti, Montanari, Monari, Gambarelli, Capitani, Parisini Detection of micro and nanosized biocompatible particles in blood. J. of Mat. Sci. Mat in Med. 15 (4): 469-472, April 2004 5 G. Barbolini, AM. Gatti, Nanopatologia. Trattato di Istopatologia. Ed. Piccin Nuova Libraria Padova ISBN 88-299-1769-9 2006, Cap.1.5 pag 75-80 6 A. Gatti., S. Montanari “Nanopathology: The health impact of nanoparticles” book, ed by PanStanford Publishing Pte.Ltd Singapore, ISBN -10981\-4241-00-8, 2008, 1-298. In order to asses the risk is mandatory to evaluate the ENMs presence and their form in the food after packaging. This concept interest the chapter 3.1.2. Characterisation of ENM in food/feed related applications 65 “If it is not possible to determine the nanoform in the food/feed 66 matrix or the form in which it is absorbed, an assumption should be made that all ENM that is added is 67 present, ingested and absorbed in the nanoform.” The presence of inorganic ENMs is possible by,means of Field Emission Gun Environmental Scanning Electromn Microscpy ., in backscattered mode and with the x.ray EDS microprobe. It is difficult with organic ENMs 3.1.3. Characterisation of ENM for toxicological testing 433 It is mandatory to characterize the ENMS also in the biological animal models when they are entrapped in the tissues. 5. Hazard identification and hazard characterisation 545 In order to assess the risk is mandatory to verify the ENMS biopersistence directly inside the animal tissues at the end of the tests. 5.4.1. Administration of ENM for ADME and toxicity studies 713 Bowel samples must be ananlyzed at the end of the chronic test in order to verify the ENMS biodistribution,exposure, persistence and cell entrapment. Protocols for the preparation of the samples can be found in A. Gatti., S. Montanari “Nanopathology: The health impact of nanoparticles” book, ed by PanStanford Publishing Pte.Ltd Singapore, ISBN -10981\-4241-00-8, 2008, 1-298. 6. Exposure assessment 857 An ENMs entrapment in the bowel mucosa must be verified as well as a crossing of the bowel barrier

28	Health Canada	Summary	Health Canada would welcome clarification from EFSA's Scientific Committee on the manner in which it would be possible to judge that the transformation of the ENM into a non-nanoform in the food/feed matrix or in gastroinstestinal fluids is complete, as stated in lines 33-34, page 2.
29	on behalf of the U.S. Government	Summary	Overall, the draft guidance is well thought-out and provides a comprehensive overview of the wide spectrum of issues and uncertainties that need to be considered in assessing the potential risks associated with the use of nanotechnology in a specific application or intended use. With respect to the use of the phrase "nano-specific properties" (p2 L28-32), we note that examples such as reactivity, mobility (although mobility is not defined), and persistence are included. An acknowledgement along with descriptions of additional nano-specific properties may be warranted. In addition, a definition or clear explanation of this phrase is needed. Further clarification on what is encompassed within this phrase seems especially important because it is proposed that in cases where the ENM loses its nano-specific properties, this guidance should not apply and conventional risk assessment protocols should be followed. The draft guidance seems to reflect a less flexible approach to the determination of data requirements and risk assessment assumptions than seems appropriate at this stage of scientific understanding of the effects of nanoscale materials. Risks associated with nanomaterials will likely vary with applications in food, feed, or pesticides. The guidance would benefit from containing more language emphasizing the need for case-by-case assessment of the data requirements and assumptions used in risk assessment. Similarly, endpoints for regulatory decision making may also vary depending on the application. Therefore, we believe decision-directed analysis would help to streamline data needs, decrease extraneous data collection, and facilitate the development of future class-based approaches. In this context, additional guidance on the utility of comparative analysis, such as relative assessments of alternative materials with varying material properties, would be helpful. (p2 L38-40) It is stated that ENMs covered by this guidance fall into two categories, the first being a nanoform of an already approved non-nanoform with the same intended use and the second a new ENM without a corresponding approved non-nanoform. We believe it is appropriate to anticipate the two general situations and analyze associated risks using different approaches. However, it is unclear why in the first situation there is an insistence on "…an already approved non-nanoform with the same intended use", especially when the use could be different. In other words, what if compound X in its nanoform is intended to serve different purpose(s) than the non-nanoform of the same compound? What if there is more than one nanoform of the compound X (e.g., different sizes or shapes of an entity having the same chemical composition)? Should additional such situations be anticipated for purposes of guidance for risk assessment? We believe additional information is needed in this regard.

30	Nanotechnology Industries Association	Summary	• lines 15-18: The NIA agrees with the statement that '[t]he general risk assessment paradigm (hazard identification and hazard characterisation followed by exposure assessment and risk characterisation) is applicable for these applications, and consequently appropriate data and information for the various steps should be made available to the risk assessor to carry out a risk assessment,' and finds that the mention of 'nano-specific risk-assessment procedures (line 32) is a contradiction of this statement. • lines 19-23: The NIA agrees with the statement that '[a]dequate characterisation of ENM is essential for establishing its identity and physico-chemical forms in food/feed products,' and recommends that the methods and equipment used to obtain the respective characterisation data is also reported. • lines 41-43: The NIA agrees with the statement that in the case of a nanoform of an already approved non-nanoform with the same intended use, only 'supplementary and specific information [...] on the potential additional hazards and risks that may arise from the nanoform [is required],' but recommends that an ADME and repeated dose 90-day inhalation study is required only after exposure assessment proves this necessary, as correctly indicated in Figure 1 (see '2. General considerations for assessing ENM').
31	UK Government Chemist	Summary	Line 63 Suggest: '... food/feed matrix. Although rapid methods are under development, currently it is ...'. The main challenges we face are the extraction of intact nanomaterials from food without their transformation and the lack of standards/materials to be used for measurement quality control.

32	ECPA-European Crop Protection Association	Summary	• The characterization of the ENM is key to identify potential risks and behavior of the material. However, the 4 criteria as identified i.e. chemical composition, physico-chemical properties, hazard characterization and potential exposure (lines 19 to 32) do not necessarily lead to a risk, but are signs to help characterize an ENM. • The "completeness" of the transformation as described in lines 33 to 37 needs to be clarified and based on scientific criteria and workable assessment methodologies. • ECPA welcomes the distinction of the 2 categories identified (lines 28 to 40). Regarding the case where a non-nanoform has already been approved with the same intended use, existing scientific assessments for the non-nanoform have already been undertaken for the non-nanoform and their results will apply in the same way to the nanoform. • ECPA welcomes the risk assessment paradigm of hazard identification and hazard characterisation and the cascade approach developed in figure 2 line 509 and in section 5. Following this approach, an ENM "not present in food" would not require a risk assessment for the nanoform and would apply the risk assessment dedicated for the non-nanoform. In practice, the risk assessment for PPP would apply. • However, the request for "genotoxicity studies, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM" as defined in lines 571-576 independent of the amount of migration is in total contradiction with the risk assessment paradigm of hazard identification and characterisation. ECPA does not support this approach and believes that this is counter-productive. Instead, additional testing requirements of ADME and 90-day studies should be determined on a case-by-case basis if the ENM is still "present" in its nano form in food/feed and only address concerns not accommodated by these. • In addition, for PPP products, a genotox battery and acute oral, dermal and inhalation toxicity studies are already mandatory with the formulated product to compare the toxicity between the pure active ingredient and the formulated product. • Regarding the case of a new ENM without approved non-nanoform, risk assessment needs to be determined on a case by case basis, depending on the risk profile of the substance. • Further in vitro and in vivo studies should only be undertaken if the concern has not yet been addressed by a previous study and if persistence of the ENM is demonstrated (Fig 2, page 16, line 509). Particularly, the benefit of further vertebrate studies should be balanced against animal welfare considerations. • ECPA welcomes that uncertainties have been well identified in the different steps pertaining to the characterization of an ENM. This will as mentioned need to be updated given further knowledge and developments in the area.

33	Pennsylvania Bio Nano Systems, LLC	Summary	EFSA is proposing a forward looking document where the categorization of new products provided in the summary and on lines 262 to 276 is: 1) nanoform of an already approved non-nanoform with the same intended use or 2) a nanoform without a corresponding nano-nanoform. There is an assumption here that the non-nanoform provides a reference point that can be used to assess the nanoform. My argument is that introducing the draft document without addressing current products and their dossiers will cause public concerns and will lose sight of current knowledge. Products already in commerce and with 'approved' use patterns are integral to the risk assessment process. The descriptors given these materials (fine, ultrafine, nano) have shifted as nanotechnology awareness broadens, but their inherent nature has not. Hence, there are nanoform products (by the proposed definition) with greater commercial volumes than the non-nanoform version, and there are data dossiers that contain more data generated for the nanoform than for the non-nanoform. Of course, all of this pivots on the definition of a nanomaterial, but not, as mentioned above, on human exposure to that same material by-another-name.
34	Eurogroup for Animal Welfare / Animalfree Research	Summary	The summary of the Draft Guidance Document reveals the extent and profoundness of the unresolved scientific questions regarding the risk assessment of ENM in food and feed that stand in the way to ensuring a high level of protection of human life and health in accordance with EFSA's mission: • It is not yet possible to routinely determine ENM in situ in the food or feed matrix (Lines (LL.) 63-64). • Therefore the exposure of humans and animals to ENM cannot be assessed with certainty (LL. 64-65). • Appropriate test models and testing protocols for the hazard assessment of ENM are still under investigation (LL. 57-59 and 70-71). Considering that exposure and hazard assessments form the main pillars of any risk assessment and that methodologies for the detection of the respective substances in the products of concern are a crucial pre-requisite for exposure and hazard assessment and also for controlling the adherence to legal requirements, it seems premature to strive laying down guidance rules for the risk assessment of ENM in food and feed. Instead of acknowledging that the field is under fast development and that the guidance document will therefore be revised with short notice as necessary (LL. 72-74), for reasons of human health protection and animal welfare the marketing of ENM in food and feed should only be permissible when the safety of ENM can be determined with reasonable scientific confidence and with ethically acceptable non-animal test methods.
35	Scientific Committee of the Belgian Food Safety Agency (FASFC)	Summary	(see also "General Comments") L11: 'ENM' is not mentioned in the list with abbreviations L27: 'ADME' is not mentioned in the list with abbreviations or the glossary
36	Cefic	Summary	• On page 2 – lines 43 – 45, it is written "For such an ENM, in vitro genotoxicity tests, ADME and a repeated dose 90-day oral toxicity study in rodents according to this ENM guidance should be provided. Depending on the outcome of these studies and on the comparison with data on the non-nanoform other in vivo studies may be needed".

37	University of Modena and ReggioEmilia	Summary	31 “A loss of nano-specific properties will move the risk assessment into a 32 conventional risk assessment and the nano-specific risk assessment procedure will no longer apply.” It is not true. Biopersistant microsized particles present in food cannot be digestible and there are no pieces of evidence of their elimination by stool. They remain on the bowel mucosa but can be entrapped in the wall and can determine a foreign body reaction. 1 M. Ballestri, A.Baraldi, A.M. Gatti, L.Furci, A.Bagni, P.Loria, R.Rapanà, N. Carulli, A.Albertazzi “ Liver and kidney foreign bodies granulomatosis in a patient with maloocclusion, bruxism, and worn dental prostheses” Gastroenterology , 2001, 121; 1234-38. 2 A.M. Gatti , F. Rivasi “ Biocompatibility of micro- and nanoparticles Part I in liver and kidney.” Biomaterials june 2002, vol 23 , issue 11 , 2381-2387. 3 AM Gatti Biocompatibility of micro- and nano-particles in the colon (part II) Biomaterials vol.25, 3, Feb 2004 385-392 4 AM. Gatti, Montanari, Monari, Gambarelli, Capitani, Parisini Detection of micro and nanosized biocompatible particles in blood. J. of Mat. Sci. Mat in Med. 15 (4): 469-472, April 2004 5 G. Barbolini, AM. Gatti, Nanopatologia. Trattato di Istopatologia. Ed. Piccin Nuova Libraria Padova ISBN 88-299-1769-9 2006, Cap.1.5 pag 75-80 6 A. Gatti., S. Montanari “Nanopathology: The health impact of nanoparticles” book, ed by PanStanford Publishing Pte.Ltd Singapore, ISBN -10981\-4241-00-8, 2008, 1-298. In order to asses the risk is mandatory to evaluate the ENMs presence and their form in the food after packaging. This concept interest the chapter 3.1.2. Characterisation of ENM in food/feed related applications 65 “If it is not possible to determine the nanoform in the food/feed 66 matrix or the form in which it is absorbed, an assumption should be made that all ENM that is added is 67 present, ingested and absorbed in the nanoform.” The presence of inorganic ENMs is possible by,means of Field Emission Gun Environmental Scanning Electromn Microscpy ., in backscattered mode and with the x.ray EDS microprobe. It is difficult with organic ENMs 3.1.3. Characterisation of ENM for toxicological testing 433 It is mandatory to characterize the ENMS also in the biological animal models when they are entrapped in the tissues. 5. Hazard identification and hazard characterisation 545 In order to assess the risk is mandatory to verify the ENMS biopersistence directly inside the animal tissues at the end of the tests. 5.4.1. Administration of ENM for ADME and toxicity studies 713 Bowel samples must be ananlyzed at the end of the chronic test in order to verify the ENMS biodistribution,exposure, persistence and cell entrapment. Protocols for the preparation of the samples can be found in A. Gatti., S. Montanari “Nanopathology: The health impact of nanoparticles” book, ed by PanStanford Publishing Pte.Ltd Singapore, ISBN -10981\-4241-00-8, 2008, 1-298. 6. Exposure assessment 857 An ENMs entrapment in the bowel mucosa must be verified as well as a crossing of the bowel barrier

38	ECPA-European Crop Protection Association	Background as provided by the European Commission	ECPA welcomes the need for an adequate science-based guidance on the risk assessment of nanomaterials. Given the current uncertainties and future developments needed in the area, the guidance document, once finalised, needs to be regularly updated to take into account of new discoveries and scientific studies.
39	CIAA	Background as provided by the European Commission	Line 139: Other nanotechnologies are referred to but this is not mentioned or addressed at any other point in the guidance.
40	Humane Society International	Background as provided by the European Commission	44 - 46 Any toxicity testing recommended and carried out as part of the risk assessment should be nano-specific. In light of the still limited understanding regarding nanomaterials and their behaviour, it is essential that testing only be carried out when the specific nature and properties of nanomaterials can be properly assessed. In situations where this cannot be assured, the safety of nanomaterials likewise cannot also be assured. Toxicity testing, as well as being nano-specific, should rely on in vitro methods where possible to ensure that results are as mechanistically informative and human-relevant as possible, and that unnecessary animal testing is avoided. 47-50 HSI supports a tiered, weight-of-evidence approach or battery of test methods based on the most relevant methods available at this time and encourages the development of appropriate in vitro human-relevant cell and tissue assays for all endpoints, instead of relying on inadequately modified, non-validated animal bioassays. This tiered approach should start with an initial characterization of the ENM, followed by in vitro basal cell and portal-of-entry toxicity assessments according to human exposure potential and a full characterization of the toxicokinetic potential; systemic or long-term in vivo tests should only be undertaken after initial tiers have been fully explored, and taking the results of initial tests into account. 56-59 Wherever possible in vitro methods should be employed to identify and characterise hazards. When ENM-specific models are not available, testing should not be performed. It is also important to highlight here the issues surrounding dose and how this is measured in the context of ENM i.e. is this by weight or surface area, taking into consideration the differences that can ensue with such issues the dosing could yield very different results. This issue is impact by the difficulties with test item preparation also. 73 It is essential that the ENM Guidance is updated regularly based on experience and acquired knowledge. However, it is also important that whilst the uncertainties mentioned exist, no vertebrate testing is carried out that will not fully address these uncertainties, as such testing would provide dubious added value from a consumer- and worker-protection standpoint. The ENM Guidance document should also encourage researchers to make use of innovative approaches as outlined by the National Research Council's "Toxicity Testing in the 21st Century: A Vision and Strategy", which it is hoped will support a move away from classical high-dose in vivo toxicology into a more human-relevant , which will lead to faster and more reliable results.
41	BEUC - The European Consumers'' Organisation	Background as provided by the European Commission	BEUC comments to lines 126 to 129: CEF and ANS panels should update the guidance documents as a matter of urgency, given the fact that applications for authorisation of products consisting of or containing nanomaterials (in particular food additives, enzymes, flavourings, food contact materials) might be submitted by industry soon and/or given the fact that substances authorised in their bulk form, might be already present on the market in their nanoform or nano formulation.

42	Federal Institute for Risk Assessment	Terms of reference as provided by the European Commission	Please note that EMEA is now EMA.
43	ECPA-European Crop Protection Association	Terms of reference as provided by the European Commission	Different scientific bodies at both EU and international levels are currently working on nanoscience. All their guidances and documents should be taken into account while developing the present guidance. Particularly the conclusions of the SCENIHR document on the Scientific Basis for the Definition of the Term “nanomaterial” of December 2010 should be applied accordingly.
44	National Institute of Advanced Industrial Science and Technology (AIST)	Terms of reference as provided by the European Commission	In a boxed article "Terms used in the ENM Guidance", there seems to be conflicting statements with regard to definitions of "nano". There are statements that "This ENM Guidance does not provide any definitions"in the 214-215 lines and "The term used in this ENM Guidance, engineered nanomaterial (ENM) is not defined in this guidance" in the 247 line. However, in the final part of this box, there is a statement that "in this ENM Guidance, the terms and definitions suggested by the SCENIHR are used, as they are considered relevant for risk assessment (SCENIHR, 2007, 2010)" in the 259-260 lines. It is contradictory with the above cited statements because SCENIHR (2010) proposed a regulatory definition of "nano", saying that "Using the number size distribution, materials might be defined as being a nanomaterial when more than 0.15% of the material has a size below 100 nm." My proposal is to delete the 259-260 lines.
45	Eurogroup for Animal Welfare / Animalfree Research	Terms of reference as provided by the European Commission	Eurogroup for Animals appreciates EFSA’s consideration of relevant documents on risk assessment in the context of nanotechnologies compiled by other scientific advisory bodies at the European level, by EU member states or third countries and also of international documents produced by the OECD Working Party on Manufactured Nanomaterials, OECD WPMN (LL. 155-159). Networking, mutual utilisation of expertise and avoidance of duplication of work are all essential in ensuring a high scientific standard of any regulatory document. Likewise, all information generated during the OECD Sponsorship Programme for the Testing of Manufactured Nanomaterials (http://www.oecd.org/document/47/0,3746,en_2649_37015404_41197295_1_1_1_1,00.html) should be made use of when assessing potential hazards of ENM in food and feed. Above all, however, we would like to encourage EFSA to play a proactive role in cooperating with the mentioned European scientific advisory bodies and, through the EU Commission’s coordinator, at the level of the OECD WPMN - a proactive role aiming at ensuring a high level of animal welfare. In order to avoid unnecessary suffering of animals and a waste of time and resources by developing scientifically flawed and outdated in vivo methods (see our general comments), such a cooperation should be dedicated to exchanging expertise between EFSA and the OECD WPMN, and specifically its Steering Group 7 (SG7 - The Role of Alternative Methods in Nanotoxicology) with the goal to set up, validate and accept comprehensive integrated non-animal testing strategies for the hazard assessment of ENM for the respective conceivable areas of application taking into account the respective modes of uptake of ENM into the human body.

46	Health Canada	1. Introduction	First, in lines 155-159 on page 5, there is no mention of ISO TC 229 work, which includes documents that describe nanomaterial risk evaluation and guidance on physico-chemical characterization of engineered nanoscale materials for toxicological assessment, among others. It is appreciated that this list of relevant documents to consider is not meant to be exhaustive; however, the ISO documents are widely used, and it may be worth acknowledging this work in your list. Second, lines 204-205 and 206-210 on page 7 further discuss the scope of the document. We suggest moving the lines (or at least lines 204-205) following line 185 to add more clarity. Finally, lines 255-257, page 8, indicate that non-nanoform materials refer to materials in the bulk form which can include aggregated nanomaterials. One question we have - is there a certain degree of aggregation necessary to differentiate between ENM and non-ENM?
47	on behalf of the U.S. Government	1. Introduction	Numerous uncertainties related to the identification, characterization, and detection of the engineered nanomaterials call into question the ease at which this guidance can be implemented. Specific methods and protocols are lacking for many of the basic measures. However, the document does identify these deficiencies and allows for updating as knowledge and technologies catch up. With respect to the use of the term “engineered nanomaterial” (p8 L227-228), in the section on definitions and related explanations on page 8, a reference is made to both the definition in the novel foods proposed directive as well as to the recently proposed overarching EC definition. It is also noted that this guidance document does not provide any definitions. We note that there are subtle but important differences between the two EC definitions and it is not clear how “engineered nanomaterial” is being used for the purposes of this document. See also our comment on this issue stated in our comments on the summary category.
48	UK Government Chemist	1. Introduction	Line 228 It might be worthwhile to add: ‘(iii) specific potentialities for biological interactions’. However, we acknowledge that the Common Position is an advanced text, and that this suggested clarification could be considered implicit in properties (i) and (ii).

49	ECPA-European Crop Protection Association	1. Introduction	ECPA welcomes that the scope of the guidance is addressing engineered nanomaterials (ENM) "as deliberately produced to be used in the food and feed area" as stated in line 248. ECPA believes that naturally occurring or unintentionally produced nanomaterials should not be included per se in the scope of the risk assessment. Indeed, they relate in the overall majority of cases to particulate substances approved following strict risk assessments and tests prescribed under EU law and already used safely on the market for many years and not having been intentionally engineered specifically for their nano properties. These technologies might lead to nanoscale structure, however this does not lead automatically neither to change in the properties of the material nor to the introduction of novel properties or hazards. This intentionally engineered distinction should be maintained in the final guidance and should not "be revised once the legal definition is agreed" as mentioned in line 250. ECPA understands that different discussions on the definition of nanomaterials are currently taking place within the European Commission services. The legal definition – once finalised- has not been designed for the food and feed area but is well an overarching definition applicable to all areas incl. electronics etc. Its scope will therefore be refined in each specific sector legislation to address the specificities of each sector such as the food and feed area. It is therefore crucial that the scope of the present guidance is specific to food and feed and continue following the EFSA and SCENIHR approach in applying to intentionally engineered/manufactured/processed nanomaterials as in lines 246 to 253.
50	Eurogroup for Animal Welfare / Animalfree Research	1. Introduction	The current version of the introduction does not make any reference to the animal welfare implications of a Guidance Document for the Risk Assessment of ENM in food and feed or to the 3Rs principle of replacing, reducing and refining animal experiments. The starting point of the Guidance Document should be revised to explicitly confirm EFSA's commitment to playing a proactive role in animal welfare. Due to the significance of animal welfare issues in ensuring a scientifically sound human health protection, concrete reference should be made to the EFSA Scientific Committee Opinion on existing approaches incorporating replacement, reduction and refinement of animal testing: applicability in food and feed risk assessment (EFSA SC, 2009). This important topic should not be subsumed with other topics in a general and unspecific reference to opinions of EFSA scientific committees (LL. 186-193). Notwithstanding Eurogroup's request to lay down tiered non-animal testing strategies for the risk assessment of ENM in food and feed and to only allow for the marketing of ENM in food and feed provided that their human health safety can be assessed with reasonable scientific confidence and in validated non-animal testing strategies, the contents of this EFSA SC Opinion should be consistently applied in the endpoint specific chapters of the Draft Guidance Document. Applied with the aim of laying down a non-animal tiered testing strategy for the testing of ENM, the respective passages should take into account that the EFSA SC opinion dates from 2009 and therefore has to be updated in accordance to the very latest scientific developments in the field of non-animal test method development and also taking into account the work of the OECD Working Party on Manufactured Nanomaterials and its Steering Group, especially Steering Group 7 - The Role of Alternative Methods in Nanotoxicology (see comments to "Terms of Reference as Provided by the European Commission).
51	CIAA	1. Introduction	Lines 204-209: It would seem appropriate to move lines 204-209 to follow line 178 to allow a better flow of the related information.

52	Scientific Committee of the Belgian Food Safety Agency (FASFC)	1. Introduction	See general comment 1. L259: For terms and definitions reference is made to SCENIHR documents. However, inclusion of most relevant terms in this guidance document will increase the readability.
53	BASF SE	1. Introduction	We would like to raise the question of the ENM definition, although we know that this question is not specifically the subject of this Guidance. However the question of which materials are relevant to this Guidance must be answered before the risk assessment methods can be given due consideration. As is evident in the contradiction of lines 571-576 with the concluding statements, there exists confusion regarding what poses a risk at all and what needs to be assessed and what not. If there is no exposure to an ENM (or any other material) there is no risk according to the state of science. In this context we want to emphasize the message that the term "nano" alone is just an indication of a size and does not imply any inherent risk. This is also acknowledged by SCENIHR and other EU bodies.
54	ELC - Federation of European Specialty Food Ingredients Industries	1. Introduction	"Assessment" – Part 1 "Introduction" Lines 199-203: it seems that EFSA intends to follow an approach according to which natural engineered nanomaterials are not differentiated specifically from artificial ones, as long as they are deliberately manufactured "to have nanoscale properties". It should be noted that staple foodstuffs such as homogenized milk would be covered by the Guidance with this approach. However, we think this goes much too far. Many food technologies such as milling, homogenization, emulsification technologies, spray-drying among others are safely used in the production of foods and food ingredients since decades. They might result in structures that are within the nano-scale, however, this is not automatically related to significant change of properties especially introducing novel properties or hazards. Lines 214 to 215: EFSA indicates that "this Guidance does not provide any definitions". However, the absence of a definition might impede a decision on whether a substance actually falls within the scope of the Guidance. This might create a situation of legal uncertainty both for the risk assessors and the applicants. It needs to be clarified, preferably on a legal basis, whether a substance falls into the scope of the guidance, before the guidance is applied. Lines 240- 241: The ELC is in agreement with the EFSA's statement and disputes that the over-arching definition prepared by the European Commission could apply to food ingredients without any adjustments, as it disregards food-specific properties, such as solubility and the case of zero exposure of nano-materials, which we believe are key for their risk assessment and to consumer understanding of "nano" as being ingredient.
55	Institute of Food Science and Technology	1. Introduction	Section 1 lines 216-239 Finally the IFST wishes to comment on the size range used in the definitions of nanostructures for assessment by the approach suggested in the guidelines. IFST believes that there is at present no scientific basis for the chosen upper and lower bounds. IFST feels that size alone is used because it provides a definition that is measurable and thus enforceable. The lower band of 1 nm is basically chosen as that above which it is relatively easy to measure size (this is true for isolated particles or their aggregates or agglomerates but not true for structures within foods, which IFST believes further justifies the use of a term particulate nanomaterials). IFST believes that the upper limit, usually 100 nm, is arbitrary, but the debate on the value of this boundary value is usually about the size range over which materials radically change their physical or chemical properties. IFST suggests that given the concern over persistence and bioaccumulation in the body, this upper boundary may be better determined by the dimension at which bioaccumulation changes radically; namely when these particles can enter cells and accumulate in regions that larger colloidal particles cannot reach.

56	Institute of Food Science and Technology	1. Introduction	Section 1 lines 246-254 IFST welcomes the statement that the actual definitions, particularly for the food industry, are still not agreed and may involve special exceptions. In this context there are discussions in the document on the use of the terms engineered, deliberately engineered and natural nanomaterials. The term engineered is taken to be equivalent to the term ”manufactured” and/or “processed” as used in other reports (e.g. SCENIHR, 2009, 2010). Thus the term engineered nanomaterials does not exclude nanostructures introduced into food through processing. IFST feels that in this case the term engineered may be open to interpretation and difficult to enforce. However, IFST believes that the term ''engineered particulate nanomaterials'' would be a useful definition for the food industry. IFST believes that the use of the term deliberately engineered is also debatable. The term does focus attention on materials that through reducing their size generates novel functionality. However if an ingredient or additive currently used, and approved for use in the food industry, results in its manufacture in the production of a fraction of the material in the nanoscale range, then one could argue that, because this is not deliberate, it is okay: most definitions based on a size range recognise polydispersity, and suggest that the definition of a nanomaterials includes distributions where a finite fraction (1%) of the number distribution lies in the nanoscale range. IFST feels that it cannot be right that the accidental introduction is okay but deliberate introduction of the same nanoparticles requires evaluation. IFST feels that in both cases they should be assessed by the same criteria.

57	Institute of Food Science and Technology	1. Introduction	A concern for IFST is the definitions used in the regulation of nanotechnology and ENMs. If this eventually leads to enforced labelling then this could affect consumer opinion, depending on whether it was seen to be proactive, demonstrating value, benefits and safety, rather than reactive and seen as a warning. IFST welcomes the use of the term nanomaterials rather than just nanoparticles: since 'materials' are defined as composed of at least one condensed phase of structures composed of atoms or molecules, this use of the term nanomaterials excludes food molecules such as carbohydrates, lipids, proteins that would be included in current definitions of nanoparticles that are based on size alone. The Joint Research Center (JRC) Reference report EUR 24403 EN emphasised the need to focus attention on particulate nanoparticles, as these are the class of nanomaterials which require new information and assessment and are believed to raise most concern. With regard to the definitions given in the document the IFST welcomes definitions of the form:- Nanomaterial: means a material that meets at least one of the following criteria:- consists of particles, with one or more external dimensions in the size range 1 nm - 100 nm for more than 1 % of their number size distribution; where the term particle is well-defined:- Particle: means a minute piece of matter with defined physical boundaries (ISO 146446:2007) IFST feels that this type of definition focuses on the importance of particulate nanomaterials and would, by definition, exclude products of processing procedures that rationally modify or create nanostructures in foods to engineer novel functionality. IFST notes that the EU has recently considered introducing additional qualifications into the definition of nanostructures or engineered nanostructures such as:- – has internal or surface structures in one or more dimensions in the size range 1 nm - 100 nm; - has a specific surface area by volume greater than 60 m2/cm3, excluding materials consisting of particles with a size lower than 1 nm. IFST accepts that these qualifiers have been added in order to pick up nanocomposites and aggregates or agglomerates of particulate nanomaterials. However, IFST feels that this has the unfortunate consequence for food that it would include a vast number of natural materials such plant cell walls, starch, protein bodies, currently accepted materials such as acid limit dextrins, resistant starches and most processed structures such as gels, foams and emulsions. In this case it would be better to define particulate nanomaterials and use this as the entry point into a tiered assessment process. If the broader term is used for the entry point then there is a requirement for extensive physical chemical characterisation of the materials, which would be extremely difficult, if not impossible to carry out for nanostructures that are intrinsic components of the food structure. IFST feels that exclusion of these nanostructures through the use of the term particulate nanomaterials would avoid the need for such characterisation, would automatically recognise that these nanostructures are covered by current assessment procedures, and could therefore exclude them from any future enforced labeling of nanomaterials. The IFST feels that it would be better to use the term particulate nanomaterials as the entry point for special consideration and require that products containing particulate nanostructures, aggregates or agglomerates should be declared in proposals for evaluation: e.g. including agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. G Lövestam, et al. JRC Reference report EUR 24403 EN.

58	Institute of Food Science and Technology	1. Introduction	Section 1 line 252 IFST is concerned that a number of mineral ENMs are being used, or suggested for use, as anti-microbial agents in food contact materials. Again IFST feels that there should be information on the consequences of release of these materials into the environment, and this should be taken into consideration when their use is evaluated for food applications. IFST feels that there is an important potential future need for anti-microbial agents, particularly at a time when there is a growing problem with the spread of antibiotic-resistant microorganisms [1]. If there is to be a use for such anti-microbials in the medical area in dressings, treatment of wounds, or generally in coating of medical implants, surgical instruments or hospital surfaces, then the IFST believes one should avoid widespread low-level exposure, which could lead to bacterial resistance to these materials. IFST suggests that this should also be considered in the evaluation of such products suggested for use as food supplements, or in directly applied coatings for natural food products to prevent spoilage, because of their anti-microbial activity. IFST notes that there are already clinical reports in the literature on bacterial resistance to nanosilver [1]. In the above examples, if approval for such materials were given, IFST feels that there is an argument for some form of selective labeling to allow consumers to exercise choice in the use of these materials, or to ensure that they undergo any necessary special recycling. [1] Fries R et al. Nanosilver. NanoTrust-Dossier No. 010en, November 2010: epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier010en.pdf
59	Institute of Food Science and Technology	1. Introduction	Section 1 line 252 IFST feels that there may be aspects of the 'whole-life' aspects of encapsulated nanoparticles in food contact composites that should be considered in their regulation or use: IFST notes that there is already evidence from studies on nanosilver [1-2] that the use of nanosilver in commercial products is increasing the level of silver in streams and rivers. It appears that most of the nanosilver particles are removed during sewage treatment, where they are converted to more stable silver sulphite nanoparticles that are considered to be less reactive. They are accumulated in sewage sludge and this could be used as fertiliser for soils, leading to the accumulation of these particles in soils. However, such nanoparticles in sludge are believed to be the normal form for which dissolved silver or silver chloride precipitates are converted to in sewage plants: nanosilver will thus just increase the level of these particles present in sludge. Disposal of food contact materials containing nanoparticles could, however on their breakdown, lead to the release of more reactive forms into the environment. IFST notes that there is recent evidence that nanoparticles can be transferred up the food chain once they are released into the environment [3-4]. IFST believes that prevention of this occurring may require specialized recycling procedures for these materials and that this should therefore be considered in their assessment. [1] Fries R et al. Nanosilver. NanoTrust-Dossier No. 010en, November 2010: epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier010en.pdf [2] http://www.sciencedaily.com/releases/2011/01/110131133005.htm [3] Judy JD, et al. Evidence for Biomagnification of Gold Nanoparticles within a Terrestrial Food Chain. Environ. Sci. Technol., 45 (2011) (2) 776–781. DOI: 10.1021/es103031a [4] Werlin R, et al. Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. Nature Nanotechnology 6 (2011) 65–71. DOI:10.1038/nnano.2010.251 WHO/FAO Report on the Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications (2010).

60	Institute of Food Research	1. Introduction	Comment 6: Section 1 line 212-260 IFR welcomes the use of the term nanomaterials in the definition of nanostructures, because the use of this term excludes food molecules such as carbohydrates, lipids, and proteins that would be included in definitions of nanoparticles based on size alone. IFR notes that the Joint Research Center (JRC) Reference report EUR 24403 EN [1] emphasised the need to focus attention on particulate nanomaterials, because these are the class of nanomaterials which require new information and assessment and are believed to raise most concern. In this context IFR welcomes the use of definitions of the form:- Nanomaterial: means a material that meets at least one of the following criteria:- consists of particles, with one or more external dimensions in the size range 1 nm - 100 nm for more than 1 % of their number size distribution where the term particle is well-defined (ISO 146446:2007) as a minute piece of matter with defined physical boundaries IFR supports this type of definition because it focuses attention on the importance of particulate nanomaterials and would, by definition, exclude most products of processing procedures that modify or create 'natural' nanostructures in foods to engineer novel functionality. IFR notes that the EU is considering more tightly defining the definition of nanostructures by introducing additional qualifications such as:- – has internal or surface structures in one or more dimensions in the size range 1 nm - 100 nm; - has a specific surface area by volume greater than 60 m2/cm3, excluding materials consisting of particles with a size lower than 1 nm. IFR appreciates that these qualifiers have been added in order to include nanocomposites and aggregates or agglomerates of particulate nanomaterials. However, IFR points out that this has the unfortunate consequence that it would include a vast number of natural plant and animal materials, common food components such as starch, and most processed structures such as gels, foams and emulsions. In this case it would be better to define particulate nanomaterials and use this as the entry point into a tiered assessment process. If the more general term is used as the entry point then there is a requirement for extensive physical chemical characterisation of the materials, which would be extremely difficult, if not impossible to carry out for nanostructures that are intrinsic components of the food structure. Exclusion of these nanostructures through the use of the term particulate nanomaterials would avoid the need for such characterisation, would automatically recognize that these are covered by current assessment procedures, and could exclude them from any future enforced labeling of nanomaterials. IFR suggests that it would be better to adopt the term particulate nanomaterials and require that products containing particulate nanostructures, aggregates or agglomerates should be declared in proposals for evaluation: e.g. including agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. 1 G Lövestam, H Rauscher, G Roebben, Bi S Klüttgen, N Gibson, J-P Putaud & H Stamm. Considerations on a Definition of Nanomaterial for Regulatory Purposes (2000).Joint Research Center (JRC) Reference report EUR 24403 EN.

61	Institute of Food Research	1. Introduction	Comment 5: Section 1 line 240-253 IFR welcomes the statement that the definitions of nanostructures to be used ultimately for assessment, regulation and possible labelling are currently under consideration. IFR notes that the eventual definition of nanostructures may involve special exceptions for the classification of food materials. In this context IFR wishes to comment on discussions in the document on the use of the terms engineered and deliberately engineered nanomaterials. The term engineered is taken to be equivalent to the term ”manufactured” and/or “processed” as used in other reports (e.g. SCENIHR, 2010). The term engineered nanomaterials would not exclude nanostructures introduced into food through processing. The term engineered may thus be fuzzy and difficult to enforce. The term engineered particulate nanomaterials would be a useful definition for the food industry. Use of the term ‘deliberately engineered’ is also debatable and would have both advantages and disadvantages. The term focuses attention on materials (particulate nanomaterials) where size has been reduced to generate novel functionality. However it would lead to certain anomalies. Ingredients or additives currently approved for use in the food industry may contain as a product of their manufacture a fraction (>1%) of the material in the nanoscale range. It could then be argued that because the generation of this fraction is not deliberate, it is acceptable. It would seem to be an inappropriate consequence that the incidental or accidental introduction is acceptable but deliberate introduction of the same nanoparticles requires evaluation. Surely in both cases they should be evaluated by the same criteria? The use of this term could provide ways of avoiding proper assessment and hinder enforcement of any assessment process.

62	Institute of Food Research	1. Introduction	Comment 4: Section 1 line 206 IFR suggests that the production and disposal of these food contact materials will lead to increased exposure to the nanoparticles and IFR is concerned about the omission of 'whole-life' aspects of ENMs from the guidelines and assessment process. IFR feels that the decision on the approval of these products should be not only based on the immediate use of these materials in food applications but should take into account the long-term social and ecological consequences of the use of these products. IFR wishes to raise concerns about the potential use of mineral nanoparticles as anti-microbial agents in food contact materials, food supplements or suggested use as direct coatings on fruits and vegetables [e.g. 6]. IFR feels that the consequences of the low level ingestion of these nanomaterials, or the consequences of low level exposure on the release of these materials into the environment, should be taken into consideration when their use is evaluated for food applications. IFR recognises that there is an important potential future clinical need for anti-microbial agents, given the growing problem with the spread of antibiotic-resistant microorganisms [1]. IFR suggests that widespread low-level exposure to the anti-microbial agents could lead eventually to microbial resistance to their use, and recognises that there are already clinical reports in the literature on bacterial resistance to nanosilver [1]. IFR suggests that this type of problem should be considered in the assessment process and, if approval for such materials were given, that appropriate risk management procedures or selective labelling is used to allow consumers to exercise choice in the use of these materials, or to ensure that they undergo any necessary special recycling. 1 Fries R, et al. Nanosilver. NanoTrust-Dossier No. 010en, November 2010: epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier010en.pdf
63	Institute of Food Research	1. Introduction	Comment 1; Section 1 The Institute of Food Research (IFR) is a Biotechnology and Biological Sciences Research Council (BBSRC) sponsored Institute. The BBSRC is the leading funding agency for academic research and training in the biosciences at universities and institutes throughout the UK. IFR is the only UK institute wholly dedicated to the food science, diet and health agenda, addressing the UK's Grand Challenges of obesity and healthy ageing by defining the relationship between food, diet and health, and making a vital contribution to the food security agenda. IFR undertakes internationally-ranked fundamental, strategic and applied research with high socio-economic impact. IFR has a track record in the use of nanoscience tools to characterise food nanostructures. Based on this remit and experience IFR wishes to make several comments on this EFSA guidelines document IFR welcomes substantial aspects of the new EFSA guidelines but wishes to raise some concerns about certain aspects of the document. These concerns are essentially about the definition of engineered nanomaterials (ENMs) used as the entry point for assessment and requiring physical chemical characterization of the ENMs, and the omission of 'whole-life' aspects of ENMs from the guidelines and assessment process.
64	Humane Society International	1. Introduction	186-188 HSI would like to challenge the statement that the test requirements stipulated in the current EFSA guidance document are applicable for engineered nanomaterials, especially considering on previous occasions a case-by-case basis of testing has been promoted by EFSA . The nature of nanomaterials and their ability to behave in novel ways deems the requirement for test requirements to be tailored specifically to the nanomaterial in question and the use of "general" guidelines is not applicable.

65	Food Safety Authority of Ireland	1. Introduction	Line 239 refers to the definition of a "Particle" as "a minute piece of matter...." Though this definition appears to be derived from an ISO standard, it is difficult to define anything with such vague terms such as "minute" and "piece" and "matter". The terms "minute" and "piece" are indefinable and without dimension. I would suggest consideration of a broader but yet more precise definition such as "a solid physical object with defined boundaries". The size range of a particle would need to be mentioned with the word particle as one can have nano-particles and sand particles which are in very different size ranges.
66	Health Canada	2. General considerations for assessing ENM	This flow chart is a great asset in the Guidance.
67	Federal Institute for Risk Assessment	2. General considerations for assessing ENM	Lines 273-276 It is not agreed that, in the case where a non-nanoform of the nanoform under assessment is not approved, the complete dataset should still be generated for this non-nanoform, while only a chemical characterisation of the nanoform is required. Please check for consistency with other parts of the document (eg. Line 565-569). EFSA may agree that risk assessment for the ENM will benefit from a full dataset for the ENM and supplemental data on the non-nanoform. Lines 320-323 Please define the following: “rapid” degradation, “good” solubility, “strongly” bound aggregates. Without minimum requirements for categorisation this part of the EFSA guidance may be misused intentionally or unintentionally.
68	Federal Institute for Risk Assessment	2. General considerations for assessing ENM	The identity of the relevant non-nanoform should be discussed, ie. bulk, molecular, or ionic. For example, for nanosilver, the nanoform may be characterised as metallic silver Ag(0), while ionic silver Ag(I) may be the relevant non-nanoform. In this section (and apparently in the whole draft document), the situation that another nanoform of the same substance is already on the market does not seem to be addressed. Please provide guidance on how to capitalise on the information from other existing nanoforms and include this information in figure 1

69	ECPA-European Crop Protection Association	2. General considerations for assessing ENM	ECPA welcomes the description made in the overall scheme in figure 1 line 267 which helps clarifying the different suggested steps. However, the first step mentions the need of a "physico-chemical characterization" to determine if the material is an ENM. It remains unclear whether this characterization will also need to be made in order to identify a material as a non-ENM. We trust that this will be based on a case-by-case approach and depending on the material at stake as well as following scientific criteria and adequate methodologies. Any additional testing requirement should address concerns which are not currently accommodated by the existing and extensive testing regimes. This is particularly the case for existing products and substances legally authorized on the market, whose safety has already been assessed and established and thus should not be subject to additional testing requirement, if the safety concerns have appropriately been addressed by the existing testing requirements. We also understand from the "appropriate EFSA guidance" mentioned in figure 1 that they refer to the existing guidance documents as developed in casu for PPP. What remains also unclear is when supplementary data is needed for the nanoform and on how to interpret where tests need to be conducted based on "the amount and quality of information available and the validity of the test originally used to generate data" and "where the information is considered insufficient" as in lines 278-283. This would need to be clarified. ECPA believes that this has to be based on a case-by-case approach depending on each substance and should take into account the scientific and practical feasibility of additional studies.
70	RIVM	2. General considerations for assessing ENM	Page 9, Figure 1. • This flowchart does not cover nanomaterials present in products already on the market which have not yet been subjected to nanospecific risk characterisation (e.g. nano silica in E551). The existence of such materials should be mentioned (Reference could be made to "Presence and risks of nanosilica in food products. Dekker et al, Nanotoxicology, 2010; Early Online, 1–13"). The decision whether such materials should be subject to RA could be described as a risk management call. • Guidance is required on how to deal with size distribution in the decision whether or not a testing as nanomaterial is required. For the risk assessment of ENM in food and feed, the SCENHIR definition for instance would include too many substances (i.e. substances that are of low or no concern). This umbrella definition is expected to be fine tuned for regulatory purposes. The document should more explicitly mention that a regulatory definition is expected to be defined elsewhere.

71	Pennsylvania Bio Nano Systems, LLC	2. General considerations for assessing ENM	Point 1: The document makes several references to “nanoproperties” (lines 49, 568, 569 and 961), but unfortunately does not specify exactly what is meant. There are “general indicators for in-depth testing” (lines 290 to 302). There are “nano-properties” on lines 316 and 317. And a new term, nanoscale properties, is introduced in lines 199 to 203, “Food and feed may contain components that have internal structures that individually could be present at the nanoscale, e.g. naturally occurring molecules, micelles or crystals. However, “natural” components are considered within the context of this ENM Guidance only if they have been deliberately used or engineered to have nanoscale properties, or used e.g. to encapsulate bioactive compounds.” Risk assessors, manufacturers and the public will probably compare nanomaterials to “natural” components on a functional basis. Low fat mayonnaise may be an example as the micelle size for such a product is likely to be well below the current 700 nm. The assessor may ask if the smaller micelle exhibits nanoproperties or nanoscale properties, which might be answered by comparing to low fat homogenized milk. The mayonnaise manufacturer may wish to emphasize that the purpose is “low fat” and not “nanoproperties.” Without an explicit listing of “nanoproperties,” there will only be size to consider. Otherwise, the “general indicators” will substitute for undefined nanoproperties, and these will be balanced against the bullets starting on line 320 to 326 (which by the way are not described and should probably be considered “indicators of low risk”). Recommend that there be properties of “high risk” and properties indicating “low risk,” rather than undefined nanoproperties. Point 2: It appears from reading Section 2 (lines 324 to 326) that (a) nanopores and (b) surface coatings (nanostructured modifications on surfaces) are examples where there is a lessening of nanoproperties as they do not release particles and are not reactive. These two forms of nanotechnology, however, may exhibit unexpected properties without particle release. For (a), pores are difficult to clean and may be havens for viruses, adsorbed species and the like, similar to the Trojan Horse concept, and may require a heightened cleaning regimen. This is especially true if the material with nanopores has surface pores or has an interconnected pore structure and if it is used under cyclical conditions such as in a batch process. For (b), the field of corrosion coatings has demonstrated that cracks, pinholes and other coating imperfections can be loci for accelerated reactions, because there is a property gradient between the intact coating region and the underlying substrate. The public consultation is correct in pointing out the lessened likelihood of particle release, but this is not the full measure of nano-specific risk assessment.
72	TNO	2. General considerations for assessing ENM	Comment to lines 280-283: “If the totality of the available information is considered sufficient at a particular stage, then a risk assessment can be performed, and no further testing would be required. However, if the information is considered insufficient…. sequence of further testing” - Is the fact that a risk assessment can be performed not a consequence of sufficient data but rather a prerequisite? - It should be noted that further testing might be required in case concern or uncertainties are identified in the risk assessment upon evaluation of all data in coherence. When a risk assessment can be performed, still a need for additional testing might be required. - Sufficiency is related to a not further defined ‘particular stage’ whereas ‘insufficient’ is not further described but can be related to more generic aspects related to indicators of potential effects [lines 296-302] and indicators for high exposure [lines 305-313]. The indicators described are indeed relevant parameters, however it would be helpful if more specific guidance was provided concerning further testing requirements.

73	Eurogroup for Animal Welfare / Animalfree Research	2. General considerations for assessing ENM	Notwithstanding Eurogroup's above general comments on the Draft Guidance Document that any attempts to lay down a concrete risk assessment testing strategy for ENM in food and feed are currently premature and that the marketing of such substances therefore should not be considered permissible at this point in time, we would like to express our approval that the risk assessment procedure as such is intended to be applied in a tiered approach, evaluating at each step what additional information and data are needed to accomplish the risk assessment (LL. 277-283) and laying down that if the totality of the available information is considered sufficient at a particular stage, then risk assessment can be performed and no further testing would be required. Likewise, we appreciate the general concept of the schematic outline for risk assessment (L. 267). It is against this background that it is not comprehensible that the hazard identification strategies depicted in Chapter 5 do not follow such a tiered assessment strategy. Evidently, the lack of a tiered hazard assessment strategy seriously compromises the scientific quality of the entire risk assessment strategy. The guidance document on the risk assessment of ENM in food and feed should be amended to contain concrete schemes for integrated tiered testing strategies for the hazard assessment of ENM.
74	CIAA	2. General considerations for assessing ENM	Lines 269-272, Fig 1: It needs to be clarified whether a non-nanoform (with a fraction in nanoform) and an ENM of the same material can have two different risk characterisation profiles. Line 305: We do not understand what this means. If production volume is low then exposure will be low and vice versa. How does purity affect this? We suggest that functionality of the ENM is of more relevance given that it may lead to its use in numerous products. Lines 315-318: The point/s at which a loss of nano-properties needs to be determined should be explained. Also, the fact that no risk assessment is required if an ENM is dissolved to a non-nanoform should be outlined clearly after lines 320-321.
75	Scientific Committee of the Belgian Food Safety Agency (FASFC)	2. General considerations for assessing ENM	Fig. 1: In order to avoid confusion, "with the same intended use" should be added to "Is there an approved non-nanoform of the food/feed substance" in the schematic outline
76	BASF SE	2. General considerations for assessing ENM	BASF applauds EFSA's pragmatic strategy of using existing information generated for an approved bulk material as part of the assessment of a newly developed ENM. This reasonable approach should also form the basis for risk evaluations in other sectors. Starting from line 261, figure 1 clearly describes the process for the risk assessment of ENM in which it is distinguished between already approved substances in food and feed in the non nano-form.

77	ELC - Federation of European Specialty Food Ingredients Industries	2. General considerations for assessing ENM	"Assessment" – Part 2 "General considerations for assessing ENM" We understand the applicability of the Guidance only for risk assessment of nanotechnologies applied to food or feed. The decision whether a substance falls under a definition of a nanomaterial has to be made under a legal framework. Therefore an upcoming definition of "nanomaterials" will have a significant impact to this guidance. Line 267 (Figure 1): it is stated that a full characterization of an ENM (or a justification to skip some characterization tests) would be required by EFSA. However, it is not clearly explained whether a complete characterization is necessary to identify a substance as a non-ENM. We understand that the decision on applicable tests have to be done case-by-case based on the properties of the test material. In addition, would existing products (whose safety has already been determined on an "as is basis") be exempted from this Guidance? We see no need for additional evaluation of currently authorized products if the former risk assessments have sufficiently answered all relevant safety concerns. The question "Is the non-nano-form food chemical/material tested according to appropriate EFSA guidance" in the decision-tree (line 267) would need to be clarified: what does "appropriate" mean precisely (e.g. does it refer to the latest guidances for evaluation of food additives, novel foods or food contact materials)? Line 282: it is mentioned that if the information is considered insufficient, a sequence of further testing would be required. Since nanotechnologies in food and feed are a developing field, it would be appreciated if applicants would get guidance by EFSA which further testing would be required for risk assessment of the ENM.
78	Institute of Food Science and Technology	2. General considerations for assessing ENM	Section 2 lines 332 IFST feels that there should be more prominence and an earlier occurrence of the criteria for persistence and bioaccumulation in the evaluation of particulate nanomaterials. IFST feels that these are the materials which cause most concern and for which there is least information and least methodology for evaluation. This has been raised in the recent House of Lords report and the WHO/FAO report. In relation to this point the IFST feels that particulate nanomaterials that can be shown to be composed of food-approved components and are completely metabolised within the body without adverse effects, may not need to undergo the proposed full physical chemical characterisation at the entry point to the tiered evaluation procedure. House of Lords Science and Technology Committee report (2010) on Nanotechnologies and Food. WHO/FAO Report on the Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications (2010).

79	Institute of Food Science and Technology	2. General considerations for assessing ENM	The IFST welcomes the suggestion of a tiered approach to the assessment for approval of uses of nanotechnology in the food industry. IFST feels this approach is important because it looks at how such materials are covered by present procedures and focuses attention on those materials that require additional information and tests and those for which there is a gap in knowledge and procedures that needs to be closed before decisions can be made. IFST believes that such a tiered approach will effectively illustrate that the vast majority of nanostructures, or applications of nanoscience in food design, are 'natural' and adequately covered by current regulatory procedures. IFST notes that a tiered approach was also suggested in the recent WHO/FAO report. IFST feels it is important that the document suggests that full toxicity data should be available for ENMs encased as composites in food contact materials, even where there is no evidence for migration of these particles into food, or the where the levels of migration are low. IFST considers that this is important because, although the direct use of these materials may not lead to significant ingestion of the particles, knowledge of the level of toxicity, or lack of toxicity, may be needed in order to assess the acceptable levels of migration. Also production and disposal of these materials may eventually lead to increased exposure to the nanoparticles.
80	MRC Human Nutrition Research	2. General considerations for assessing ENM	1) We support EFSA's definition of engineered nanomaterials (ENM) as 'intentionally produced/engineered materials with at least one dimension less than 100 nm or composed of discrete functional parts of less than 100nm (agglomerates/aggregates)'. Biologically this makes sense because, as a rule of thumb, particles below 100 nm diameter tend not to trigger active uptake mechanisms (i.e. macro-pinocytosis and phagocytosis) but instead tend to be taken up through more constitutive endocytic mechanisms. Nonetheless we wish to point out that the gut is heavily exposed to fine particles (i.e. particles > 100 nm diameter) and that these should be considered in the overall picture. We also support EFSA's view that 'natural' components such as micelles should only be considered when they have been deliberately used or engineered to have nanoscale properties or used to encapsulate bioactives.

81	Institute of Food Research	2. General considerations for assessing ENM	Comment 7: pp 332-337 IFR recognises the importance given to the concept of persistence and bioaccumulation of particulate nanoparticles in the WHO/FAO document [1] and the recent House of Lords Science and Technology committee report [2]. IFR feels that, in addition to the use of the definition particulate nanoparticles, more prominence should be given to the criteria for persistence and bioaccumulation at an earlier stage in the evaluation of particulate nanomaterials. IFR believes these are the materials which cause most concern and for which there is least information and least methodology for evaluation. IFR suggests that in this context most particulate nanomaterials that can be shown to be composed of food-approved components, which are completely metabolised within the body without adverse effects, may not need the proposed full physical chemical characterization at the entry point to the tiered evaluation procedure, in order to enable their evaluation. Finally IFR wishes to comment on the size range (including fractions of the number distribution) used in the definitions of the nanoscale. There is at present no scientific basis for the chosen upper and lower bounds and size alone is used because it provides a definition that is measurable and enforceable. The lower band of 1 nm is basically chosen as that above which it is relatively easy to measure size (but, whereas this is true for isolated particles, or their aggregates or agglomerates, it is not true for structures within foods - further justifying the use of a term particulate nanomaterials). The upper limit, usually 100 nm, is arbitrary. IFR notes that the debate is usually about the size range within which materials radically change their physical or chemical properties. However, IFR suggests that, in view of the concern over persistence and bioaccumulation in the body, that this upper boundary may be chosen as the dimension at which bioaccumulation changes radically, and reflect the size boundary below which these nanoparticles can enter cells and accumulate in regions that larger colloidal particles cannot reach? Such a biological definition may be easier to define and measure. 1 WHO/FAO Report on the Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications (2010). 2 House of Lords Science and Technology Committee report (2010) on Nanotechnologies and Food.

82	Institute of Food Research	2. General considerations for assessing ENM	Comment 3: Section 1 line 206 IFR welcomes the suggestion in the document that is important that toxicity data should be available on engineered nanoparticles which are to be encased as composites in food contact materials, even where there is no evidence for migration of these particles into food, or the where the levels of migration are low. IFR feels that this is important because, although the direct use of these materials may not lead to significant ingestion of the nanoparticles, it may be necessary to have knowledge of the toxicity, or lack of toxicity for nanoparticles in order to set acceptable levels of migration into foods. IFR suggests that the production and disposal of these food contact materials will lead to increased exposure to the nanoparticles and IFR is concerned about the omission of 'whole-life' aspects of ENMs from the guidelines and assessment process. IFR feels that the decision on the approval of these products should be not only based on the immediate use of these materials in food applications but should take into account the long-term social and ecological consequences of the use of these products. IFR notes that use of nanosilver in commercial products appears to be increasing the level of silver in streams and rivers [1-2]. IFR notes that most of the nanosilver particles appear to be removed during sewage treatment, where they are accumulated in sewage sludge in a less reactive, more stable form as silver sulphite nanoparticles. Thus the use of the sludge as fertiliser for soils, leading to the accumulation of these particles in soils, may not pose a significant problem. However the disposal of food contact materials containing nanoparticles, such as nanosilver, could lead to the release of more reactive forms into the environment. IFR notes there is recent evidence that nanoparticles can be passed up the food chain once they are released into the environment [3-4]. Thus IFR suggests that the assessment process should consider steps to prevent such release, either through failing to approve such materials, or through ensuring appropriate risk management procedures such as labelling, to enable consumer choice, or to ensure specialized recycling procedures for these materials. 1 Fries R, et al. Nanosilver. NanoTrust-Dossier No. 010en, November 2010: epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier010en.pdf 2 http://www.sciencedaily.com/releases/2011/01/110131133005.htm 3 Judy JD, et al. Evidence for Biomagnification of Gold Nanoparticles within a Terrestrial Food Chain. Environ. Sci. Technol., 45 (2011) (2) 776–781. DOI: 10.1021/es103031a 4 Werlin R, et al. Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. Nature Nanotechnology 6 (2011) 65–71. DOI:10.1038/nnano.2010.251
83	Institute of Food Research	2. General considerations for assessing ENM	Comment 2: Section 2 (261-343) IFR welcomes the suggestion of a tiered approach to the assessment of uses of nanotechnology in the food industry. It is felt that this approach, as also suggested in the recent WHO/FAO report [1], is important because, by looking at how nanomaterials are covered by present procedures it focuses attention on those materials that require additional information and tests and, ultimately, those for which there is gap in knowledge and procedures which needs to be filled before decisions can be made. Such a tiered approach should effectively demonstrate that the vast majority of food nanostructures are 'natural' and adequately covered by current regulatory procedures. 1 WHO/FAO Report on the Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications (2010).

84	Humane Society International	2. General considerations for assessing ENM	Assess – General considerations for assessing ENM 273-283 A tiered, weight-of-evidence approach or battery of test methods based on the most relevant methods available at this time and encourages the development of appropriate in vitro human-relevant cell and tissue assays for all endpoints, instead of relying on inadequately modified, non-validated animal bioassays. This tiered approach should start with an initial characterization of the nanomaterial, followed by in vitro basal cell and portal-of-entry toxicity assessments according to human exposure potential and a full characterization of the toxicokinetic potential; systemic or long-term in vivo tests should only be undertaken after initial tiers have been fully explored, and taking the results of initial tests into account.
85	BEUC - The European Consumers'' Organisation	2. General considerations for assessing ENM	BEUC comments to lines 264 to 266: A different situation to consider is when a nanoform of an already approved substance in food/feed is engineered for a different intended use. BEUC considers that such a case should be explicitly mentioned, whereas as for the requirements, they should be the same as those for a new ENM (ENM for which a corresponding non-nanoform does not exist/is not approved). BEUC comments to lines 290 to 326: Amongst the general aspects to be considered, any change of the physico-chemical characteristics of the bulk substance compared to the non nanoform should be taken into account (e.g. increased/decreased solubility) as well as any impact that these changes might have on the bioavailability and on the ADME.
86	Food Safety Authority of Ireland	2. General considerations for assessing ENM	In the second box down on the right of Figure 1 after line 266 that reads "If evidence demonstrates no exposure there is no need for further testing". I suggest the first "no exposure there is..." to be changed to "no or very limited exposure there is....". Line 310 reads "Targeted release" and I would suggest "Targeted or controlled release".
87	Health Canada	3. Characterisation of ENM	Of particular interest for organizations such as Health Canada, who has recently initiated nanomaterials research or characterization, this section of the Guidance pertaining to the characterization of ENM is considered very comprehensive and identifies all possible parameters and analytical techniques available for their characterization. Given the state of the science, we recognize and support EFSA's approach to place the emphasis on the description of all ENM parameters that should be understood and the various approaches currently available to measure a particular parameter. We have some further suggestions for your consideration. First, lines 356-359, page 11, discuss the importance of determination of shape in characterizing ENMs. The determination of shape is indicated in Table 1 (Line 412) as being "essential." Perhaps a less critical physical/chemical property could be used to illustrate this point. Second, regarding lines 360-364, page 11, the selection of analytical methods for physico-chemical methods being dependent on the nature of the material is not unlike that for non-ENMs. We recommend that this statement either be removed from the guidance or it be added as this is also true for the characterization of non-ENMs. Finally, we expect that with experience and as the science evolves, certain key parameters will be identified and this Guidance will be updated accordingly. It might also be useful for recipients of this Guidance to provide further details regarding the known research groups who are developing methods for identification and characterization of ENM in complex matrices as well as to identify in a more comprehensive manner institutions that establish nano-size reference materials.

88	ECPA-European Crop Protection Association	3. Characterisation of ENM	• The characterization of the ENM is key to identify potential risks and behavior of a substance. Again, we believe that this should be done on a case-by-case basis depending on each concerned substance. ECPA acknowledge that EFSA recognizes that knowledge gaps exist (such as in line 355), which will make it very difficult to identify the different parameters for the characterization of many substances. ECPA also agrees that an optimal method will depend on the type of ENM, its measurement environment and thus is only possible on a case by case basis. • With the current lack of clarity regarding adequate methods for characterization of the nano-material form in the various matrices (as in lines 373 and 374) and as stated on page 11, line 360-361 "the selection of an optimal method will be dependent on the type of ENM", the decision on what constitutes suitable methods to choose from, their development and their technical validation, should be a joint state-of-the-art exercise between industry, academia and authority-related laboratories. The technical method validation, including the method performance criteria, can be a joint inter-laboratory/interagency project in collaboration with international agencies (e.g. ILSI, ICVAM, ECVAM,...). Laboratories performing the studies then need to demonstrate proficiency in meeting the requested standards. Once an appropriate analytical method has been developed and validated as described above, this will enable the applicant to provide the methods of analysis as described in the guidance.
89	RIVM	3. Characterisation of ENM	P 11-15, section 3: Although the section appears to be sufficiently comprehensive in mentioning the different properties that may be important and how to characterise these, some further guidance on when to measure which property and how appears necessary (despite the fact that it may depend on the specific nanomaterial). P 13, Table 1 • Is the log kow meaningful for nanoparticles? The log Kow does not seem to be meaningful for non-soluble or poorly soluble nanomaterials, e.g. nanosilica, nanosilver etc. Partitioning of these nanomaterials is not driven by chemical potential like the soluble forms of these compounds. • The Description of the Parameter “Particle and mass concentration” would be clearer if it ends like “[...] and particle number per mass when as dry powder.” P 14, section 3.1.3 • Special attention should be paid to changes in the particle during the food product manufacturing and preparation. • An additional issue for consideration is that the physicochemical properties of nanomaterials in food and feed may deviate from the physicochemical properties of the ENM formulation prior to the application in food/feed which is the form that is used in hazard assessment. At present, it is unclear if this matters and how to deal with that. Characterization of the ENM formulation as manufactured and of the particle in food/feed is required to start this discussion.
90	CIAA	3. Characterisation of ENM	Line 373: Delete ‘manufactured’.

91	ELC - Federation of European Specialty Food Ingredients Industries	3. Characterisation of ENM	"Assessment"- Part 3 "Characterisation of ENM" Lines 365 - 368: it is proposed that the physical-chemical characterisation of the ENM has to be considered in various stages including its fate in the food matrix. It should be clear that food matrices vary very much and that this can only be done in some exemplary matrices. Similarly, we would warn against a request for characterisation that would apply to "formulations delivered for use in food/feed products": there may be dozens of commercial formulations derived from a single material and it would neither make sense nor be feasible to test them all: here again the request for characterisation shall be limited to relevant typical formulations. Lines 373 - 374: it is indicated that methods to determine ENM in the food matrix are under development and not available yet. Line 411: it is pointed out that a separation of the ENM from the matrix may influence the ENM-properties. These statements tend to show a gap in detection of ENM as well as an impossibility to determine properties of the ENM in food. Therefore, one might question the feasibility to implement the Guidance on imported foods. Any discrepancy between controls conducted on imported and EU-produced foods would create a discrimination, particularly against foods produced in the EU. How does EFSA assess the safety level of imported foods?
92	MRC Human Nutrition Research	3. Characterisation of ENM	2) We agree with the need for a thorough characterisation (including but not limited to size, shape, solubility, surface charge, surface reactivity) of the ENM in the different scenarios i.e.: as manufactured, in formulations, in the food matrix (as and when analytical methods become available), as used in toxicity testing and as present in tissues and biological fluids. We would like to emphasize the need to include physical characterisation of materials after a simulated gastrointestinal digestion, i.e. in gastric and intestinal simulated fluids. The report, rightly, highlights that if nanoparticles become solubilised under gastrointestinal conditions the risk will be equivalent to that of soluble species originated from non-nanoparticulate materials. However, a further, more worrying possibility is that the ENM become(s) more toxic upon digestion. This may happen via different routes such as size reduction (e.g. dispersion of primary particles of the ENM or partial dissolution), changes in surface reactivity, change in zeta potential (e.g. pH driven change or due to the adsorption of gastrointestinal species) to name a few. To be able to truly assess risk it is, therefore, important that these nanomaterials are physicochemically characterised in digestion assays as well as the proposed characterisation in food or food simulants.
93	Humane Society International	3. Characterisation of ENM	380 It is important to stress at this juncture that when it comes to ENM characterisation the scientific community is still establishing the best practices. It is also apparent from recent studies that when single particles are in suspension it is possible to determine their size, however, a mixture of two or more particles can give inaccurate measurements. Therefore, mixtures should be treated with caution, and until validated methods are established the size distribution cannot be performed with scientific accuracy. 449 The consistency regarding use of methods and even use of protocols for the same method is of paramount importance when studying nanomaterials. In order to ensure that accurate, reproducible results are being generated, harmonised testing protocols should be developed and utilised for all relevant endpoints.

94	University of Modena and ReggioEmilia	3. Characterisation of ENM	3.1.2. Characterisation of ENM in food/feed related applications 65 “If it is not possible to determine the nanoform in the food/feed 66 matrix or the form in which it is absorbed, an assumption should be made that all ENM that is added is 67 present, ingested and absorbed in the nanoform.” The presence of inorganic ENMs is possible by,means of Field Emission Gun Environmental Scanning Electromn Microscpy ., in backscattered mode and with the x.ray EDS microprobe. It is difficult with organic ENMs 3.1.3. Characterisation of ENM for toxicological testing 433 It is mandatory to characterize the ENMS also in the biological animal models when they are entrapped in the tissues.
95	Health Canada	3.1. Requirements for identification, detection and characterisation of ENM	Lines 380-381, page 12, discuss measuring the size parameter. When asking for two different methods for characterization of size, this may result in disagreement between the two methods. In this case, which size measurement should be considered to be the “correct” one for regulatory purposes? If one method must be electron microscopy, perhaps this should be the only method that is required to eliminate any ambiguity. Otherwise, this could lead to selective characterization of ENM vs non-ENM (i.e. choosing being non-ENM so that data requirements are reduced) at the applicant’s discretion.
96	on behalf of the U.S. Government	3.1. Requirements for identification, detection and characterisation of ENM	(p12 L381-386): The guidance states the particle size parameters should always be measured by at least two independent methods (one being electron microscopy) as the results obtained from different measurement techniques may differ because of the physical principles applied in the measurement method. Additional guidance may be needed on the concordance of results obtained by the two methods. It would also be important to clarify that discordant results from two methods could require additional measurements/methods and, conversely, whether and when a single method might be sufficient.
97	ECPA-European Crop Protection Association	3.1. Requirements for identification, detection and characterisation	See ECPA comments on section 3.
98	Scientific Committee of the Belgian Food Safety Agency (FASFC)	3.1. Requirements for identification, detection and characterisation of ENM	Table 1: • In Table 1 the parameters for characterisation and identification of ENM are described. The available methods are provided in a table in Appendix A. However, for the particle size some information on the methods is given between brackets. This information is confusing and not in line with the information provided in Appendix A (EM appears to be mandatory from Table 1). + “primary/secondary” should be explained in e.g. the glossary • Surface charge: as the zeta potential depends on pH, this should be measured at different pH values (formulation, food/feed matrix, stomach: acid vs. gut: alkaline) – See also general comment 2; a similar remark can be made for particle size, surface chemistry, etc. • Chemical reactivity/catalytic activity: it should be clarified that the surface coating refers to the coating of the ENM.

99	BEUC - The European Consumers'' Organisation	3.1. Requirements for identification, detection and characterisation of ENM	BEUC comments to Table 1: Parameters for characterisation and identification of ENM: We believe that “dispersibility” should be added to the list as a distinct parameter from “solubility”. Insoluble materials might in fact show an increased dispersibility when in nanoform, which might be confused with an increased solubility. The resulting stable dispersion (colloidal dispersion) might be (wrongly) considered a true solution rather than a dispersion where the solid and the liquid phase co-exists, thus it might be difficult to distinguish between a dissolved and a dispersed nanomaterial. Methods like laser scattering are therefore required to detect nanoparticles and measure their size in order to be able to determine whether the nanomaterial in question yielded a (true) solution or a colloidal suspension. The term “dissolved” should be then used only to indicate true solutions while the term “dispersed” should be used when both liquid and nanoparticulate phases are present. Any increase in solubility or dispersibility might affect bioavailability, ADME, exposure.
100	Health Canada	3.1.1. Characterisation of ENM prior to use in food/feed related applications	In Lines 392-395, page 12, we suggest amending this sentence to the following: “Information from non-nanoform guidance that could be used to characterize the ENM includes the name (generic or proprietary), CAS number (if available), method of production….and stability/shelf life” to add clarity.
101	UK Government Chemist	3.1.1. Characterisation of ENM prior to use in food/feed related applications	Line 412 In Table 1, it would be helpful to clarify any intended distinction between a suspension and a dispersion, particularly in view of the distinction drawn between a dispersion and a solution by footnote a). Possibly, ‘dispersion’ covers a wider range of phase systems, or ‘suspension’ carries a distinct meaning as regards the homogeneity or stability of the mixture.
102	UK Government Chemist	3.1.1. Characterisation of ENM prior to use in food/feed related applications	Line 391 Suggest appending: ‘Applicants should consider the need to develop, maintain (including by calibration), and apply routine QC methods for checking compliance with these specifications.’
103	CIAA	3.1.1. Characterisation of ENM prior to use in food/feed related applications	Line 392: Although the term non-nanoform is explained in the glossary, it is not always clear when used in the document. In particular, at line 392 it is unclear as to whether it refers to the bulk or molecular/ionic form of the material.
104	Food Safety Authority of Ireland	3.1.1. Characterisation of ENM prior to use in food/feed related applications	Line 345: I would suggest the following alteration to read" In addition to the relatively small size, which is the main physical characteristic of ..."

105	Health Canada	3.1.2. Characterisation of ENM in food/feed related applications	It might be beneficial to enhance the clarity of what is meant by “aging variations” as stated in line 432, page 14.
106	on behalf of the U.S. Government	3.1.2. Characterisation of ENM in food/feed related applications	We agree with the distinction that appears to be made in the document (page 8, lines 252-253 and page 12, lines 399-401) between materials naturally occurring in the nanoscale and those that are intentionally added or engineered. Similarly, it is helpful to emphasize the actual characteristics and properties of the ENM rather than an artificial threshold solely based on particle size for risk assessment purposes (page 8, lines 213-214). In this regard, a more risk-based approach may be appropriate rather than premature grouping or focus on engineered nanoparticles alone
107	UK Food Standards Agency	3.1.2. Characterisation of ENM in food/feed related applications	Line 412 - Table 1 was very useful
108	ECPA-European Crop Protection Association	3.1.2. Characterisation of ENM in food/feed related applications	See ECPA comments on section 3.
109	TNO	3.1.2. Characterisation of ENM in food/feed related applications	Comment to table 1 [line 412]: The parameters considered as an essential requirement for the chemical characterization and identification of the ENM are given in table 1. Within these requirements also the ‘nature of any impurities’ should be identified. In this respect, it is important to consider that two types of impurities may be concerned: 1) impurities in the ENM by other nano materials, e.g. as a result of cross-contamination, and 2) impurities within the ENM (impurities already present in the starting chemicals and/or impurities introduced upon production of the ENM). It seems important from a hazard point of view to discriminate between these potential impurities. Furthermore, the distribution (homogeneous or not) of any impurity in an ENM may be relevant for the hazard profile of the ENM as well.
110	CIAA	3.1.2. Characterisation of ENM in food/feed related applications	Line 408: It is acknowledged that separation of the ENM from the food matrix may be needed but also that the separation methods may affect the ENM properties. However, there are no suggestions of appropriate separation methods or how best to determine whether the properties have been affected, which does not help with interpretation. Table 1 - The list on characterisation requirements is very prescriptive and does not explain clearly why some parameters are essential (only given for catalytic properties). This also needs to be taken into account in the decision tree approach according to importance.

111	Scientific Committee of the Belgian Food Safety Agency (FASFC)	3.1.2. Characterisation of ENM in food/feed related applications	L418: 'Any catalytic activity of ENM needs to be measured': it is not clear if all these effects should be evaluated. When formulated this way, the catalytic activities which have to be evaluated should be specified, instead of listing some examples. Therefore, it is suggested to replace "any" with "possible" or "relevant". (based on the description of the parameter "chemical reactivity/catalytic activity" (surface coating'), this parameter seems to be closely related to the parameter "surface chemistry". It is suggested to elaborate a little more the description of this parameter in the table.)
112	on behalf of the U.S. Government	3.1.3. Characterisation of ENM for toxicological testing	(p14 L427-430): For toxicological assessment of an ENM, it is essential to know in which form the tested ENM is present in the test systems. Characterization of the ENM in the test system is relevant to determine the effect of the test medium/formulation (and its constituents) on the characteristics and properties of the ENM, in order to determine the validity of the toxicity test outcome. We believe it is important to clarify the extent of similarity between the test system and the application system. It is also noted that additional information may be needed to address the concern for aging variations. Furthermore, toxicity testing of non-nanoscale materials as the basis for baseline information would call for bridging type studies between non-nanoscale material and nanoscale counterpart before relying on one set of toxicity data (e.g., with non-nanoscale materials) to help inform the other set (e.g., with nanoscale materials) of potential toxicity.
113	ECPA-European Crop Protection Association	3.1.3. Characterisation of ENM for toxicological testing	See ECPA comments on section 3.
114	on behalf of the U.S. Government	3.1.4. Uncertainties in characterisation of ENM	(p14 L456-463): The guidance states characterization of ENMs in food/feed matrices may be insufficient due to the current limited availability of analytical methods. It is suggested that possible food/feed matrix interactions of the ENM may be determined using food simulants (e.g. water, oil, alcohol, or simulants representing the characteristic composition of the target food, e.g. starch for carbohydrate-rich foods). It is unclear whether a "food simulant" could serve as proxy for the food matrix and whether additional justification may be needed on the appropriate testing capabilities for the detection and characterization of ENMs.
115	ECPA-European Crop Protection Association	3.1.4. Uncertainties in characterisation of ENM	ECPA welcomes the recognition of uncertainties and the absence of a gold standard method applicable for characterizing all ENM as addressed in lines 435-436. This should be better addressed during the different steps suggested by the present guidance. See also ECPA comments on section 3.
116	TNO	3.1.4. Uncertainties in characterisation of ENM	Comment to lines 456-463 A few remarks are provided on the use of food simulants in order to characterize the ENM. However, these remarks do not lead to clear guidance when simulants should be used and how these results should be interpreted.

117	Eurogroup for Animal Welfare / Animalfree Research	3.1.4. Uncertainties in characterisation of ENM	Eurogroup acknowledges the prevailing uncertainties depicted in Chapter 3.1.4 concerning the characterisation of ENM as such as well as specifically their detection in food and feed. We would like to emphasize that the lack of reliable and relevant methods for the detection and characterisation of ENM seriously compromises the relevance of any exposure or hazard assessment and in consequence of a risk assessment that reliably protects humans from unwanted effects by eating ENM that have been intentionally added to their food. It also compromises the possibility to enforce any risk assessment rules since their compliance cannot be monitored objectively and reliably. Therefore we firmly believe that it is currently premature to lay down a guidance document for the risk assessment of ENM in food and feed aimed at marketing such products.
118	CIAA	3.1.4. Uncertainties in characterisation of ENM	Lines 454-455: It needs to be clarified if a non-nanoform (with a fraction in nanoform) and an ENM of the same material can have two different risk characterisation profiles.
119	Scientific Committee of the Belgian Food Safety Agency (FASFC)	3.1.4. Uncertainties in characterisation of ENM	L479: the paragraph concerning available reference materials should be actualized
120	BEUC - The European Consumers'' Organisation	3.1.4. Uncertainties in characterisation of ENM	BEUC comments to lines 456 to 463: The level of uncertainty and the extent as to whether results obtained using food simulates can be extrapolated must be carefully evaluated and clearly reported on a case-by-case basis.
121	Nanotechnology Industries Association	3.2. Performance criteria for characterisation methods	• lines 479-484: In addition to the reference materials validated for size calibration on the nanometre scale (i.e. silica: IRMM-304, and gold: NIST RM 8011, 8012, and 8013), the ENM Guidance should mention here the repository of nanomaterials with a representative range of 25 different types of reference nanomaterials, which has just been launched by the JRC European Commission''s Joint Research Centre.
122	UK Government Chemist	3.2. Performance criteria for characterisation methods	Line 484 Suggest appending: 'The lack of suitable validated reference materials should not impede progress. In fact, where methods of analysis are still relatively weak, there has to be a greater reliance on standard materials. In the absence of certified reference materials, self-generated standards may be required. Steps should be taken to maximise the value of working standard materials, e.g. by sharing data on their performance between methods and laboratories.'
123	ECPA-European Crop Protection Association	3.2. Performance criteria for characterisation methods	See ECPA comments on section 3.
124	TNO	3.2. Performance criteria for characterisation methods	Comment to lines 469-470 and 473-474 '…the applied methods are fit for purpose and deliver reliable results'. It is suggested to include minimum criteria for analytical methods (e.g. >70% recovery of ENM in feed/food matrices, etc.).

125	Eurogroup for Animal Welfare / Animalfree Research	3.2. Performance criteria for characterisation methods	The caveat spelled out in LL. 467-468 confirms Eurogroup"s comments to Chapter 3.1.4.
126	BEUC - The European Consumers" Organisation	3.2. Performance criteria for characterisation methods	BEUC comments to lines 465 to 478: We suggest replacing the word “criteria” referred to methods’ performance with the word “characteristics” or “parameters”. In fact, while performance characteristic are functional quality that can be attributed to an analytical method (e.g. specificity, repeatability, reproducibility, recovery, etc…), performance criteria can be defined as requirements for a performance characteristic according to which it can be judged that the analytical method is fit for the purpose and generates reliable results. Moreover, in order to increase confidence that newly developed methods can deliver reliable results, it should be stressed that single-laboratory validation according to international recognised guidelines (e.g. IUPAC) is to be considered as a minimum requirement.
127	Health Canada	4. Exposure scenarios	Health Canada fully supports EFSA’s statement in lines 494-497, page 15, suggesting that “where it can be demonstrated that the ENM are solubilized in the food/feed matrix, or digested in gastrointestinal fluids, no specific testing for the nanoform is required, but there may be a need to assess the resulting substances”. We share the Scientific Committee’s opinion that in such a case, the likelihood of the ENM maintaining its nano-properties is low.
128	UK Government Chemist	4. Exposure scenarios	Line 509 Where not possible to identify an ENM in a food or feed matrix, it is logical to seek evidence relating to fate. This may be a distinct, and perhaps more conventional, measurement problem. The two boxes halfway down Figure 2 could therefore read: ‘Where possible, identification of material or its breakdown/transformation products in ...’. Moreover, ‘identification’ could be replaced by ‘quantitation’, if and when this is a realistic goal.
129	ECPA-European Crop Protection Association	4. Exposure scenarios	ECPA welcomes the approach followed by EFSA, by which the general risk assessment paradigm of hazard identification and hazard characterization followed by exposure assessment and risk characterization is applied for the evaluation of ENM in food and feed. In that sense, we welcome the cascade approach followed in figure 2 line 509. Particularly, we agree that, should “the ENM not be present in the food”, there is “no need to consider the nanoform” in the risk assessment as no exposure would occur. We understand that, once this has been determined, the substance at stake would follow “risk assessment of any non-nanoform fraction” and in casu the risk assessment designed for PPP.
130	BEUC - The European Consumers" Organisation	4. Exposure scenarios	BEUC comments: Consideration to the potential enhanced bioavailability derived from an increased dispersibility or solubility should be given when outlining oral exposure scenarios.

131	ECPA-European Crop Protection Association	5. Hazard identification and hazard characterisation	ECPA welcomes the risk assessment paradigm of hazard identification and hazard characterisation and the cascade approach developed in figure 2 509 and in section 5. Following this approach, an ENM "not present in food" would not require a risk assessment for the nanoform and would apply the risk assessment dedicated for the non-nanoform. However, the request for "genotoxicity studies, ADME and repeated-does 90-day oral toxicity study in rodents on the ENM" as defined in lines 571-576 independent of the amount of migration is contradiction with the risk assessment paradigm of hazard identification and characterisation. ECPA does not support this approach and believes that this is counter-productive. Instead, these additional studies should be undertaken once it is proved that the ENM is still "present in food" and therefore once an exposure to an ENM has been encountered. It has also to be mentioned that, for PPP products, a genotox battery and acute oral, dermal and inhalation toxicity studies are already mandatory with the formulated product to compare the toxicity between the pure active ingredient and the formulated product.
132	BASF SE	5. Hazard identification and hazard characterisation	BASF also appreciates very much the statement on page 17, lines 534 – 537 that unrealistic high dosing can lead to outcomes that may not be related to the inherent toxicity of the material but to the high amount of the material administered and that the choice of dose levels should therefore be carefully considered and a justification on the selected doses should be provided.
133	ELC - Federation of European Specialty Food Ingredients Industries	5. Hazard identification and hazard characterisation	"Assessment" – Part 5 "Hazard identification and hazard characterisation" Lines 572-576: ELC supports the CIAA views that from a risk assessment perspective, the statement that information on genotoxicity as well as ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration cannot be supported. The amount of migration contributes to a possible exposure. If migration is negligible, there is no exposure and thus no risk. In this case, we do not agree with the statement that a 90 day repeated dose study and ADME studies will be required. Moreover, this is not in line with figure 2 (line 267) where it is stated that "If evidence demonstrates no exposure there is no need for further testing". In addition, the demand for tests is also not in line with the existing regulation. If a substance has already been approved for use in food contact materials (e.g. by EC/2002/72) and has specific migration limits, we do not agree that ADME, repeated dose 90 day and in vitro genotoxicity studies are required.
134	University of Modena and ReggioEmilia	5. Hazard identification and hazard characterisation	5. Hazard identification and hazard characterisation 545 In order to assess the risk is mandatory to verify the ENMS biopersistence directly inside the animal tissues at the end of the tests. 5.4.1. Administration of ENM for ADME and toxicity studies 713 Bowel samples must be ananlyzed at the end of the chronic test in order to verify the ENMS biodistribution,exposure, persistence and cell entrapment. Protocols for the preparation of the samples can be found in A. Gatti., S. Montanari "Nanopathology: The health impact of nanoparticles" book, ed by PanStanford Publishing Pte.Ltd Singapore, ISBN -10981\-4241-00-8, 2008, 1-298.

135	Health Canada	5.1. General considerations	Health Canada supports the suggestion, in lines 538-539, page 17, that the effects of the increased bioavailability in terms of toxicity of food components, such as vitamins, through ENM used as a carrier system should be considered. We suggest also that the hazard from potentially reduced efficacy of food components carried by the ENM be considered. Further, the efficacy of nutrients supplied as nanocompounds should also be assessed. For instance, research is currently being conducted on developing nanocompounds containing minerals such as zinc and iron for fortification of foods.* The current Guidance describes a comprehensive set of tests to assess the toxicity of ENM but there is limited information on the characterization of the nutritional effectiveness of nutrients supplied in nanoform. Dose-response studies of depletion in animals measuring appropriate biomarkers of nutritional status may be appropriate for assessing efficacy of nanonutrient (s). Similar studies with the non-nanonutrient (s) could be conducted in parallel for comparison. We would note, however that improbable high dosing may not be the approach we would recommend as it may lead to an output that is not necessarily due to the toxicity of the ENM but rather to the body's mechanism for handling the presence excess of the ENM. The observed effect would therefore be irrelevant to the actual safety assessment of the ENM in question. Further, we would recommend that the terminology "choice of dose levels", line 356, be clarified to provide stronger guidance as studies conducted could use amount of test material administered that is inadequate to assess its safety. *Hilty FM, Arnold M, Hilbe M, Teleki A, Knijnenburg JT, Ehrensperger F, Hurrell RF, Pratsinis SE, Langhans W, Zimmermann MB. Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nat. Nanotechnol. 210 May;5(5):374-80. Epub 2010 Apr 25.
136	on behalf of the U.S. Government	5.1. General considerations	(p2 L56-59; p17 L523-524): The guidance states that appropriate in vitro and in vivo studies on the ENM should be undertaken to identify hazards and obtain dose-response data to characterize the hazards. Some test models and standard testing protocols used for non-nanoform substances may not necessarily be appropriate or optimal for the testing of ENMs, and efforts in the research community are currently underway to address these issues. We believe that additional guidance is needed to determine appropriate in vitro and in vivo studies and validation methods.
137	Nanotechnology Industries Association	5.1. General considerations	• lines 524-526: The NIA welcomes the statement that '[s]ome test models and standard testing protocols used for non-nanoform substances may not necessarily be appropriate or optimal for the testing of ENM, and ongoing efforts in the research community are currently addressing these issues. Therefore the recommendations for approaches to toxicity testing in this ENM Guidance will be updated as necessary in the light of future, emerging information,' and commends the ongoing work at the OECD WPMN Sponsorship programme for a regular update on the latest globally agreed results in nanomaterial measurement and safety testing. • lines 571-576: The NIA does not agree with the unconditional statement that, in the case of food contact material, '[...] information on genotoxicity, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration.' moreover, this statement represents a clear contradiction to the exposure scenario assessment outlined in Figure 2 (see '4. Exposure Scenarios').
138	UK Government Chemist	5.1. General considerations	Line 542 Suggest: '... free form in the food; and - particularly for trace elements - the chemical forms or species of a nutrient likely to be presented to sites of contact with the body by the carrier. For this, the ...'.

139	AESGP - Association of the European Self-Medication Industry	5.1. General considerations	Lines 534 – 537: AESGP supports the warning against studies based on tests performed with very high doses
140	AESGP - Association of the European Self-Medication Industry	5.1. General considerations	Lines 529 – 533: AESGP supports this statement concerning dosimetry and is of the view that mass is the correct metric
141	ECPA-European Crop Protection Association	5.1. General considerations	See ECPA comments on sections 5 and 5.2
142	TNO	5.1. General considerations	Comment to lines 529-533 It is noted that choices in dosing regime, e.g. related to surface area and/or number of particles, may significantly reduce possibilities for comparison (lines 556-564) of non-nano substances to ENM. As conversion of mass dose to other metrics (and vise versa) is likely to be batch specific, a generic conversion factor might not be feasible. Instead a conversion factor for each batch or the mass dose next to the other metric(s) should be specified in reports where the mass dose is not considered in the dosing regime.

143	Eurogroup for Animal Welfare / Animalfree Research	5.1. General considerations	Eurogroup for Animals acknowledges the prevailing uncertainties depicted in Ch. 5.1 regarding the hazard identification and characterisation of ENM in food and feed. We would like to emphasize the important role that appropriate dose metrics play in any reproducible, relevant and reliable hazard characterisation procedure for ENM. The respective prevailing uncertainties (LL. 529-531) seriously compromise the relevance of any hazard assessment and in consequence stand in the way of a risk assessment that reliably protects humans from unwanted effects by eating ENM that have been intentionally added to food. Furthermore, evidently, existing knowledge gaps regarding the appropriateness of test methods and testing protocols for hazard characterisation (LL. 524-525) further diminish the value of any hazard characterisation. It is not scientific state-of-the-art to implement testing strategies with unvalidated test methods, nor would such an attempt be able to ensure a high level of human health protection as called for by EFSA's mission. For reasons of human health protection, the conclusion to be drawn from the knowledge gaps depicted in Chapter 5.1 should not be to lay down guidance rules with the aim to update them on short notice (LL. 527-528). Instead, as already depicted in our comments to 3.1.4, the conclusion should be that it is currently premature to allow the marketing of ENM in food and feed and, therefore, to lay down a guidance document for a risk assessment of ENM in food and feed aimed at marketing such products. Additionally, considering the intrinsic scientific deficiencies of animal test methods as such, any efforts to develop appropriate test methods and testing protocols for ENM hazard identification should strive to develop non-animal test methods and testing strategies: The National Academy of Sciences (2007) points to the scientific deficiencies of animal test methods – already for the testing of conventional "bulk" chemicals: "Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects which provide little information about biological changes leading to such changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues." Accordingly, the US National Research Council has spelled out a paradigm change from in vivo to in vitro testing strategies as a vision for the 21st century (CTTAEA and NRC, 2007): "The committee envisions a new toxicity-testing system that evaluates biologically significant perturbations in key toxicity pathways by using new methods in computational biology and a comprehensive array of in vitro tests based on human biology." In consequence, as regards the safety testing of nanomaterials, where new test methods and testing strategies are required in the first place, instead of adapting scientifically flawed test methods to new application areas, scientific and political efforts should set out to develop and validate scientifically sound non-animal testing strategies making use of modern toxicological test methods and technologies from the beginning.
144	CIAA	5.1. General considerations	Lines 534 -537: We appreciate the statement that unrealistic high dosing can lead to outcomes that may not be related to the inherent toxicity of the material but to the high amount of the material administered and that the choice of dose levels should therefore be carefully considered and a justification on the selected doses should be provided.

145	Scientific Committee of the Belgian Food Safety Agency (FASFC)	5.1. General considerations	L534: It is useful to mention that at high concentrations, reduced toxicity can be observed due to the formation of agglomerates of ENM. This might complicate the interpretation of the results of toxicity studies.
146	Cefic	5.1. General considerations	On page 17 – lines 534-537 – Cefic appreciates the statement that unrealistic high dosing can lead to outcomes that may not be related to the inherent toxicity of the material but to the high amount of the material administered and that the choice of dose levels should therefore be carefully considered and a justification on the selected doses should be provided.
147	Henkel AG & Co KGaA	5.1. General considerations	Chapter 5.1, lines 534-537 Henkel appreciates the statement that unrealistic high dosing can lead to outcomes that may not be related to the inherent toxicity of the material but to the high amount of the material administered and that the choice of dose levels should therefore be carefully considered and a justification on the selected doses should be provided.
148	VCI (German Chemical Industry Association)	5.1. General considerations	Chapter 5.1, lines 534-537 VCI appreciates the statement that unrealistic high dosing can lead to outcomes that may not be related to the inherent toxicity of the material but to the high amount of the material administered and that the choice of dose levels should therefore be carefully considered and a justification on the selected doses should be provided.
149	Humane Society International	5.1. General considerations	523-528 The key term here is 'appropriate': studies that do account for the novel nature of the nanomaterials will not provide a clear picture of the particle's behaviour and/or toxicity. Reliance on such studies will not be of benefit to the hazard or risk assessment , . In circumstances where no difference has been identified between nanomaterials and conventional substances it is not appropriate to default to in vivo guideline tests when in vitro alternatives may exist or could be developed to ensure the data obtained is human relevant and does not rely on animal tests with inherent uncertainties. HSI would like to urge efforts to into creating a toxicity testing strategy akin to the National Research Council's Toxicity Testing in the 21st Century: A Vision and Strategy, relying on high-throughput, advanced techniques with direct human and environmental relevance. Additionally, in such conditions where "no difference" is suggested it is impossible to draw this conclusion when taking into consideration the lack of clarity on issues such as dosing and handling procedures for nanomaterials. Without such information toxicity tests will not produce reproducibly robust data. Sampling and handling as well as definitions and variability between nanomaterials are all major players in the performance of nanomaterials at the toxic level. It is therefore naive to assume standard toxicological tests for endpoints are suitable or are producing relevant results when nanomaterials are deemed to be the "same" as conventional substances. 534-537 HSI agrees that previous studies have used very high doses for hazard characterisation which have led to potential unrealistic outcomes; however we question the reliance on existing OECD test guidelines that prescribe testing to such doses.

150	BEUC - The European Consumers'' Organisation	5.1. General considerations	BEUC comments to lines 520 to 522: Particular attention should be given not to consider as soluble an ENM which in reality shows just an increased dispersibility compared to its bulk form. See comments on paragraph 3.1 on the difference between solubility and dispersibility and the risks of confusion if not ascertained by appropriate analytical methods. BEUC comments to lines 538 to 545: Attention should be paid also to carrier systems which are not ENM but are able to deliver the food ingredients or components in nanoform or at nanosize.
151	Food Safety Authority of Ireland	5.1. General considerations	I agree with the stipulation articulated in line 523 and subsequent lines, but I think such research work should be publicly funded so that the consumer and regulator alike can have full confidence in the data provided and thus preempt and label or accusation at a later stage that it was industry funded and therefore of dubious quality etc.
152	Health Canada	5.2. Testing outline	In regards to the toxicity testing strategy, there is agreement that the testing approach should have a comparison of information between the non-nanoform and nanoform. The in vitro genotoxicity, ADME and repeat 90-day studies are a good starting point. However, it is suggested to conduct in vitro studies such as the digestion, barrier integrity, permeability (blood brain barrier, placental barrier), or immunotoxicity studies up front and have these studies as a requirement. Results from the in vitro studies may trigger additional components to the repeated 90-day study, such as a neurotoxicity or immunotoxicity portion, or trigger additional toxicity studies. Furthermore, there is a need to develop new toxicity testing methods or adapt existing methods to nanoform materials that behave differently to their non-nanoform counterparts.
153	on behalf of the U.S. Government	5.2. Testing outline	(p17 L546): In section 5.2, under (1), reference is made to ENM transformation. In addition to timing and location, we believe it is important to note the issue of transformation itself and the need for well-designed studies to clearly determine if and how specific nanomaterials are transformed through the “shelf life” of the product. This would provide useful data and ensure that toxicity testing adequately reflects any potential transformation product(s).
154	UK Government Chemist	5.2. Testing outline	Line 564 Suggest appending: ‘In the event that these “first instance” tests indicate that the nanoform is significantly less hazardous than the non-nanoform, then any request to waive further toxicity testing must be scientifically justified.’
155	AESGP - Association of the European Self-Medication Industry	5.2. Testing outline	Lines 571 – 576: AESGP believes that the information mentioned in lines 574-576 is not necessary in case no migration of the engineered nanomaterial occurs. The statement that "information on genotoxicity, ADME and a repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration" can not be supported from a risk assessment perspective. The amount of migration contributes to possible exposure: if migration is negligible, there is no exposure and therefore no risk. In such cases, we believe that the information mentioned above should not be required. The request does also not seem to be in line with Figure 1 (Schematic outline for risk assessment of ENM) of the Draft Guidance, which indicates that "if evidence demonstrates no exposure there is no need for further testing". In addition, it appears to be not in line with the existing regulatory framework – if a substance has already been approved for use in food contact materials (e.g. pursuant to Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs) and has specific migration limits, AESGP does not believe that in vitro genotoxicity studies, ADME studies and a repeated-dose 90-day oral toxicity study should be required.

156	ECPA-European Crop Protection Association	5.2. Testing outline	On pages 17 and 18 lines 547 to 576 are described 3 general cases for toxicity testing strategy. Overall it is unclear and leads to many questions on what is understood by " the transformation of the ENM into a non-nanoform in the food and feed matrix or in the gastrointestinal fluids is judged to be complete" as mentioned in line 550-551. It raises particularly uncertainties on which bases and potential criteria on which the "completeness" of the process would be based upon. This should be clarified in the final guidance. In addition lines 556 raises also a need for clarification as to the identification of whether "some or all of ENM" persist. This should also be clarified in the final guidance. ECPA welcomes the risk assessment paradigm of hazard identification and hazard characterisation and the cascade approach developed in figure 2 and in section 5. Following this approach, an ENM "not present in food" would not require a risk assessment for the nanoform and would apply the risk assessment dedicated for the non-nanoform. However, the request for "genotoxicity studies, ADME and repeated-does 90-day oral toxicity study in rodents on the ENM" as defined in lines 571-576 independent of the amount of migration is in total contradiction with the risk assessment paradigm of hazard identification and characterisation. ECPA do not support this approach and believes that this is counter-productive. Instead, these additional studies should be undertaken once it is proved that the ENM is still "present in food" and therefore once an exposure to an ENM has been encountered. On top of this the demand for these tests is also not in line with the existing regulation. If a substance has already been approved for use in food contact materials (e.g. by EC/2002/72) and has specific migration limits, ADME, repeated dose 90 day and in vitro genotoxicity studies should not be required.
157	RIVM	5.2. Testing outline	P 17, lines 556 – 564: Please refer to this as 'sameness' in parallel to REACH.
158	TNO	5.2. Testing outline	Comment to lines 556-576 In case a non-nanoform application is approved with the same intended use as a new ENM, a reduced testing package with the ENM, covering in vitro genotoxicity tests, ADME and a repeated dose 90-day oral toxicity study in rodents is required. Depending on the outcome of these studies and on the comparison with the toxicity data of the non-nanoform, additional in vivo studies may be needed which are indicated in general in Table 2 with reference to specific sections. Considering lines 562-563, where it is stated that 'If there are differences, e.g. in distribution, or effects… then more toxicity tests will be required'. However, in case the test results of ENM indicate a lower toxicity in the 90-day study than the non-nano substance, the relevance of additional testing might be questionable. It would be welcomed to get some more guidance on how to interpret the comparison/differences between non-nano and ENM test results with respect to the need for additional testing.

159	Eurogroup for Animal Welfare / Animalfree Research	5.2. Testing outline	For ENM that persist in the food/feed matrix and in gastrointestinal fluids and information on the non-nanoform of the same substance is available, the testing outline foresees comparing information on ADME and toxicity of the non-nanoform with ADME, rodent repeated-dose 90-day oral toxicity and genotoxicity information of the ENM (as of L. 556). Due to the limited knowledge on the effects of ENM, a limited dataset is not considered appropriate at this point in time (LL. 573-574). Eurogroup for Animals has serious scientific and ethical concerns against this proposal – as well as against the other ENM testing scenarios depicted (as of LL. 556 and 565, respectively). Whereas we agree that the limited knowledge on the effects of ENM requires specific measures to be taken, we do not agree with the conclusion to persistently prescribe a full in vivo data set of ADME, 90 day and in vivo genotoxicity studies. Firstly, adequate testing protocols for the mentioned in vivo tests are still under investigation as regards their application for ENM (see comments to 5.4.3). The respective test methods are far from even being submitted to a validation process. It is not state-of-the-art to lay down a testing strategy making use of unvalidated test methods. Furthermore, at a time where international efforts are striving for a paradigm change from in vivo to in vitro toxicology, the current testing outline does not stand in line with EFSA's commitment to playing a proactive role in animal welfare. As already has been accomplished for the risk assessment procedure as such, a tiered scheme should be laid down for the hazard assessment of ENM in food/feed. Tier 1 of such a scheme should prescribe collecting all available data on the respective substance (in its nanoform and, if available, in its non-nanoform) and then performing simple in vitro test methods in the 2nd tier. The 3rd tier consists of specific in vitro test methods. After each tier, all information gathered so far is evaluated to determine if a scientifically sound safety assessment is already possible. For the assessment of repeat dose toxicity and toxicokinetics of non-nanoform substances, Grindon et al. (2008) and Combes et al. (2008) present concrete schemes on how such integrated decision-tree testing strategies should be performed. For ENM, the EU Commission's Joint Research Centre JRC, together with the Netherlands Environmental Assessment Agency RIVM and BASF SE have put forward a tiered testing strategy that meets the above-mentioned requests (Sauer, 2010; see also comments to 5.3). Such a tiered testing strategy should be accepted and thus become applicable for regulatory purposes on a step-by-step basis over the course of time whilst the respective in vitro test methods become validated and accepted. As regards ENM in food and feed, an application mainly driven by economic and life-style and not medical motivations, we can see no justification whatsoever to call for the routine performance of animal tests, tests that cause sentient beings harm, suffering and distress (see comments to 5.4), let alone the routine performance of unvalidated test methods whose manifold scientific deficiencies are well known. Both for reasons of human health protection and animal welfare, the marketing of ENM in food and feed should only be permissible if their safety can be determined in scientifically validated non-animal testing strategies. If the safety assessment of ENM in food/feed is not possible in vitro - also taking into account the abundance of other uncertainties in regard to the risk assessment of ENM, the knowledge gaps regarding characterisation methods, dose metrics exposure assessment, the respective substance should be considered not ready for use.

160	CIAA	5.2. Testing outline	Lines 554-555: The point/s at which a loss of nano-properties needs to be determined should be explained in addition to the fact that no risk assessment is required if an ENM is dissolved to a non-nanoform. Line 564: This should read "more toxicity testing may be required" - the nanoform may increase bioavailability leading to a lower NOAEL which should be revealed by the ADME work rather than leading to further testing. Lines 572-576: From a risk assessment perspective, the statement that information on genotoxicity as well as ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration cannot be supported. The amount of migration contributes to a possible exposure. If migration is negligible, there is no exposure and thus no risk. In this case, we do not agree with the statement that a 90 day repeated dose study and ADME studies will be required. Moreover, this is not in line with figure 2 (line 267) where it is stated that "If evidence demonstrates no exposure there is no need for further testing". In addition, the demand for tests is also not in line with the existing regulation. If a substance has already been approved for use in food contact materials (e.g. by EC/2002/72) and has specific migration limits, we do not agree that ADME, repeated dose 90 day and in vitro genotoxicity studies are required.
161	Scientific Committee of the Belgian Food Safety Agency (FASFC)	5.2. Testing outline	L571 : “independent of the amount of migration” appears to be very restrictive; it is suggested that when it can be demonstrated that there is no migration, the amount of required tests can be reduced.
162	BASF SE	5.2. Testing outline	As BASF we also strongly support EFSA’s concluding statement, that the general risk assessment paradigm (hazard identification and hazard characterisation followed by exposure assessment and risk characterisation) is applicable for the evaluation of ENM in food and feed and consequently appropriate data and information for the various steps should be made available to the risk assessor to carry out a risk assessment. This conclusion however is not consistently followed in the Guidance. In figure 2, starting from line 509, EFSA proposes that if the nanomaterial cannot penetrate into the food – that means no relevant exposure occurs – there is no specific need to consider the nanoform in the risk assessment. It is our opinion that this would also exclude the further testing of the nanoform. At a later stage however (line 571-576) EFSA requires genotoxicity studies, ADME and a subchronic oral study in rodents for ENM even in the case of non-exposure. This is a fundamental contradiction to the risk assessment paradigm with severe financial as well as animal welfare consequences. We firmly believe that the risk assessment paradigm is applicable to ENM and should be consistently followed in the Guidance. We therefore do not support the assertion, that studies are required in any case even if there is no exposure to the specific nanomaterial.
163	Cefic	5.2. Testing outline	• On page 18 – lines 572-576 - The statement that information on genotoxicity, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration cannot be supported from a risk assessment perspective. The amount of migration contributes to a possible exposure. If migration is negligible, there is no exposure and thus no risk. In this case, we do not agree with the statement that a 90 day repeated dose study and ADME studies will be required. Moreover, this is not in line with figure 2 (line 267) where it is stated that "If evidence demonstrates no exposure there is no need for further testing". On top of this the demand for tests is also not in line with the existing regulation. If a substance has already been approved for use in food contact materials (e.g. by EC/2002/72) and has specific migration limits, we do not agree that ADME, repeated dose 90 day and in vitro genotoxicity studies are required.

164	MRC Human Nutrition Research	5.2. Testing outline	3) We support the requirement for more in-depth testing of persistent ENM of high reactivity or 'mobility'. 4) We approve the move to conventional risk assessment if there is a complete loss of the nano-specific properties in the food matrix or during digestion of the ENM such that the gut mucosa is not exposed to the ENM per se.
165	Henkel AG & Co KGaA	5.2. Testing outline	Chapter 5.2, lines 572-576 The statement that information on genotoxicity, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration cannot be supported from a risk assessment perspective. The amount of migration contributes to a possible exposure. If migration is negligible, there is no exposure and thus no risk. In this case, we do not agree with the statement that a 90 day repeated dose study and ADME studies will be required. Moreover, this is not in line with figure 2 (line 267) where it is stated that "If evidence demonstrates no exposure there is no need for further testing".
166	VCI (German Chemical Industry Association)	5.2. Testing outline	Chapter 5.2, lines 572-576 The statement that information on genotoxicity, ADME and repeated-dose 90-day oral toxicity study in rodents on the ENM is required independent of the amount of migration cannot be supported from a risk assessment perspective. The amount of migration contributes to a possible exposure. If migration is negligible, there is no exposure and thus no risk. In this case, we do not agree with the statement that a 90 day repeated dose study and ADME studies will be required. Moreover, this is not in line with figure 2 (line 267) where it is stated that "If evidence demonstrates no exposure there is no need for further testing".
167	Humane Society International	5.2. Testing outline	Researchers should ensure they have exhausted all possibilities using in vitro genotoxicity testing before in vivo testing is pursued. Where mature guidance is not available, an approach representing the state-of-the-art should exist. However, this should only be recommended if demonstrated to be sufficiently specific and robust for nanomaterials hazard and risk assessment. Guidance should not rely on existing methodologies simply because they are available.

168	Humane Society International	5.2. Testing outline	570 The ENM toxicity testing strategy should be based on the most nano-specific methodologies and ensure that the results obtained are representative of the nanoparticles in question. A toxicity strategy should also assume that appropriate and adequate protocols for sample preparation and characterisation are also in place. Where tests are labelled as “might be necessary”, strict guidelines need to be in place to ensure tests are not carried out unnecessarily. The base set of toxicological endpoints is a comprehensive and important suggestion; however, given the lack of certainty regarding the nano-specificity of these tests for nanoparticles, it is inappropriate to use them at this stage. Nano-specific methods focusing on advanced in vitro alternatives should be used where possible and developed where further work is required. 571-576 The requirement for an extended dataset is due to the uncertainties surrounding nanomaterials but with further research and reliance on more no-specific methods the answers to these questions can be approached without the requirement for extensive inappropriate test methods. 676 Before any in vivo studies are carried out researchers must have exhausted the in vitro approaches to determine endpoints. In a recent publication by the Royal Commission on Environmental Pollution it was acknowledged that "...the scientific basis to fully understand all properties and risks of nanomaterials is not sufficiently available at this point in time” . In accordance with this, HSI further believes that animal testing of nanomaterials is scientifically highly questionable. We would prefer to see an acknowledgement that, in concordance with the mention that some specified in vitro methods are not yet validated, existing animal tests are also not validated for this application (indeed, in some cases, existing animal tests have not been formally validated to modern standards for any application), and greater emphasis to be placed on the development, validation and use of non-animal test methods. All test methods come with uncertainties, and in the case of in vivo models these include the difficulties of extrapolating test data between species, genders and breeds of animals including humans (due to anatomical, physiological, biochemical, metabolic and pharmacological differences). There are major uncertainties in interpreting information from high-dose animal tests with single chemicals in ways that are relevant to low-dose human exposures to chemical cocktails. There are also problems with mimicking human routes of exposure in animal tests, and with scaling up from small animals with a short lifespan to larger humans who may be exposed to chemicals over decades. Even for data-rich chemicals, these uncertainties often delay rather than facilitate regulatory decision-making, prolonging risks of damage to human health and the environment. With a new field such as nanomaterials, the full range of potential toxicities is not known. Using standard animal toxicity tests, which are little more than ‘black box’ methods, would risk overlooking novel unwanted effects. Human cell-based assays, in contrast, would allow the study and elucidation of a range of molecular and cellular mechanisms of toxicity. For example, human cell culture assays can be used to monitor the oxidative stress responses of cells exposed to nanoparticles. 678 Human-specific alternatives should be sought for ADME and toxicity studies, and efforts fostered into validating in silico and in vitro test methodologies . We wholly agree with the recommendations into furthering the currently limited knowledge and understanding of ENM behaviour and toxicokinetics through in silico and in vitro methods.

169	BEUC - The European Consumers'' Organisation	5.2. Testing outline	BEUC comments to line 554: '…or of the delivered food ingredient' should be added after 'the nanoform nature of the ENM' for the reasons explained in the previous comment.
170	Health Canada	5.3. In vitro studies	Health Canada recognizes that this section provides guidance on in vitro testing to determine, for example, potential effects on the immune cells. In particular, this section of the Guidance could benefit from additional information pertaining to another potential trigger for in vitro test for effects on immune cells which could come from the repeated dose 90-day oral toxicity study in rodents. The triggers would include changes to leucocyte count, alteration of thymus or spleen weight, or histopatholigical changes in the bone marrow, thymus, spleen, intestine, or mesenteric lymph node. In the case where those parameters are modified in the 90-day rodent study, the in vitro whole blood assay in section 5.3.3.2 of this guidance will be a useful screen for changes to some of the pathways of immunostimulation or immunosuppression.
171	on behalf of the U.S. Government	5.3. In vitro studies	(p18 L588-589): The guidance states that there may be a need to consider whether impurities may be present in the ENM that are known to be toxic. Additional information and reference material on the potential impact upon impurities and by-products as a source of toxicity is recommended.
172	ECPA-European Crop Protection Association	5.3. In vitro studies	See ECPA comments on sections 5 and 5.2
173	RIVM	5.3. In vitro studies	P 19, section 5.3.1. Solubility/digestibility is now positioned as a critical factor that determines further data requirements. This requires a good definition of solubility: it would also be useful to have a recommendation of methods to test this and a description of results that would justify the conclusion that a NM is soluble. What time span should be considered? Page 18, section 5.3, line 587: please also refer to the paper published by Park et al. (Park MV ; Lankveld DP ; Loveren H van ; De Jong WH. The status of in vitro toxicity studies in the risk assessment of nanomaterials. Nanomedicine 2009; 4(6):669-85). This paper clearly indicates the (im)possibilities of in vitro assays in relation to risk assessment. P 20, section 5.3.3.2, line 652: The whole blood cytokine release model is a useful test for immune reactivity but it is not generally accepted as the test for immunotoxicity. It may not even be suitable for NMs.
174	TNO	5.3. In vitro studies	Comment to lines 583-589 The suitability of the test system is amongst others highly depending on adequate cellular uptake of ENM. As no definition of 'adequate' [line 586] is provided, this may raise discussions on the suitability of the tests performed. Is it possible to further define what is considered as 'adequate'?. Some in vitro studies are specifically designed for screening purposes. As in vivo information on ADME, repeated dose and genotoxicity is required in all cases, it can be debated whether in vitro screening data (other than that derived from in vitro genotoxicity tests or to provide information on the mechanism of action) has substantial added value.

175	Eurogroup for Animal Welfare / Animalfree Research	5.3. In vitro studies	Instead of presenting in vitro test methods as an option to be taken on a voluntary basis (LL. 578-582, e.g. “may provide information”, “may be helpful”), Eurogroup for Animals would like to see in vitro test methods firmly incorporated into a tiered testing strategy, such as the one put forward by JRC/RIVM/BASF (see comments to 5.2). In the 1st tier of the JRC/RIVM/BASF testing strategy, in vitro local effects and primary biological effects on the one hand and the kinetic translocation of ENM on the other hand are tested separately. Due to this splitting, in vivo testing can be avoided in the initial stage of the testing strategy. If ENM do not show any biological effects in vitro, they are unlikely to have effects in vivo; and further toxicity testing is considered unnecessary. Likewise, if tier 1 kinetic evaluations do not reveal ENM translocation, they are assumed to be unlikely to have systemic effects, and again further studies for systemic effects are not necessary (Sauer, 2010). The testing components of non-animal test batteries can be combined to fulfil all steps of the risk assessment process — hazard identification, dose-response assessment, exposure assessment, and the integrated process of risk characterization (Krewski et al., 2011). Panels of in vitro assays for the respective specific toxicity pathways provide a point of departure for the risk assessment. Computational systems biology modelling of pathway circuitry and dynamics indicates the shape of the dose response at lower doses, leading to an acceptable concentration proposed for a human population. The acceptable concentration is then converted to an exposure level through techniques of reverse dosimetry implemented by pharmacokinetic modelling (http://alttox.org/ttrc/overarching-challenges/way-forward/; adapted from: Boekelheide & Andersen, 2010). As regards ENM in food and feed, evaluation of the information gathered should always make use of the precautionary principle. As soon as in vitro test methods indicate that the ENM might have adverse effects, for the protection of the humans and the animals that might be exposed to ENM in food and feed, i.e. by eating them, the use of such ENM should not be considered permissible.
176	CIAA	5.3. In vitro studies	Line 577 5.3. In vitro studies For the in vitro studies it is essential to be clear on the form of the ENM in the studies – e.g. free particles (unlikely), agglomerates, aggregates etc. In addition, it is important to be clear that these studies are only relevant for pristine ENM so do not take into account any secondary effects of food matrix.
177	Eurogroup for Animal Welfare / Animalfree Research	5.3.1. In vitro digestion studies	In vitro digestion studies (L. 591) should form part of a first tier of a non-animal integrated testing strategy.
178	BEUC - The European Consumers'' Organisation	5.3.1. In vitro digestion studies	BEUC comments to lines 591 to 598: characteristics which might determine an increased biovailability of the substance in nanoform compared to the non nanoform should be appropriately considered and their potential consequences should be appropriately addressed.

179	on behalf of the U.S. Government	5.3.2. In vitro genotoxicity testing	(p19 L608-610): Information on the mode of action of the ENM is recommended, e.g. if reactive oxygen species are generated then genotoxic effects can be anticipated, which can be detected in the comet assay (Karlsson, 2010). Identifying the mode of action, if determinable, is important. This information may guide toxicity studies, as appropriate. More broadly, the guidance implies a "one-size-fits-all" requirement for in vitro genotoxicity testing, a 90 day oral toxicity study, and ADME information. It is unclear why genotoxicity testing and 90 day oral toxicity studies are necessary, particularly as it is not clear that current test methods work for nanoparticles.
180	TNO	5.3.2. In vitro genotoxicity testing	Comment to lines 600-601 For genotoxicity the endpoints gene mutations, structural aberrations and numerical aberrations should be considered. The required tests specified under 2 are 'An in vitro chromosomal aberration test (OECD 437) or in vitro micronucleus assay …'. It is noted that the in vitro chromosome aberration test is not designed to cover aneugenicity. Therefore numerical aberrations are not covered adequately when performing this test. Furthermore, in the micronucleus test only in case centromere/kinetocore staining is performed upon a positive response, aneugenicity can be covered. Within the context of the current OECD guidelines, discrimination between aneugenicity and clastogenicity cannot be guaranteed within the proposed test set-up. Detection of numerical aberrations is not specifically performed in the current testing strategy.
181	Eurogroup for Animal Welfare / Animalfree Research	5.3.2. In vitro genotoxicity testing	Eurogroup for Animals disagrees with the testing strategy outlined in 5.3.2. Information on the mode of action of ENM (L. 608) should always be gathered in vitro in a first tier of the non-animal testing strategy (after collection of all available information). If generation of reactive oxygen species is detected in mode of action evaluations, the respective ENM should be considered genotoxic, which should lead to its rejection for application in the area food and feed. If no such unwanted effects are observed in mode of action evaluations, the ENM should proceed to the second tier, in which in vitro genotoxicity tests are performed. These in vitro genotoxicity tests should be supplemented with in vitro genotoxicity tests making use of complex metabolic activation systems as such methods become available for regulatory purposes. For the safety of humans and for the prevention of animal testing, ENM that reveal genotoxic effects in vitro or whose safety cannot be established in vitro should not be considered adequate for application in food or feed.
182	CIAA	5.3.2. In vitro genotoxicity testing	Lines 599-619: It should be kept in mind that for in vitro genotoxicity testing the choice of suitable test doses is very important and might be challenging. We suggest adding one sentence to acknowledge this fact in the Guidance Document.
183	Scientific Committee of the Belgian Food Safety Agency (FASFC)	5.3.2. In vitro genotoxicity testing	L603: nanoparticles as 1 word L608-610: This paragraph might fit better after the required in vitro tests for ENM (after L615) instead of before them. Suggestion: 'In addition to these tests, the comet assay might be used. In this regard, information on the mode of action of the ENM may be helpful, e.g. if reactive oxygen species are generated then genotoxic effects can be anticipated, which can be detected in the comet assay (Karlsson, 2010).
184	University of Porto	5.3.2. In vitro genotoxicity testing	line 610: Because reference nanomaterials are not available to use as a positive control, a recognized genotoxic compound will be selected with basis on the mechanism of cytotoxicity evidenced by the nanomaterial under study.
185	Cefic	5.3.2. In vitro genotoxicity testing	• On page 19 – lines 599-619 - It should be kept in mind that for in vitro genotoxicity testing the choice of suitable test doses is very important and might be challenging. We suggest adding one sentence to this fact in the Guidance Document.

186	Henkel AG & Co KGaA	5.3.2. In vitro genotoxicity testing	Chapter 5.3.2, lines 599-619 It should be kept in mind that for in vitro genotoxicity testing the choice of suitable test doses is very important and might be challenging. We suggest adding one sentence to this fact in the Guidance Document.
187	VCI (German Chemical Industry Association)	5.3.2. In vitro genotoxicity testing	Chapter 5.3.2, lines 599-619 It should be kept in mind that for in vitro genotoxicity testing the choice of suitable test doses is very important and might be challenging. We suggest adding one sentence to this fact in the Guidance Document.
188	TNO	5.3.3. Other in vitro studies	Within the hazard identification/characterization: the possible load of macrophages as a result of removing ENM from the blood/tissues, in relation to the influence on the normal functioning of the macrophages (eg. removal of bacteria, bacterial infections): is this sufficiently covered in the testing strategy?
189	Eurogroup for Animal Welfare / Animalfree Research	5.3.3. Other in vitro studies	Eurogroup for Animals disagrees with the notion that "other" in vitro studies may be performed in order to provide additional information (L. 621). Instead, adequate in vitro studies should form the first and second steps in a tiered-testing strategy. Likewise, their performance should not be laid down on a voluntary basis (L. 623 "may be applied"), but the performance of such tests should become mandatory. After each step of the tiered testing strategy, the information gathered should be evaluated based upon weight-of-evidence and making use of the precautionary principle. Making use of non-animal test methods, such a tiered testing strategy should be accepted and thus become applicable for regulatory purposes on a step-by-step basis over the course of time whilst the respective in vitro test methods become validated and accepted. For the safety of humans and for the prevention of animal testing, ENM that reveal hazardous effects in vitro or whose safety cannot be established in vitro should not be considered adequate for application in food or feed. No animal testing should be considered ethically acceptable in the case of ENM in food and feed (see comments to 5.3).
190	CIAA	5.3.3. Other in vitro studies	Lines 620-672: We would like to draw attention to an additional in vitro test - the uptake and persistence of particles in macrophages. This investigation would demonstrate how macrophages deal with nanoparticles. This would contribute to a better understanding of internal exposure.
191	Cefic	5.3.3. Other in vitro studies	On page 19 – lines 620-672 - We would like to draw attention to an additional in vitro test which is the uptake and persistence of particles in macrophages. This investigation would show how macrophages deal with nanoparticles. This would contribute to a better understanding of internal exposure.
192	Health Canada	5.3.3.1. Gastrointestinal barrier integrity and inflammatory response	In line 640, page 20, we suggest revising to "Barrier integrity and permeability, as assessed by the….marker phenol red" to add clarity. In lines 640-642, page 20, we recommend providing further details on the positive control and including a literature reference to support the selection of the positive control to give it more comprehensiveness. For example, the last sentence of this bullet (lines 641-642) could be revised to: "Twenty percent of ethanol was reported to increase permeability (Catalioto et al., 2009)**, and therefore can be used as a positive control." ** Catalioto R.M., Festa C., Triolo A., Altamura M., Maggi C.A. & Guiliani S. (2009) Differential effect of ethanol and hydrogen peroxide on barrier function and prostaglandin E2 release in differentiated Caco-2 cells: selective prevention by growth factors. Journal of Pharmaceutical Sciences, 98 (2), 713-727.

193	MRC Human Nutrition Research	5.3.3.2. Effect on immune cells	7) When addressing potential effects of ENM on immune cells the following should be observed and /or considered: (i) More than one cytotoxicity assay should be employed, and the possible adsorption of the molecules used in the assay to the ENM should be investigated (e.g. when LDH is assessed from supernatants, it would advisable to carry out controls where ENM have been incubated with LDH to check for adsorption). This should equally be applied when measuring the release of inflammatory mediators by ELISA. (ii) Different incubation time points should be investigated: importantly prolonged in vitro cellular exposure to nanoparticles might not be physiological hence observed cytotoxic effects might be a result of particle gorging rather than inherent to the ENM. Investigators should also consider pulse and chase experiments to find out how cells behave beyond continuous exposure to ENM. (iii) Primary investigations using cells derived for the periphery may be informative but, for the gut, may be misleading. In instances where ENM are likely to be translocated across the GI tract, ENM exposure to gut mucosal immune cells should also be considered (especially when considering the differences in phenotype and response properties of these cells when compared to their peripheral counterparts).
194	Health Canada	5.4. In vivo studies	The Scientific Committee suggests that a modified 90-day oral toxicity study is necessary to assess an ENM in a thorough manner. Health Canada supports the Committee's statement unless it can be demonstrated scientifically that the ADME results show that the ENM is not absorbed, in which case the 90-day study would not be considered essential.
195	ECPA-European Crop Protection Association	5.4. In vivo studies	Overall, further in vivo studies should only be undertaken if the concern has not yet been addressed by a previous study. Particularly, the benefit of additional vertebrate studies should be balanced against animal welfare consideration. See ECPA comments on sections 5 and 5.2
196	RIVM	5.4. In vivo studies	P21, section 5.4.2, line 714: The importance of tissue kinetics, accumulation/persistency and elimination from tissues should be stressed as this is considered to be more relevant than plasma levels. Special attention should be paid to the typical target organs: liver, spleen, kidney, and lungs, i.e. organs with increased capacity for uptake of particles. P22, section 5.4.2, line 746: persistence should be considered in ADME studies, to correlate accumulation to long term toxicity if possible. If it can be demonstrated that the NM does not accumulate, no further long term testing should be required. P 23, section 5.4.4. lines 779-798: • The addition of tests for cardiovascular and inflammatory parameters may be considered. • Due to the large surface area of nanomaterials resulting in reactive molecules on the surface, such materials may have antibacterial properties. Antibacterial effects on the gut microbiota may be considered as well as effects on environmental bacteria as the NM present in food may be excreted and enter the waste sewage system.
197	Proefdiervrij (ds RAT)	5.4. In vivo studies	678-830 In vivo methods for hazard identification are precribed, not taking into account that in the field of toxicology a lot of work is going on to replace animal testing and to achieve a paradigm shift to non-animal testing. No data sharing is prescribed in case any animal testing needs to be done.

198	Eurogroup for Animal Welfare / Animalfree Research	5.4. In vivo studies	In acknowledgement of the scientific deficiencies of in vivo toxicity test methods depicted in our General Comments and of the fact that appropriate testing protocols for the testing of ENM are still under investigation (see comments to Ch. 5.1), Eurogroup for Animals can recognize no justification for suggesting that ENM tested on a fixed set of in vivo test methods could be considered safe for consumption by humans – or safer than if tested making use of a non-animal testing strategy. – Such an attempt would fail to address the fundamental scientific limitations of animal tests depicted above. Furthermore in the case of animal testing for risk assessment of ENM in food and feed, the harm-benefit analysis prescribed in Article 38(2)(d) of Directive 2010/63 leads to the conclusion that these tests should not be considered ethically acceptable. A comprehensive evaluation of the "benefit" of a test should not only take into account the appropriateness of the test method to achieve the goal of the test and its reliability and relevance, but also, in the case of regulatory testing, the necessity for the product or substance in question. In the case of ENM in food and feed, an area of application driven by economic and life-style motivations and not medical demands, these provisions lead to the conclusion that the benefit of such studies would be low. The harm inflicted upon research animals due to in vivo ENM safety testing, however, is to be considered at least moderate, if not high. In accordance with Annex VIII of Directive 2010/63/EC on the severity classification of animal experiments, procedures are classified as moderately severe, if "animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress as well as if they are likely to cause moderate impairment of the well-being or general condition of the animals". Procedures are classified as severe, if the animals are likely to experience "severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress as well as if they are likely to cause severe impairment of the well-being or general condition" (Section 1 of Annex VIII of Directive 2010/63). One example for a moderately severe procedure (as provided for in Section 2 of Annex VIII) is "acute dose-range finding studies, chronic toxicity/carcinogenicity tests, with non-lethal end-points". One example for a severe procedure is "toxicity testing where death is the end-point, or fatalities are to be expected and severe patho-physiological states are induced". It cannot be disputed that in vivo toxicity tests for ENM would fall under one of these two categories. Thus, in balancing a "low benefit" against a "moderate to high harm", in the case of ENM in food and feed, the harm-benefit-analysis leads to the conclusion that such animal testing should not be considered ethically acceptable.
199	Federal Institute for Risk Assessment	5.4.1. Administration of ENM for ADME and toxicity studies	Lines 698-701 It is not agreed that absorption of ENM from the GI tract is "likely" higher following gavage than after administration with food or drinking water. If you have supporting evidence, please include the appropriate reference. Otherwise, EFSA may wish to change the text from likely higher to "may be different".
200	UK Government Chemist	5.4.1. Administration of ENM for ADME and toxicity studies	Line 713 It might be reiterated that as much relevant information as possible on the parameters discussed in this paragraph should be obtained by in vitro digestion studies.

201	Eurogroup for Animal Welfare / Animalfree Research	5.4.1. Administration of ENM for ADME and toxicity studies	The issues depicted in Ch. 5.4.1 further underline that it is currently premature to lay down testing strategies for the risk assessment of ENM in food and feed and hence to permit the marketing of such products: LL. 683-684 describe the limitations regarding the amounts of ENM that can be administered and in administering the ENM as such. LL. 697 – 701 question the relevance of forms of application. In consequence, we do not agree with the conclusion formulated in L. 707 that the limitations of the bolus administration may be accepted when performing in vivo toxicity tests. Instead, it should be acknowledged that the scientific basis for determining possible risks of ENM is currently insufficient for ensuring a responsible protection of humans from unwanted effects by eating ENM that were intentionally added to their food.
202	UK Government Chemist	5.4.2. ADME studies	Line 727 Suggest: '... be detected), but online coupling with separation techniques such as FFF looks set to overcome this problem. Radioactive ...'.
203	Eurogroup for Animal Welfare / Animalfree Research	5.4.2. ADME studies	The issues depicted in Ch. 5.4.2 further underline that it is currently premature to lay down testing strategies for the risk assessment of ENM in food and feed and hence to permit the marketing of such products: LL. 717-718 note that the difficulties of undertaking ADME studies on ENM should not be underestimated (!). In addition, particular difficulties in measuring the amounts of ENM in blood, tissues and excreta and in establishing the form in which they are present in the body are acknowledged (LL. 719-721). These difficulties and unresolved scientific problems significantly reduce the meaningfulness of the data obtained from such studies. Therefore it should be acknowledged that the scientific basis for determining possible risks of ENM is currently insufficient for ensuring a responsible protection of humans from unwanted effects by eating ENM that were intentionally added to their food.
204	CIAA	5.4.2. ADME studies	714 5.4.2. ADME studies: Although entitled ADME, this section really concerns 'Absorption' and 'Distribution'. Little consideration of metabolism or excretion is given. There is little guidance on appropriate techniques to use. A link to the need for appropriate projects at DG Research would be helpful. Furthermore, it would be useful to have clear links to EMEA on metabolism.
205	BEUC - The European Consumers'' Organisation	5.4.2. ADME studies	BEUC comments to lines 715 to 722: Bioavailability is an important factor to be considered when evaluating ADME. Bioavailability studies should always be required, including when the ENM studied exists also in the corresponding non nanoform in order to detect any increase of bioavailability determined by the nano structure. Bioavailability versus absorption is a pivotal element of biokinetics, toxicity testing, and risk assessment.
206	Eurogroup for Animals / Animalfree Research	5.4.2.1. ADME pilot study	The elicitation of harmful effects during in vivo toxicity studies, e.g. by administration of highly toxic doses (L. 753), but also by administration of moderately toxic doses over a longer period of time, must always be expected. Hence, the harm-benefit analysis of such in vivo testing leads to the conclusion that the harm expected to be inflicted upon the animals outweighs the benefit from the respective studies (see comments to Ch. 5.4).
207	Health Canada	5.4.3. In vivo repeated dose 90-day oral toxicity study	In lines 772-774, we suggest revising the last sentence of the first paragraph to the following: "The results from the repeated-dose 90-day oral toxicity can be used to identify a Benchmark Dose Lower Confidence Limit (BMDL) or a No-Observed-Adverse-Effect-Level (NOAEL)."

208	Eurogroup for Animals / Animalfree Research	5.4.3. In vivo repeated dose 90-day oral toxicity study	Eurogroup for Animals can find no scientific evidence to support the request to always perform a repeated-dose 90-day oral toxicity study in rodents, let alone making use of a modification that appears to have been designed by means of an armchair decision. The fundamental scientific limitations of in vivo studies have been discussed above (see comments to 5.1). However, also the scientific relevance of a 90-day duration for the general toxicological study has never been established: As regards the inhalational toxicity of ENM, the scientific relevance of choosing a 90-day duration for the main toxicity test is being questioned. Evidence suggests that short-term 5-day inhalation studies may provide comparable prediction of respiratory tract toxicity to 90-day studies, presenting the opportunity to save time and resources in screening inhalation toxicity of test substances (Ma-Hock et al., 2009). These ongoing scientific discussions further reveal that it is currently premature to lay down testing strategies for the risk assessment of ENM in food and feed and hence to permit the marketing of such products. Furthermore, while we agree that toxicological data derived from laboratory species may not be directly applicable for ENM foreseen to be administered in feed (LL. 775-776) – nor, in fact, for ENM foreseen to be administered in food(!), these problems in performing meaningful extrapolations should not lead to the conclusion to request further in vivo testing with further animal species, but that the respective in vivo testing strategies are not adequate in ensuring a sound human health protection. At best they can ensure that a company meets its warranty deeds, which however should not be the driving force for EFSA to compile a risk assessment Guidance Document. Finally, again, as regards the safety testing of ENM in food and feed, an application area without medical motivations, we can see no justification whatsoever to request the performance of in vivo test methods that cause sentient animals pain, suffering, distress or lasting harm.
209	TNO	5.4.4. Other in vivo toxicity tests	Comment to lines 783-785 Further testing for chronic toxicity and carcinogenicity is required only related to accumulation. Although accumulation is an important observation to consider further testing, other effects might also be of relevance, e.g. observation of morphological changes as critical endpoint in the 90 day study, etc.
210	Eurogroup for Animals / Animalfree Research	5.4.4. Other in vivo toxicity tests	Eurogroup disagrees with the request to consider in vivo chronic toxicity testing in case of evidence of accumulation in organs and tissues (L. 783-784). Instead, such evidence should lead to the conclusion that the respective ENM should not be permissible in food and feed. Likewise if available toxicity data lead to the assumption that developmental toxicity cannot be excluded (LL. 791-792), for the protection of humans, the ENM should not be permissible in food and feed without further – scientifically questionable – testing.
211	Federal Institute for Risk Assessment	5.4.5. In vivo genotoxicity testing	Lines 805-820 Please clarify which tissues should be evaluated for in vivo genotoxicity, except where this is obviously the bone marrow (line 809-810). Does EFSA recommend to include the site of contact, the liver, … ?
212	UK Food Standards Agency	5.4.5. In vivo genotoxicity testing	1.Line 803-804: EFSA advises: In vivo genotoxicity testing may also be considered where there is evidence for a prolonged inflammatory response from in vivo studies. This needs care, since it is not a general genotoxicity testing trigger. 2. Line 814-815: Also, conducting a liver unscheduled DNA synthesis test may not be too relevant unless there is liver inflammation. More clarification of the circumstances triggering this type of testing would be helpful, and also which assay and which organ would be studied.

213	TNO	5.4.5. In vivo genotoxicity testing	Comment to lines 803-804 'In vivo genotoxicity testing may also be considered where there is evidence for a prolonged inflammatory response from in vivo studies'. The ratio of performing genotoxicity testing in case of an inflammatory response in vivo is not understood. Furthermore it is unclear which in vivo tests (and for which endpoint) the additional information should be considered? It is noted that non-genotoxic mechanisms for carcinogenicity are not covered by genotoxicity tests. Furthermore, already a core set of 2 genotoxicity tests are required. Comment to line 811 The comet assay, performed upon a positive in vitro test, should be performed in vivo and using relevant organs of choice (e.g. target organs based on other in vivo data, first contact sites, etc.).
214	Eurogroup for Animals / Animalfree Research	5.4.5. In vivo genotoxicity testing	Please refer to our comments to 5.3.2 regarding a genotoxicity testing strategy.
215	University of Porto	5.4.5. In vivo genotoxicity testing	line 820: Even when data obtained in the in vitro genotoxicity tests are negative, in vivo necessary assays, as ADME, can be used, without additional animals, to search for micronucleus or other endpoints in the peripheral blood, giving additional important information.
216	Federal Institute for Risk Assessment	5.5. Uncertainties in toxicity testing of ENM	LInes 821-830 Please also discuss additional uncertainties from using established test species despite lack of knowledge regarding their suitability / sensitivity (in addition to the uncertainties already mentioned arising from using test protocols established for conventional chemicals). For example, immunotoxicological mechanisms were discussed in chapters 5.3.3 and 5.4.3 and some may therefore propose other species than the rat.
217	Nanotechnology Industries Association	5.5. Uncertainties in toxicity testing of ENM	lines 824-827: In relation to the correct statement that '[c]urrent toxicity testing approaches used for conventional materials are recommended as a suitable starting point for case-by-case risk assessment of ENM. Toxicity testing methods may need methodological modifications (e.g. regarding sample preparation and characterisation). Specific uncertainties arise due to limited experience of testing ENM in currently applied standard testing protocols,' and advises to specifically consider the globally agreed published results of the OECD WPMN Sponsorship Programme, such as (i) 'Number 15: Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials' (ENV/JM/MONO(2009)21), and (ii) 'Number 24: Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials' (ENV/JM/MONO(2010)25). lines 829-830: The NIA advises against the premature focus on '[a]dditional endpoints not routinely addressed [that] may need to be considered in addition to traditional endpoints.' This statement is in contradiction with the otherwise recommended application of and comparison with (presumably validated) data and information on the approved non-nanoform of a given nanomaterial. The investigation of unconventional endpoints, under application of non-validated test methods should be avoided at this stage.

218	ECPA-European Crop Protection Association	5.5. Uncertainties in toxicity testing of ENM	ECPA welcomes the consideration of uncertainties in the toxicity testing of ENM and the fact that "current toxicity testing approaches used for conventional materials are recommended as a suitable starting point for case-by-case risk assessment of ENM" and agree that "toxicity testing methods may need methodological modifications" as in lines 824-827. This will need to be undertaken as well on a case-by-case approach. However it must be acknowledged that acceptable uncertainties are associated with the toxicity testing of conventional materials as they are with any scientific undertaking. Therefore, ECPA does not support the approach according to which "there may be additional toxic effects caused by ENM that are not readily detectable by current standard protocols" and that "additional endpoints not routinely addressed may need to be considered in addition to traditional endpoints" as in lines 828-830. Adding specific additional endpoints for ENM must be supported by scientific evidence and will depend on the specific material at stake. Therefore, this should be done and updated depending on new future scientific information available as mentioned in lines 527-528.
219	Eurogroup for Animals / Animalfree Research	5.5. Uncertainties in toxicity testing of ENM	The issues depicted in Ch. 5.5 further underline that it is currently premature to lay down testing strategies for the risk assessment of ENM in food and feed and hence to permit the marketing of such products: In conclusion of the plethora of uncertainties in toxicity testing of ENM (LL. 824, 826, 827-828, 829), just as the difficulties in characterising, detecting and measuring ENM in food and feed (L. 822), it should be acknowledged that the scientific basis for determining possible risks of ENM is currently insufficient for ensuring a responsible protection of humans from unwanted effects by eating ENM that were intentionally added to their food.
220	CIAA	5.5. Uncertainties in toxicity testing of ENM	Lines 829-830: It would be helpful to state the additional endpoints that may be required.
221	BASF SE	5.5. Uncertainties in toxicity testing of ENM	We concur with EFSA that current toxicity testing approaches used for conventional materials are suitable for the assessment of ENM and also that methodological modifications may be necessary in some cases (lines 824-827). Published literature has demonstrated that current toxicity testing approaches are sufficient to detect toxic effects with ENM. The need for specific additional endpoints relevant to ENMs must be conclusively supported by scientific evidence and in this regard we support the statement in lines 527-528 that the Guidance will be updated in the future with emerging information.
222	Federal Institute for Risk Assessment	6. Exposure assessment	Line 854 For risk assessment it must also take into account the consumer exposure of ENM as residues in food of animal origin, as indicated in the line 854, page 25 of DRAFT Guidance for risk assessment of nanomaterials. How can this be done? Maybe with an assessment of consumer exposure by using metabolism and residue studies similar as mentioned in the "Technical Guidance for establishing the safety of additives for the consumer" (EFSA Journal (2008) 801).
223	on behalf of the U.S. Government	6. Exposure assessment	(p24 L834-835): The guidance states that issues like food/feed sampling, variability within composite samples and variation in concentrations between samples are not different from the exposure assessment of micro/macroscale or dissolved chemicals. Additional factors such as the impact of food handling, processing and storage conditions on the overall potential exposure, and cumulative effects of testing such as feeding nanomaterial to non-lab animals vs. application to humans due to the variability in the feeding practices may provide for variation in exposure. For example, animals may be fed the same ration day after day, for long periods of time.

224	ECPA-European Crop Protection Association	6. Exposure assessment	The principles of exposure assessment should remain the same for both non-nanoform as well as nanoform. This goes in particular for the risk assessment paradigm and cascade approach as mentioned in figure 2. This approach should be continuously followed in the entire guidance in order to avoid contradictions as mentioned in ECPA comments on sections 5 and 5.2.
225	RIVM	6. Exposure assessment	P 24, lines 843 – 848: Please recommend a dose metric for exposure assessment
226	TNO	6. Exposure assessment	Comment to line 854 'For ENM added to feed, the potential carry over to food should be considered for human exposure'. It is noted that ENM in feed indicate livestock feeding studies in relevant species in case of absorption of the ENM by livestock. ADME data etc. will not sufficiently cover edible matrices as milk (ruminants) or eggs (poultry). In case worst case estimations (all ENM absorbed are transferred to a single matrix) do not indicate a health risk, performance of livestock feeding studies are not necessary.
227	BEUC - The European Consumers'' Organisation	6. Exposure assessment	BEUC comments to lines 832 to 842: When the considered ENM exists also in non nano-form consumption data should detail intakes of both forms separately, especially when the two forms shows different bioavailability.
228	BEUC - The European Consumers'' Organisation	6. Exposure assessment	BEUC comments to lines 715 to 722: Bioavailability is an important factor to be considered when evaluating ADME. Bioavailability studies should always be required, including when the ENM studied exists also in the corresponding non nanoform in order to detect any increase of bioavailability determined by the nano structure. Bioavailability versus absorption is a pivotal element of biokinetics, toxicity testing, and risk assessment.
229	University of Modena and ReggioEmilia	6. Exposure assessment	To add 6. Exposure assessment 857 An ENMs entrapment in the bowel mucosa must be verified as well as a crossing of the bowel barrier and also extravasation.
230	Federal Institute for Risk Assessment	7. Risk characterisation	Line 858 ff. There is the first, and apparently only, mention in line 861 that read-across from one ENM to another may be possible (nano-to-nano extrapolation). EFSA may whish to clarify under which circumstances such bridging may be possible and what kind of data / bridging studies should be generated / performed. The procedure suggested by in this draft guidance to be applied for non-nanoform to nanoform read-across may be adopted (ie. 90d study plus ADME and additional considerations).
231	ECPA-European Crop Protection Association	7. Risk characterisation	See ECPA comments on section 7.1.
232	Eurogroup for Animals / Animalfree Research	7. Risk characterisation	Eurogroup for Animals agrees with the provisions laid down in Chapter 7 to call for a tiered approach for generating information required for risk assessment (LL. 868-876). However we would like to repeat our concern that this tiered approach has not yet been applied as regards integrated testing strategies for hazard assessment. Finally, again, as regards the safety testing of ENM in food and feed, an application area without medical motivations, we can see no justification to request the performance of in vivo test methods that cause sentient animals pain, suffering, distress or lasting harm. Therefore all efforts should strive to develop, validate and implement a tiered non-animal testing strategy for the hazard assessment of ENM in food and feed.

233	Federal Institute for Risk Assessment	7.1. Uncertainties in the ENM risk characterisation	Lines 921-922 It is stated that interspecies and intraspecies variability should be addressed using the established default factors of 10 and 10. It is agreed that this currently appears to be the only sensible option, but it may be added that use of these factors is increasing the overall uncertainty of the assessment. EFSA may also note that the factors were validated over year using data almost exclusively generated for non-nanoforms (ie. were not validated for nanoforms). In addition, some of the (sub)factors are based on specific considerations not applicable to nanomaterials. For example, the interspecies factor for toxicokinetics of 4 may be viewed as based on allometric scaling (cf. eg. REACh guidance).
234	ECPA-European Crop Protection Association	7.1. Uncertainties in the ENM risk characterisation	ECPA welcomes the consideration of uncertainties in ENM risk characterisation. We also appreciate the application of conventional risk assessment methods and in particular the fact that the “conventional defalt uncertainty factor of 10 for inter- and 10 for intra-species differences” would apply as mentioned in lines 917 to 922.
235	RIVM	7.1. Uncertainties in the ENM risk	P 26, line 920: With regard to risk assessment: which dose metrics should be used for assessment factors (AFs)? If surface area is taken, the usual factor 10 is probably insignificant. Are conventional AFs suitable for nanomaterials?
236	TNO	7.1. Uncertainties in the ENM risk characterisation	Comment to lines 911-913: ‘in some circumstances, only a qualitative ENM risk assessment may be possible’. In view of the data requirements specified in this guidance, it appears that a quantitative approach should be possible, taking into account the associated level of uncertainty. It should be made clear in what cases a qualitative approach is acceptable. Comment to lines 914-915 ‘The absence of data essential for the risk assessment should be indicated…’. If essential data for risk assessment are lacking, one cannot draw conclusions from the risk assessment. Performing a risk assessment in case of lacking essential data is not in line with section 2 [lines 280-283], where it is stated that insufficient data requires further testing. ‘…. the quality of the existing data should be indicated…’. Please provide guidance on this aspect.
237	Eurogroup for Animals / Animalfree Research	7.1. Uncertainties in the ENM risk characterisation	The issues depicted in Ch. 7.1 summarize the extent of uncertainties prevailing in current ENM risk characterisation and underline just how premature it would be to lay down a Guidance Document for the risk assessment of ENM in food and feed and hence to permit the marketing of such products. General uncertainties are acknowledged (LL. 888-895), just as the fact that there are difficulties in characterising the form in which the ENM is present in the test system or in food and feed (LL. 896-897). Not even the dose administered can be determined with certainty (LL. 899-900). Specific protocols for toxicity tests for ENM are lacking (L. 901), and fundamental mechanistic questions on how ENM interact with biological systems remain unresolved (LL. 905-906). Therefore, instead of proceeding with laying down a guidance document based on assumptions, it should be acknowledged that the scientific basis for determining possible risks of ENM is currently insufficient for ensuring a responsible protection of humans from unwanted effects by eating ENM that were intentionally added to their food.
238	CIAA	7.1. Uncertainties in the ENM risk characterisation	Lines 920-922 We appreciate the statement that the conventional default uncertainty factors of 10 for inter- and 10 for intra-species differences should be applied if not otherwise indicated by consideration of the data. Currently, there are no indications of a need to modify these factors.

239	Scientific Committee of the Belgian Food Safety Agency (FASFC)	7.1. Uncertainties in the ENM risk characterisation	L921: The reliability of the uncertainty factors for inter- and intra-species differences (10x10x) should be validated for the specific case of nanomaterials.
240	BASF SE	7.1. Uncertainties in the ENM risk characterisation	BASF supports the conclusion in line 921-922 that the currently accepted uncertainty factors of 10 for inter- and 10 for intra-species differences should be applied as there are no indications for a need to modify these factors.
241	Cefic	7.1. Uncertainties in the ENM risk characterisation	On page 26 – lines 920-922 - Cefic appreciates the statement that if not indicated otherwise by consideration of the data, the conventional default uncertainty factors of 10 for inter- and 10 for intra-species differences should be applied as currently there are no indications for a need to modify these factors.
242	Cefic	7.1. Uncertainties in the ENM risk characterisation	On page 26 – line 912 - it is written “As for conventional non-nanoforms of substances in food/feed, risk assessment should preferably be quantitative, but at present, in some circumstances, only a qualitative ENM risk assessment may be possible” Question: What constitutes a qualitative ENM risk assessment?
243	Henkel AG & Co KGaA	7.1. Uncertainties in the ENM risk characterisation	Chapter 7.1, lines 920-922 Henkel appreciates the statement that if not indicated otherwise by consideration of the data, the conventional default uncertainty factors of 10 for inter- and 10 for intra-species differences should be applied as currently there are no indications for a need to modify these factors.
244	VCI (German Chemical Industry Association)	7.1. Uncertainties in the ENM risk characterisation	Chapter 7.1, lines 920-922 VCI appreciates the statement that if not indicated otherwise by consideration of the data, the conventional default uncertainty factors of 10 for inter- and 10 for intra-species differences should be applied as currently there are no indications for a need to modify these factors.
245	UK Government Chemist	Appendix A - Currently used characterisation methods	Line 1087 (2nd comment) In the penultimate line of the table, we suggest either: ‘Kinetic measurements of the stoichiometric and/or catalysed reactions’ or ‘Kinetic measurements of the chemical, biochemical and/or catalysed reactions’. Either alternative would reflect the main text more completely.
246	UK Government Chemist	Appendix A - Currently used characterisation methods	Line 1087 A few more of the abbreviations in the table may need spelling out below line 1114 - for example: MALS - multi-angle light scattering SLS - static light scattering NTA - nanoparticle tracking analysis.

247	Health Canada	Conclusions	Health Canada is committed to fostering the responsible development and introduction of nanotechnology and its applications in the food sector to ensure safe and nutritious food for Canadians. Maintaining and improving high standards for food safety while enabling innovative food products with high foreseen benefits remains our priority. Health Canada recognizes that standardized measurement techniques and well defined reference materials, together with instrumentation for measuring and characterising nanomaterials are required to address existing knowledge gaps. We applaud EFSA's effort to shed some light in the area of risk assessment for nanotechnology applications in the food and feed sectors despite the limited scientific information available to date. We again thank you for the opportunity to review and provide comments on EFSA's Guidance and look forward to continued collaboration between our organizations on this issue.
248	on behalf of the U.S. Government	Conclusions	(p27 L953-958): Where there is an approved non-nanoform of a substance with the same intended use in food/feed, in vitro genotoxicity tests, ADME, and repeated-dose 90-day oral toxicity study in rodents are recommended to assess the potential additional hazards and risks that may arise from the nanoform. Depending on the outcome of these studies and comparison with data on the non-nanoform, other in vivo studies may be needed. We recommend additional clarification on the most appropriate in vitro and in vivo studies as well as a discussion on the absence of Ames test under the in vitro genotoxicity testing and use of 90-day study in rodents and whether this applies to one or two species.
249	ECPA-European Crop Protection Association	Conclusions	• ECPA welcomes the application of the general risk assessment paradigm as in lines 927 to 930. • The characterization of the ENM is key to identify potential risks and behavior of the material. However, the 4 criteria as identified i.e. chemical composition, physico-chemical properties, hazard characterization and potential exposure (lines 936-943) do not necessarily lead to a risk, but are signs to help characterize an ENM. We also appreciate that ENM entails particular nano-specific properties and that "a loss" of these will mean the application of a conventional risk assessment as explained in lines 943-944. • Scientific criteria on how to determine the "completeness" of the transformation of the ENM as mentioned in lines 945 to 949 need to be set in order to clarify when the "completeness" of the transformation will occur. • Additional "genotoxicity studies, ADME and repeated-does 90-day oral toxicity study in rodents" as cited in lines 953 to 958 for the case where a non-nanoform exists, should only be undertaken if the ENM is still present in food and thus once an exposure to an ENM has been encountered. ECPA do not support this approach and believes that this is counter-productive. Instead, these additional studies should be undertaken once it is proved that the ENM is still "present in food" and therefore once an exposure to an ENM has been encountered. For PPP products, a genotox battery and acute oral, dermal and inhalation toxicity studies are already mandatory with the formulated product to compare the toxicity between the pure active ingredient and the formulated product. Therefore additional studies should only address concerns not accommodated by these existing risk assessment requirements. • ECPA appreciates that the determination of the nanoform as detailed in lines 972 to 979 has to consider the amount of ENM added/in contact with the food. However, we believe that the assumption mentioned in lines 977-979 that all ENM added are "present/ingested/absorbed in the nanoform" would need to be based on a case-by-case approach and not as a general approach. • ECPA welcomes that uncertainties are recognized in lines 980-986 and that the guidance will need to be revised and updated in view of new and future knowledge on the field.

250	Eurogroup for Animals / Animalfree Research	Conclusions	Eurogroup for Animals would like to end the comments to the EFSA Draft Guidance Document on the Risk Assessment of ENM in Food and Feed to repeat our conclusion that it is currently premature to lay down testing strategies for the risk assessment of ENM in food and feed and hence to permit the marketing of such products. Instead, it should be acknowledged that the scientific basis for determining possible risks of ENM is currently insufficient for ensuring a responsible protection of humans from unwanted effects by eating ENM that were intentionally added to their food.
251	Cefic	Conclusions	Questions for the Committee Apart from the above comments and remarks, Cefic would also appreciate clarification on the following issues; • How will this process be implemented? • In what matrix will the physico-chemical characteristics of ENMs be initially evaluated? • Are the ADME studies with ENMs sufficiently reliable upon which to draw any meaningful conclusions? • Is the prescribed process more of a “pass/fail” system – relative to a quantitative evaluation? • How should the dose levels be selected for the in vitro genotoxicity tests, ADME tests, and 90-day oral toxicity studies? • Is there a basis for which scientific interpretations can be made?
252	MRC Human Nutrition Research	Conclusions	5) We welcome the division of ENM in 2 categories: (i) nanoform of an already approved non-nanoform with the same intended use, and (ii) new ENM without a corresponding non-nanoform. In many cases we agree that in scenario (i) the risk assessment should be less exhaustive than in (ii) but alert EFSA to careful characterisation(s) of the additional risk that may arise from the nanoform in cases where it would increase the exposure in relation to the non-nanoform, for example by increasing absorption or alter the “chemistry” (by implication biochemistry) of the material such that this is now driven by its new physical characteristics. For example, the antacid “magnesium silicate” tells us little about the particulate (fibrous) magnesium silicate termed asbestos. Ditto for soluble silica versus quartz silica or amorphous nanosilica particles. In the other direction the established safety of microparticulate TiO2 tells little about the toxicity of nano TiO2. 6) Furthermore, we would like to urge caution when dealing with applications where, in EFSA’s views, it can be established that the ENMs are soluble or biodegradable. It is very important to define clearly what solubilisation or biodegradation mean and the site and timing of these are also very important. It should be established if the ENM would persist in the GI tract tissue for a significant period of time (even if it is not absorbed systemically) or if after solubilisation in the gastric environment the ENM would ‘re-form’ in the lower GI tract (at neutral pH for example). With the thorough definition of ADME (absorption, distribution, metabolism and excretion) this should be possible.
253	BEUC - The European Consumers'' Organisation	Conclusions	BEUC comments to lines 935 to 944: See comment on ADME studies. BEUC comments to lines 945 to 949: See comments on Requirements for identification, detection and characterisation of ENM and also on ADME studies. BEUC comments to line 956: bioavailability studies should be included and a comparison between the bioavailability of both forms should be performed. BEUC comments to lines 963 to 966: If the ENM shows an increased bioavailability compared to the non nanoform, its contribution to the exposure scenario should be accurately assessed.

254	UK Government Chemist	Glossary	Line 1152 With reference to the entry 'Non-nanoform': As this term includes aggregated nanomaterials, should there be a new paragraph in section 1 stating that the guidance is also available for consideration by risk assessors of non-nanoforms, where there is a possibility that the corresponding nanoforms will be generated in the gastrointestinal tract, e.g. by means of disaggregation?
256	Eurogroup for Animals / Animalfree Research	References	As cited in the respective comments, Eurogroup for Animals would like to invite EFSA to take into account information from the following publications in their decisions regarding the compilation of a Guidance Document on the Risk Assessment of ENM in Food and Feed. Boekelheide, K. & Andersen, M.E. (2010). A Mechanistic Re-definition of Adverse Effects - A Key Step in the Toxicity Testing Paradigm Shift. ALTEX. 27, 243-252. Combes R, Grindon C, Cronin MTD, Roberts DW, Garrod JF (2008). Integrated Decision-tree Testing Strategies for Acute Systemic Toxicity and Toxicokinetics with Respect to the Requirements of the EU REACH Legislation. ATLA 36, Suppl. 1, 91–109, EFSA SC (2009) Existing approaches incorporating replacement, reduction and refinement of animal testing: applicability in food and feed risk assessment. Adopted on 8 April 2009. The EFSA Journal (2009) 1052, 1-77. http://www.efsa.europa.eu/en/scdocs/scdoc/1052.htm Grindon C, Combes R, Cronin MTD, Roberts DW, Garrod JF (2008). An integrated decision-tree testing strategy for repeat dose toxicity with respect to the requirements of the EU REACH legislation. ATLA 36 Suppl. 1, 139-147. Krewski, D., Westphal, M., Al-Zoughool, M., Croteau, M.C. & Andersen, M.E. (2011). New Directions in Toxicity Testing. Annu. Rev. Public Health. 32, in press. Ma-Hock L, Burkhardt S, Strauss V, Gamer AO, Wiench K, van Ravenzwaay B, Landsiedel R. Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. Inhal Toxicol. 21(2), 2009, 102-18. Sauer UG. Conference Report. Session Nanotoxicology. Linz 2010. 16th Congress on Alternatives to Animal Testing, 3rd September 2010. ALTEX 27(4), 318-322.