The Synthesis Report on the public consultation of the SCENIHR opinion on

The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies

EXECUTIVE SUMMARY

1. Background 5
2. Public consultation 6
2.1 Responses to the Consultation 6
2.2 General observations made by respondents 7
2.3 Key Issues arising from the public consultation 9
2.3.1 Appropriateness of existing risk assessment methodologies 9
2.3.2 New and modified methodologies for risk assessment 10
2.3.3 Information gaps 11
3. Conclusions 12

ANNEX I A Summary of the comments and the SCENIHR responses

EXECUTIVE SUMMARY

This report provides an overview on the comments and recommendations of various stakeholders, received during the public consultation of the SCENIHR opinion on how to assess potential risks of nanotechnology products. The on-line consultation took place from 20 October to 16 December in 2005.

The objective of the SCENIHR consultations is in general to foster involvement of various interested parties and solicit comments on the SCENIHR opinions. This will ensure the wider understanding and use of the SCENIHR recommendations as well as facilitate timely identification of issues of concerns to stakeholders.

With regard to the nanotechnology opinion, the responding stakeholders included representatives of manufacturers and users of nanotechnology products as well as their European associations, research institutions, academia, public authorities, NGOs and individuals. Comments were received both on-line and in letters differing to some extent from the format designed for the Internet consultation. The comments received were taken into consideration in the preparations of this report. Some of the respondents did not want their comments to be published on the Internet.

This document outlines stakeholder proposals for the further development of the assessment of the hazard characteristics of NPs, exposure evaluation and overall risk assessment. In general, feedback on the SCENIHR opinion was very constructive. Stakeholders were supportive of the overall assessment of the appropriateness of the existing risk assessment strategies and offered many detailed comments relevant to further fine-tuning of the opinion and/or further work on the issue in specific fields. The proposals also strongly emphasized the need for international coordination in the field of environmental and health and safety of nanotechnology products.

The main points of the responses and specific recommendations from the public consultation have been presented in a concise manner. This report summaries the proposals, while the special annex to this document addresses the stakeholder proposals in detail. The SCENIHR has also modified its opinion in order to better response to the stakeholder concerns. The Commission services will further consider how to take into consideration the issues related to international co-operation and legislative work which were raised in the consultation, but which are not included in the SCENIHR mandate.

1. Background

The Commission Strategy[1] and Action Plan[2] on Nanotechnologies underline the importance of a safe and responsible approach and integration of risk assessment into every step of the life cycle of nanotechnology-based products. The Commission requested the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) for an opinion on the following questions:

1. Are existing methodologies appropriate to assess potential and plausible risks associated with different kinds of nanotechnologies and processes associated with nanosized materials as well as the engineered and adventitious products of nanotechnologies?
2. If existing methodologies are not appropriate to assess the hypothetical and potential risks associated with certain kinds of nanotechnologies and their engineered and adventitious products, how should existing methodologies be adapted and/or completed?
3. In general terms, what are the major gaps in knowledge necessary to underpin risk assessment in the areas of concern?

The SCENIHR opinion[3] concluded that nanomaterials may have different (eco-) toxicological properties than the substances in bulk form and therefore their risks need to be assessed on a case by case basis. The SCENIHR also foresaw that current risk assessment methodologies require some modification in order to deal with the hazards associated with nanotechnology. In particular, the existing toxicological and ecotoxicological methods may not be sufficient to address all of the issues arising with nanoparticles. For dose evaluation information on the number of nanoparticles and/or their surface area in addition to traditional mass concentration characterization is needed. Equipment for routine measurements in various media for representative exposure to free nanoparticles is inadequate. In addition, existing exposure assessment methods may not be appropriate to determine the environmental fate of nanoparticles.

Nanotechnologies are expected to bring significant improvements to the quality of life of European citizens as well as offers a new competitive edge to European businesses. The SCENIHR opinion provides an authoritative and wide ranging review of the appropriateness of existing risk assessment methodologies. This evaluation allows the Commission to assess the adequacy of existing procedures and technical requirements and indicates to industry and to the research community additional research and development areas in regard to potential risks to human health and the environment.

The priority for the Commission is to ensure a high level of consumer safety in relation to highly innovative nanotechnologies. Therefore the SCENIHR, in order to ensure that the potential concerns of various stakeholders' are sufficiently addressed, decided to launch a public consultation on the opinion.

[1] COM(2004) 338 Final
[2] COM(2005) 243 final
[3] SCENIHR/02/2005

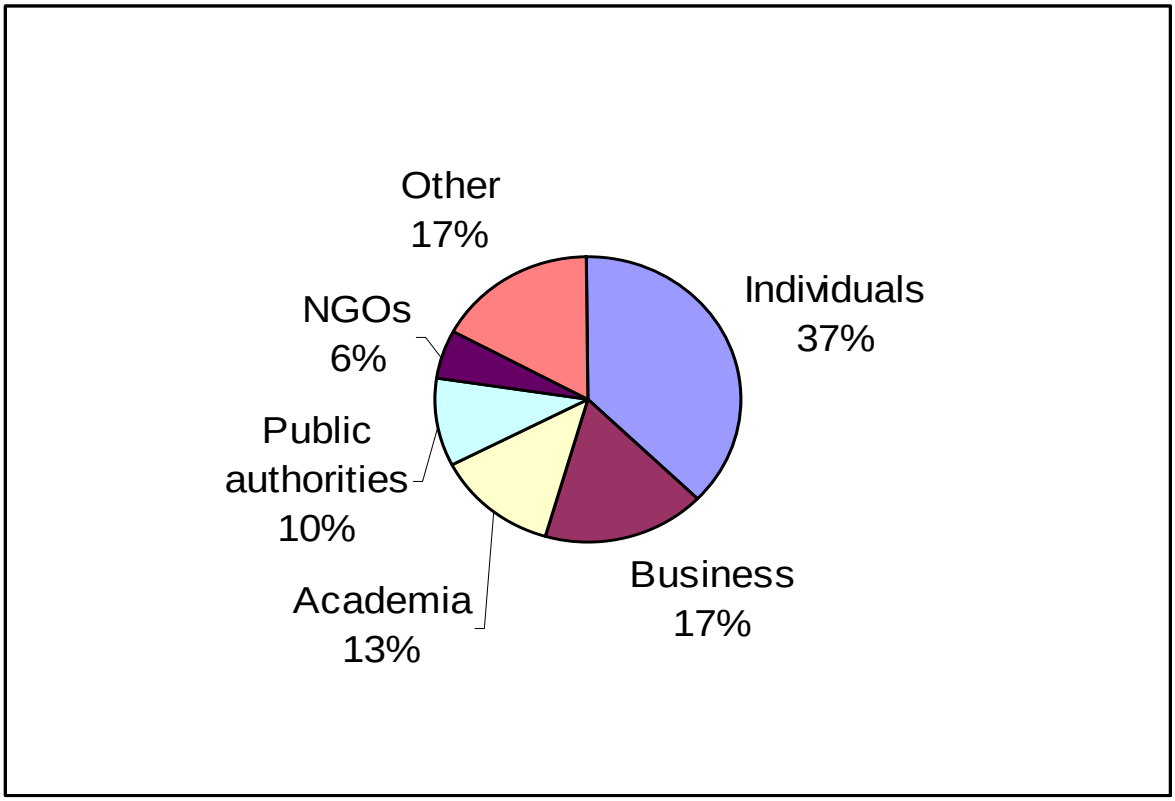
2. Public consultation

2.1 Responses to the Consultation

On 20 October 2005 the Commission, in consultation with the SCENIHR, launched a public consultation on the SCENIHR opinion on 'the appropriateness of existing methodologies to assess the potential risks associated with the products of nanotechnologies'. The objective of this consultation was to collect views of various interested parties with regard to the scientific opinion.

A total of 70 contributions were received from a broad range of representatives of manufacturers and users of nanotechnology products as well as their European associations, research institutions, academia, public authorities, NGOs and individuals. From business responses, the majority came from either producers of nanotechnology products or from users of nanotechnology products in their manufacturing processes. More than half of the comments from business came from SMEs.

Figure 1. Responses to the public consultation – Stakeholder groups



The majority of the contributions were received from the EU countries, three from the United States, two from Switzerland and one from Belarus.

Table 1. Responses to the public consultation by countries

Country	Total	Individuals	Academia	Business	Public authorities	NGOs	Other
EU countries	**64**	**25**	**9**	**11**	**5**	**3**	**11**
Austria	2			1	1		
Belgium	8	3				1	4
France	5		1	2			2
Germany	12	6	1	5			
Greece	1					1	
Ireland	1	1					
Italy	9	5	2			1	1
Netherlands	7	3	2		1		1
Poland	1				1		
Portugal	1	1					
Spain	1	1					
United Kingdom	16	5	3	3	2		3
Non-EU countries	**6**	**1**		**1**	**2**	**1**	**1**
Belarus	1	1					
Switzerland	2			1	1		
United States	3				1	1	1

This report provides a synthesis of the recurrent positions most frequently advanced by respondents with regard to the SCENIHR opinion. It does not reflect any judgement on the part of the SCENIHR as regards the different comments made in response to the consultation. In drawing up this summary, the SCENIHR has been guided not only by the number of respondents expressing a particular point of view, but also by qualitative considerations such as the extent to which the respondents are representative and the arguments advanced by respondents in support of their views. For this reason, the report does not present a systematic statistical analysis, but rather a qualitative assessment of the responses received and of the main arguments underpinning these responses. What follows, therefore, should be regarded as a summary of statements provided by respondents in respect of their perceived priorities on the issues covered in, or relating to, the Consultation Document.

2.2 General observations made by respondents

The respondents highlighted several general observations that are outside the scope of the SCENIHR mandate, but have common relevance for the Commission work on safe, responsible and integrated nanotechnologies. They refer to the development needs for :

- better coordination on nanotechnology issues in the EU and internationally,
- international consensus over the testing and risk assessment methodologies (procedures, devices and reference materials as well as databases) of nanotechnology products

- integration of the risk assessment demands of nanotechnology products into the existing legal frameworks and defining when these products are considered new substances and when not,
- initiation of a dialogue between the Commission and the stakeholders, especially industry, on the safety of nanotechnologies
- international standardisation of terminologies and methodologies of risk assessment of nanotechnology products,
- further work on the risk-benefits assessment of nanotechnology products especially in regard to nanomedicine and nanofood; and
- measures endeavouring public perception and consumer confidence in nanotechnologies.

A majority of respondents agree or mostly agree with the SCENIHR assessment of the situation with respect to the risk assessment methodologies and the need for case by case evaluation of risks of nanotechnology products. They consider it important to enhance the efforts for better understanding of the mechanistic and toxicological properties of nanoparticles and their behaviour in varying conditions. Furthermore, the need for internationally harmonised terminology and standardised (and validated) testing methods is broadly recognised.

The main body of partial disagreement relates to the appropriateness of existing testing methods. The SCENIHR opinion recognised the value of the current methodologies, but due to the novel properties of nanotechnology products, highlighted potential needs for modification and/or further development of methodologies. This was misunderstood by some respondents from the chemical industry as neglecting the current methodologies.

In general, the responses from the academia (universities and research institutions) as well as from several individual researchers, agree or mostly agree with the SCENIHR opinion. Nevertheless, they highlight the need for further work on the appropriate characterization of physico-chemical properties of nanoparticles (especially in regard to their toxicity and dose evaluation), hazard evaluation, exposure evaluation and in the overall risk assessment. Long-term effects on the distribution of nanoparticles in nature, in the living environment and in occupational settings are considered equally important for long-term risks. Some researchers warn also about oversimplification of the situation and highlight need for further developed and preferably step-by-step risk assessment methodologies for nanoparticles. The latter concern was also the reason for a few 'mostly disagree' comments from individual researchers, who felt that advancements are not taking place at an appropriate speed and/or scope compared to the potential risks of the expanding use of nanotechnology products.

Most of the responses from SMEs in the nanotechnology business concur on the SCEHNIHR opinion, but several of the large enterprises and the European associations of the chemical industry are concerned about the demands for modified or new risk assessment methodologies. They rather foresee development demands for appropriate risk assessment methodologies relevant in regard to the characterisation of physico-chemical properties of nanoparticles, better data on nanoparticles characteristics for the assessment of biological effects rather than for new testing methods for hazard evaluation. Business considers international coordination and standardisation in risk assessment also very important.

Public authorities agree or mostly agree with the SCENIHR opinion. However, they highlight the need for further work on better guidance and tools for risk assessment as well as on legal

aspects and international coordination (non-SCENIHR work areas). The former relate to the uncertainties of the novel properties and consequent behaviour of nanoparticles. The latter, on the other hand, relates to the current situation in national authorities who feel that current legislation may impede, according to one response, demands from authorities for appropriate information on the characteristics of nanoparticles. Furthermore, there is a demand to extend the scope of risk assessment to nanotechnology products and applications in food industry.

NGO responses, as well as some individual responses, also agree or mostly agree with the SCENIHR opinion, but highlight the need for the assessment of long-term risks and a shift from animal testing to alternative methods. In particular, the respondents representing people concerned about animal testing consider the latter more relevant for human toxicology of nanoparticles.

The other organisations in this consultation refer to scientific networks, Technology Platform on Industrial Safety (ETPIS) and the business associations of the chemical industry. The former two concur on the SCENIHR opinion, but the representatives of the chemical industry mostly disagree, mainly due to the demands for modified and new testing of novel properties of nanotechnology products.

2.3 Key Issues arising from the public consultation

2.3.1 Appropriateness of existing risk assessment methodologies

The SCENIHR stated in its opinion that

"Although the existing toxicological and ecotoxicological methods are appropriate to assess many of the hazards associated with the products and processes involving nanoparticles, they may not be sufficient to address all the hazards. Specifically, particular attention needs to be given to the mode of delivery of the nanoparticle to the test system to ensure that it reflects the relevant exposure scenarios. The assays may need to be supplemented by additional tests, or replaced by modified tests, as it cannot be assumed that current scientific knowledge has elucidated all the potential adverse effects of nanoparticles.
For exposure, the use of mass concentration data alone for the expression of dose is insufficient, and the number concentration and/or surface area need to be included. Equipment that enables routine measurements in various media for representative exposure to free nanoparticles is not yet available. The existing methods used for environmental exposure assessment are not necessarily appropriate for determination of the distribution, partitioning and persistence of nanoparticles in the various environmental compartments.
Given the above uncertainties, the current risk assessment procedures require modification for nanoparticles."

The respondents strongly support the SCENIHR opinion that recognises the value of the existing risk assessment methodologies and highlights the potential demands for modified and new risk assessment methodologies in regard to nanotechnology products. The current methodological approaches apply, but practical methods may not be sufficient for the assessment of the novel properties of nanotechnology products and their short and long-term behaviour in varying conditions and in varying industrial formulations and applications.

While partly agreeing to the SCENIHR assessment results, some business respondents and their European associations considered that the existing risk assessment methodologies with

minor modifications are also sufficient for nanotechnology products. The industry also opposes that for hazard evaluation and risk assessment it should be taken into consideration that nanoparticles could exacerbate pre-existing medical conditions. They consider that those features are not specific to nanoparticles.

2.3.2 New and modified methodologies for risk assessment

The SCENIHR stated in its opinion the following:

Three different situations can be identified where existing methodologies are considered unsuitable:
- *Routine methodologies have not yet been made available and / or have not been included in the testing guidance and/or achieved regulatory acceptance.*
- *Scientific research has identified a phenomenon to be evaluated and existing methodologies need to be adapted.*
- *Advances in nanotechnology may require additional methodological principles and developments.*

Included in the areas of requirements for new or modified methodologies are:
- *Appropriate methodologies must be made available for the routine and careful characterisation of the physico-chemical properties of nanoparticles.*
- *Methodologies and equipment need to be developed that enable routine measurements, in various media, of representative exposure to free nanoparticles.*
- *Although conventional toxicity and ecotoxicity tests have been shown to be useful in evaluating the hazards of nanoparticles, some methods may require modification and some new testing methods may also be needed in order to optimise this process of hazard evaluation, including the assessment of whether nanoparticles can exacerbate pre-existing medical conditions.*
- *In this context, although again some potentially suitable methods exist for the detection of nanoparticle translocation, these need to be developed further and incorporated into new testing strategies and guidelines for the assessment of the systemic distribution of nanoparticles.*

More specifically the above mentioned methodologies need to provide information on how nanoparticles distribute in human tissues and in environmental compartments. This information can then be used in the exposure assessment algorithm provided in figure 6 in section 3.10.5 of this opinion.

The respondents from academia, public authorities and NGOs supported the above-mentioned conclusions requesting further adjustments for nanotechnology products in order to gain the benefits of rapidly evolving technologies. However, the business representatives were convinced about the appropriateness of existing risk assessment methods and foresaw only the need for slight modifications of risk assessment methodologies for nanomaterials. All respondents emphasised the need for standardisation of risk assessment methodologies in order to enable comparability of studies, efficient use of resources and compliance with existing legislative requirements.

Nevertheless, the other stakeholders, especially from academia and individual researchers in research institutions, highlighted the need for modified and new methodologies due to novel properties of nanotechnology products. The key additional areas compared to the SCENIHR opinion are the following:

- Better routine as well as non-routine methods for the diversified characterisation of nanoparticles, including aggregates, embedded nanomaterials and the potential of insoluble nanoparticles to act as carriers of other (possibly toxic) chemicals;
- Better understanding of the mechanisms and toxicokinetics of NPs, including the structure/function relationships, potential immunological responses, bioaccumulation

and novel mechanisms and end points as well as advancement in instrumentation and testing methods making use of e.g. suitable reference materials and experiences in in-vitro methods (both in nanotechnology applications in non-food and food area);

- Better knowledge of the relevant exposure scenarios, 'bottom-up' exposures and improved analytical methods, including personal sampling devices for exposure monitoring;
- Means and methods to assure sufficient safety of nanomaterials, including further evaluation of appropriateness of existing testing guidelines e.g. in chemicals, medicinal products and in medical devices, ;
- Efficient mechanisms for the information exchange internationally on nanosafety studies;

2.3.3 Information gaps

The SCENIHR stated in its opinion that:

In general, and in spite of a rapidly increasing number of scientific publications dealing with nanoscience and nanotechnology, there is insufficient knowledge and data concerning nanoparticle characterisation, their detection and measurement, the fate (and especially the persistence) of nanoparticles in humans and in the environment, and all aspects of toxicology and environmental toxicology related to nanoparticles, to allow for satisfactory risk assessments for humans and ecosystems to be performed.

The major gaps in knowledge that need to be filled in relation to improved risk assessment for the products of nanotechnology include:

- *The characterisation of the mechanisms and kinetics of the release of nanoparticles from a very wide range of production processes, formulations and uses of the products of nanotechnology.*
- *The actual range of exposure levels to nanoparticles, both to man and to the environment.*
- *The extent to which it is possible to extrapolate from the toxicology of non-nano sized particles and other physical forms e.g. fibres of the same substance to the toxicology of nanosized materials, and between nanoparticles of different size ranges and shape*
- *Toxicokinetic data following exposure, so that target organs can be identified and doses for hazard assessment determined. This includes dose response data for the target organs, and knowledge of the subcellular location of nanoparticles and their mechanistic effects at the cellular level.*
- *Information from the occupational exposure and associated health effects on workers involved in the manufacture and processing of nanoparticles*
- *The fate, distribution and, persistence and bioaccumulation of nanoparticles in the environment and environmental species including micro-organisms*
- *The effects of nanoparticles on various environmental species, in each of the environmental compartments and representative of different trophic levels and exposure routes.*

In addition, there are several aspects of the fundamental properties of nanoparticles that require elucidation, including the ability of nanoparticles to act as vectors of chemicals, micro-organisms and interactions with other stressors.

The respondents pointed out the importance of coordinated and global actions when coping with demands for extensive information environmental and health aspects of nanotechnology products. A step-wise process may prove very feasible both in regard to filling of the knowledge gaps and in the further adaptations of the current testing strategies and practical guidance documents to nanomaterials.

In addition to the SCENIHR list of knowledge gaps listed above, the respondents highlighted information demands in the following areas:

- Sophisticated characterisation of NP in regard to both toxicological behaviour, dose evaluation and both environmental and human exposure conditions and better instrumentation (e.g. monitoring equipments, sensors, control devices);
- Greater understanding of all the toxicological pathways, toxico-kinetic processes, feasible end points, including accumulation, inter and intra-species differences etc. enabling also the development of hazard profiles and determination of the differentiated susceptibility of individuals;
- Monitoring of the effects of exposure to NPs via various exposure routes;
- Internationally comparable and/or standardised methods for measurement and toxicological assessment of NPs

3. Conclusions

The public consultation brought out the enormously complex nature of the demands related to nanotechnology products, their characterisation, toxicological properties, exposure scenarios and environmental fate. It highlights safety demands for businesses as they endeavour to keep up their competitive edge in these dynamically evolving technologies while simultaneously ensuring consumer perception of upcoming nanotechnology products and meeting their legal obligations.

The consultation highlights the need for pragmatic actions in regard to safety of nanotechnology products. When doing so, it is essential to recognise the value of a case by case risk assessment. Furthermore, despite the fact that respondents had different approaches and priorities to risk assessment, several points of convergence were identifiable and it will be possible to take considerable advantage of the experiences gained with the existing risk assessment methodologies.

The public consultation of the SCENIHR opinion sought stakeholder involvement and in doing so succeeded in obtaining useful advice both in regard to the current opinion and the future work in the field of risk assessment methodologies. Both are considered most valuable and make stakeholders recognise that they had been adequately consulted and that their views had been taken into account in the modified opinion and will be taken into account in the forthcoming work of the SCENIHR.

Annex 1

The Stakeholder comments and the SCENIHR responses

1. Introduction 2
2. Stakeholder comments and responses from the SCENIHR 2
2.1 General comments outside the SCENIHR mandate 2
2.2 Appropriateness of the risk assessment methodologies 3
2.2.1 Scope of the SCENIHR opinion 3
2.2.2 Characterisation 4
2.2.3 Hazard evaluation 5
2.2.3.1 General approach 5
2.2.3.2 Toxicology of nanomaterials 6
2.2.3.3 Toxicity testing 8
2.2.4 Exposure evaluation 11
2.2.4.1 General approach 11
2.2.4.2 Exposure Scenarios 11
2.2.4.3 Exposure monitoring methods 12
2.2.5 Risk Assessment 13
2.2.5.1 General approach 13
2.2.5.2 Practical guidance 14
2.3 Further development of risk assessment methodologies 15
2.3.1 Characterisation 15
2.3.2 Hazard evaluation 16
2.3.2.1 Toxicology of nanomaterials 16
2.3.2.2 Toxicity testing 17
2.3.3 Exposure evaluation 18
2.3.4 Risk assessment 18
2.4 Information gaps 19
2.4.1 General considerations 19
2.4.2 Characterisation 19
2.4.3 Hazard evaluation 22
2.4.3.1 Toxicology of nanomaterials 22
2.4.3.2 Toxicity testing 23
2.4.4 Exposure evaluation 23
2.4.4.1 Environmental exposure 23
2.4.4.2 Human exposure 24
2.4.5 Risk assessment 25
2.5 Responses from the SCENIHR 27
2.5.1 Scope of the opinion 27
2.5.2 Characterisation 27
2.5.3 Hazard evaluation 27
2.5.4 Exposure evaluation 28
2.5.5 Risk Assessment 28
2.5.6 Further development of risk assessment methodologies 28

List of abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
BET	Brunauer, Emmett and Teller method, which permits a determination of the surface area of e.g. catalyts
CEFIC	European Chemical Industry Council
CIA	Chemical Industry Association in the United Kingdom
DEFRA	Departement of Environment, F, Agency
ECETOC	European Chemical Toxicologist
EMEA	European Medicinal Agency
FDA	Food and Drug Agency
ICCA	International Council of Chemical Associations
LC50	Median lethal concentration
NONS	Notification of New Substances
NPs	Nanoparticles
PBT	Persistent Bioaccumalative Toxic
PNEC	Predicted No-Effect Concentration is the concentration below which exposure to a substance is not expected to cause adverse effects.
REACH	New chemicals legislation (Registration, Evaluation and Authorisation of Chemicals
RIVM	(Rijksinstitut vor Volksgezondheid en Milieu) Dutch Institut for Health and the Environment
SME	Small and Medium size Enterprise (number of employees 1-250)
TGD	Technical Guidance Document
UBA	(Umweltbundesamt) Environmental administration in Austria
URPL	(Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych) Office for Registration of Medicinal Products, Medical Devices and Biocidal Products in Poland

Countries	AT Austria, BE Belgium, FR France, DE Germany, GR, Greece, IRL Ireland, IT Italy, NL The Netherlands, PL Poland, PT Portugal, SP Spain, UK United Kingdom, CH Switzerland and USA United States of America

1. Introduction

The opinion of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) on how to assess the potential risks of nanotechnology products was adopted on 28-29 September 2005. In the same meeting the SCENIHR decided to make use of public consultation in order to collect views and recommendations from stakeholders. Public consultation was considered a useful tool to further adapt scientific opinions towards the special, pragmatic or legislative demands and aspirations of the stakeholders as well as to collect recommendations for possible further work in the SCENIHR.

In the following, the proposals from stakeholders are provided in a compressed manner and grouped according to key issues and commented in detail (except the General Recommendations that concern issues outside the SCENIHR mandate) by the SCENIHR. In case of confidential responses, the identification of the stakeholders, an interest group and country are given. Links to commercial web sites are not included in the report, but are available in the individual responses.

2. Stakeholder comments and responses from the SCENIHR

2.1 General comments outside the SCENIHR mandate

International co-operation

Support the call for co-operation between member states, the Commission and Industry, as well as wider international co-operation. In the view of the UK, the OECD could usefully play this role internationally [DEFRA, UK]. Coordination and communication between the different activities is needed and we consider it is essential to initiate a dialogue between different stakeholders [UBA, AT].

International consensus over the testing and risk assessment of NPs is essential to the future of nanotechnologies and the potential benefits they could offer to populations worldwide. The European Commission is asked to ensure that work carried out in the 'better regulation' initiative is not duplicated in other parts of the world [CIA, UK]. Over–arching requirement for the development of nomenclature and definitions is a major priority [DEFRA, UK].

Risk management should focus on harmonising already accepted testing strategies and work on worldwide accepted protocols [CEFIC, BE].

The information from the research exercises on selected reference materials should be made available to an international information exchange platform (and/or database or alert system) [RIVM, NL].

Legislation

The coverage of nanotechnology products under the existing chemicals legislation and REACH needs to be clarified. Information specific for nanotechnology products in order to identify and evaluate the environmental and health hazards and to assess the risks needs to be defined. The SCENIHR opinion could be a starting point, but needs to be clarified if this information can be requested on the basis of legislation in force [UBA, AT]. Additional regulations on NPs are not necessary, since NPs are covered by the existing chemicals legislation (NP-specific CAS numbers questionable) [Henkel, DE].
Regulatory aspects (p 55) on labelling by means of a different CAS number or additional code would be, within a juridical system, a different substance and thus a new substance with all consequences. Another option (Article 7.1 of Existing Substance Regulation) "important new information should be reported" also on different forms of the same chemical species [TNO Occupational Health, NL].

Commission and Industry

The European chemical industry wishes to enter into a dialogue with the Commission on the very complex issue of nanomaterials [CIA, UK].

Funding

Funding of research on NPs characteristics and risks instead of funding of product development [C.Raab-Heine, IRL].

Risk-benefit assessment

In the Committee's opinion there is a strong focus on methodologies regarding risks. In our opinion, this focus needs to be extended to adequate risk-benefit analyses regarding human health and the environment as many of the nanotechnology products will have the potential to provide substantial benefits [RIVM, NL]. Methods and considerations for balancing risks and benefits need to be developed [W. Passchier, NL].

Although it is mentioned that a transparent framework for risk benefit analysis should be developed, the perception and appraisal of nanotechnology or NPs by the general public (or consumers) is not mentioned. The opinion focuses on exposure routes other than the possible exposure to NPs via food. It is, however, reasonable to expect ingestion of NPs from novel foods, NPs in the food production or in packaging material, as an enrichment of foods from packaging of micronutrients, environmental contamination of food (directly or indirectly). Sound communication on the impacts of possible risks and advantages of nanotechnology products is crucial for the public perception. Technically identified risks and benefits should be evaluated in parallel with evaluation of social/economical impact of the identified risks and/or benefits. Important lessons could and should be learned from GMO case. Many cross links between the GMO case and the introduction of NPs public concern, technical and scientifically developments and the (lack) of communication between these bodies can be drawn [RIKILT-Institute of Food Safety, NL].

2.2 Appropriateness of the risk assessment methodologies

2.2.1 Scope of the SCENIHR opinion

Nanomedicine and medical devices

It would be useful to explore literature on new pharmaceutical products in development in which nanotechnology is applied, in order to see whether it is now already possible to identify properties of (some types of) these products which might need specific attention and in order to assess whether new guidance documents or amendments of existing guidance documents would be useful. However, this is the responsibility of the EMEA and should be done by experts involved in assessment and authorization of medicines [RIVM, NL].

Since the SCENIHR opinion mentions human medicinal products in different places, it is not clear whether it is intended to cover medicines or not. If this is not the case, this should be mentioned more clearly e.g. in the introduction. If medicines are intended to be covered, the text should also refer to existing guidance in the area of human medicines and section 3.10 should cover the specific aspects of human medicines assessment more explicitely [RIVM, NL].

Food ingredients and food contact materials

Although the SCENIHR opinion distinguishes between risks associated with free NPs and NPs encased in solid matrices, it does not specifically consider food ingredients or food contact materials from which a degree of migration into food may occur. It may therefore be necessary to consider ingestion, as well as inhalation of free NPs, and to draw a distinction between free particles which will dissolve into 'harmless molecules' (e.g. as typical soluble food components) and those that will not dissolve or disperse following ingestion. Consequently, we believe it may be necessary to consider the potential food / food contact use of nanoscale particles as a separate category of materials, on a case-by-case basis, within the existing frameworks for the safety approval of novel foods, food additives and packaging additives, respectively [Institute of Food Science and Technology, UK].

Industrial safety

The Report does not discuss in detail the fire and explosion hazards of nanoparticles (which would appear to be covered in the terms of reference). There is merit in characterising these properties of nano-scale particles and to do this, new methods will need to be developed [DEFRA, UK].

2.2.2 Characterisation

Characterization of nano-sized materials will be a key activity and non-routine methods may need to be used. Consideration of these factors may lead to appropriate modifications of the risk assessment process, but existing procedures and guidelines should be the starting point [CHEMSTAR Nanotechnology Panel, USA]. Parameters to be measured (mass / number / surface area / shape) and the development of measurement technologies in various media are priorities: Understanding solubility, particle size distribution, agglomeration and the absorption of other chemicals are important factors to consider within any screening / prioritisation process [DEFRA, UK].

Solicit a determination of a set of standard parameters to describe the NPs properties (e.g. ISO norms) [Henkel, DE]. NPs should be well characterized. Particle size is only one of many decisive properties (aggregates, surface functionalization, etc.). Risk assessment should be based on detectable particle properties which can be reproducibly measured [Business, AT]. Photon correlation spectroscopy (p 19) is the generic name for the technique in measuring particle size from a change in the interference patterns with time during Brownian motion (Ref 16). Shape and particle size can also be measured by the more traditional forms of light and low angle x-ray scattering (Rayleigh & Mie laws, Zim plots –Ref 17) [URPL, PL]. More emphasis needs to be placed on assessing the consistency during production of nanoproducts and stability of tested nanomaterials [FDA Nanotechnology Panel, USA].

Knowledge of the aggregation state of the NPs in the exposure evaluation is considered essential and relating to the release behaviour, the transport position in the appropriate fluid medium (e.g. gas or fluid) and the aggregation behaviour within the biological target. An understanding of particle transport and fate in disaggregated and aggregated forms is essential in the biological target and the environment more generally [Univ. of Cambridge, UK]. Agglomerates behave differently from single nanoparticles, but not like one large particle (p. 10) [TNO Occupational Health, NL].

Product specification (p 49): to reflect the aqueous conditions found in the human body, an estimate of the nanoparticle charge, (ie. the zeta potential) as well as the Hamaker constant, (total energy of inter-particle interaction), could be made under standard conditions at various concentrations of nanoparticles. Techniques for routine assessment of both are widely available (Ref 1,2,3) [URPL, PL].

The definition of dose is rightly highlighted as a key and problematic issue. There is also a need to more fully understand the mechanistic basis of exposure to determine a credible dose calculation: for

example, the non-monotonic capture efficiency of different sized particles in the airway/lung. This is particularly important in understanding the effect of aggregation/agglomeration or aerosolisation of materials containing nanoparticles [Scientific Liaison Advisory Services, UK].

The number concentration and/or surface area are considered insufficient for the evaluation of dose. Instead it is recommended that overall chemical composition of the particles, modification/lattice structure of NPs, homogeneous or inhomogeneous structure of the particle: internal gradients; composite particle, the oxidation state/ionization state/charge, the state of the surface (for instance: hydrophilic, hydrophobic) and behaviour (solubility in aqueous media needed for physiological relevance) would be decisive characters [Fraunhofer IKTS, DE].

Measurement of mass concentration as single parameter might not be sufficient to assess effects of NPs. Also the surface area as single parameter is not suitable to characterize the toxicological potential. The effect of the chemical composition, the number concentration, the surface and the morphology has to be further investigated [BASF, DE].

Challenge in viewing the results of well-conducted studies with carefully characterized particles with the goal of understanding the impact of particle property modifications on toxicity [ECETOC, BE].

Important parameters to be considered for the nanosafety are: size, morphology, chemical composition and speciation [Individual, IT]. Agglomerates behave differently from single nanoparticles, but not like one large particle (p. 10). Toxic aspects of nano-aggregates are omitted except on p21: it's crucial that NP aggregation in inhaled air be taken into account and the risk assessment be directed on the form and size of the NPs in practical situations [TNO Occupational Health, NL].

A classification of nanoparticles on the basis of its origin/synthesis is necessary for the risk assessment. For instance, NPs obtained by wet mode represent less of a risk than the others. For exposure, it is necessary to know the size, morphology, chemistry, physical state (hot or cold, electrically charged or not, etc) and the concentration in the unit time [Academia, IT].

2.2.3 Hazard evaluation

2.2.3.1 General approach

Existing, modified and/or new methodologies?

No general statements on hazards of nanomaterials to humans can be made and risk analysis can only be performed on a case by case basis [CEFIC/BE, Bayer/DE, ECETOC/BE, Henkel/DE, ICCA/BE]. Potential hazards of NPs can be derived from the data from bulk material: chemical nature is a major hazard factor also for NPs [Henkel, DE]. As for other xenobiotics that are not nanoscaled materials all of the potential adverse effects are not necessarily known in advance of testing but that doesn't erode confidence in the ability of testing to illuminate those effects. When discussing tests that have proven to be capable of revealing the toxicological effects of a wide variety of materials acting by various mechanisms (many of which are largely unknown), it can be anticipated that significant toxicological effects of nanoscaled particles will also be demonstrated by these existing test methods [Degusa, DE]. The existing toxicological and ecotoxicological test methods in principle appropriate to assess the health and environmental effects of NPs. To consider the specific nature of NPs those tests have to be modified and validated accordingly. Particular attention needs to be given in applying these methods to the mode of delivery of the NPs to the test system to ensure that it reflects the relevant exposure scenarios [BASF, DE].

It may in fact be that toxicological methods might not need to be changed in a fundamental way (notably in ecotoxicology), other than a) to account for the need to use appropriate dose metrics, with in particular a need to measure surface area and particle number as well as mass during the test; b) a need to be flexible in design and allow follow-up, additional endpoints which may be informed by screening studies. SCENIHR may be able to provide some clarity on this issue and in light of these considerations develop a strategy for assessing the potential hazards of nanomaterials in the context of existing protocols (e.g. NONS and REACH) [DEFRA, UK].

The current approach/structure for the environmental risk assessment and classification and labelling in general is suitable for nanoparticles. Also the testing strategy, e.g. the need for having various trophical levels (e.g. algae, daphnids and fish for water) in the base set, may also hold for nanoparticles. (Some people argue the need for acute toxicity test for nanoparticles owing to their release/exposure characteristics) [RIVM, NL].

Current test methods need to be modified particularly to reflect the risk aspects of nano particulates. Due to quantum, electrostatic and other effects related to size, the toxicity of NPs cannot be deduced from larger particulates or bulk materials. In this respect, they must be treated as New Substances under current legislation. Equally, data on the NPs toxicity must not be used in the classification of larger particulates [European Powder Metallurgy Association, UK].

Improved or novel methods may help to further elucidate mechanisms of toxicological impact and therefore may add to the improvement of screening tests characterizing the hazard potential of nanomaterials [CEFIC/BE, CIA/UK]. Correlations between the physical-chemical properties of the particles and their physiological or biological behaviour are not well understood [Fraunhofer IKTS, DE]. Particular attention could be given to tests verifying impact of long-term exposure on organs like liver, brain, etc. by checking and quantifying the presence of NPs inside these organs [Individual, IT]. Disagrees with the SCENIHR assessment (Chapter 4, paragraph 7 and chapter 3.4.7) stating that NPs of considerable solubility in the physiological environment show interactions with living systems which are close to bulk chemical justify the use of well established toxicological testing procedures and approaches. The toxicity of NPs may differ from its core chemical if the dissolution is slow or needs specific pH condition allowing prior translocation to other targets [Swiss Federal Office of Public Health, CH]. More fundamental assessment of mechanism of particles with cells, and beyond would be most valuable [Individual, UK]. It will be important to fund research into the mechanistic basis of nanoparticle interaction with body tissues: the surface nature of particles significantly below 100 nm is potentially very different from "bulk" surface properties [Scientific Liaison Advisory Services, UK].

2.2.3.2 Toxicology of nanomaterials

General remarks

As a general comment, it would be helpful to have more of the statements supported by references. As one example, the important statement on page 43 that nanoparticles are able to pass via the olfactory nerve to the brain could have been referenced (to work by Oberdörster and others for example) [DEFRA, UK].

Chemical/biochemical modifications (p 7) to enzymes, antibodies, drugs and many other biological molecules, encompassing the nanoscale, (Ref 19), have been commonplace in medicine, biochemistry/molecular biology and in the pharmaceutical industry for many years (Ref 20). This is seen in standard textbooks on the subjects. Many biochemical reactions in living organisms fall within the nanoscale range and the structure, function and mechanisms are mostly very well defined, as is the classical case for enzymes. An example of the exquisite control achieved by enzymes is the molecular re-arrangements and self assembly at the micro-nanoscale level observed in the process of DNA

transcription, translation, (Ref 21). The 'Nanotechnology' definition provided at this point may therefore overlap with essential biological processes [URPL, PL].

Dosing

Particle size -surface to volume ratio (p 22): the severity in responses disappeared when the dose was expressed as surface area. For instance, nano-sized particles were as toxic as fine particles of the same composition (Oberdörster et al., 2000), and nano-sized TiO2 was also as toxic as nano-BaSO4 (Tran et al., 2000). When based on similar mass, the number of nano-sized particles is 1000-times higher (10 to the power of 3) when compared to a 10-fold large particle of the same composition, which may explain in such a case the higher toxicity of the nanoparticles. In a study by Arts et al. (2000), it was shown that at a similar total particle number, the 10-fold larger carbon particles (ca. 300 nm) were more toxic than the same nano-sized particles (c. 30 nm; i.e. about 1000-times less mass). Consequently, no intrinsic risk is associated with the nanoscale per se (larger-sized fibres an exception) [TNO Occupational Health, NL].

Perhaps there should be a general disclaimer (p 22) that many toxicology studies to date (that were relied on to make conclusions in the report) did not adequately characterize the test materials in their studies, so in many cases the test materials may not have been representative of, or generally applicable to, "nanoparticles" in use by industry that humans and animals are likely to be exposed to [FDA Nanotechnology Panel, USA].

Particles for Drug Delivery (p 29) Ramesh et al 2004 used lipids (DOTAP:chelesterol) for the delivery of DNA plasmid in a mouse study. We do not think that this type of lipid contains the property typical for a nanoparticle product [FDA Nanotechnology Panel, USA].

Most toxicity studies with NPs carried out at unrealistically high mass concentrations in intratracheal instillation studies and the toxicity of aggregated NPs in suspension rather than single NPs have been studied [TNO Occupational Health, NL].

Delivery routes

Special attention in toxicological testing should be given to the mode of delivery (reflecting exposure scenarios). Furthermore, the dosage should be critically evaluated (putative relevance of the high specific surface of NPs; possibly higher ability of certain NPs to cross biological barriers) [Henkel, DE] Mode of delivery in test systems needs to be relevant for the exposure [TNO Occupational Health, NL].

Adverse health effects

Questions naturally arise as to whether the features (p 13) pose any inherent threats to humans and the environment. Bearing in mind that naturally occurring processes, such as volcanoes and fires, in the environment have been generating nanoparticles and other nanostructures for a very long time, it would appear that there is no intrinsic risk associated with the nanoscale per se. The text can be interpreted as: nanoparticles are not more hazardous than larger particles: this cannot be stated with the present level of knowledge [TNO Occupational Health, NL].

Central to the health risk concerns (p 8) is the fact that evolution has determined that the human species has developed mechanisms of protection against environmental agents, either living or dead, this process being determined by the nature of the agents commonly encountered, within which size is an important factor. The exposure to nanoparticles having characteristics not previously encountered may well challenge the normal defence mechanisms associated with, for example, immune and inflammatory systems [TNO Occupational Health, NL].

Hazard considerations (p 44): Examples already exist of harmful nanosized particle interactions with biological molecules through self-assembly which cause a range of disease and syndromes–namely prions. This is an emerging experimental field with a number of contending technologies to inactivate these extremely infectious entities (Ref 7). This perhaps should be taken into account with nanoparticles. Within a chemistry context, self assembly and template patterning with nanoparticles are well documented (Refs 8, 9). Prions and related fragments can be nanosized protein particles which are responsible for interfering with the natural folding of endogenous protein leading to the formation of fibrils and amyloidosis. This giving rise to a number of medical conditions such as the spongiform encephalopathies, Creutzfeldt-Jacob disease, systemic senile amyloidosis etc. (Refs 10,11,12). Also unlike viruses but in common with nanoparticles, prions do not provoke an adaptive immune response (Ref 13). Current state of the art detection methods for prions require specialised assays (Ref 14) or direct implantation of treated and untreated surfaces (eg. wires) into the brains of laboratory animals (Ref 15) [URPL, PL].

Novel mechanism of toxicity

Unclear statements (p 33 and p 44) on impaired macrophage phagocytosis by NPs (Renwick et al., 2000). NPs in general are too small to alveolar macrophages and translocate directly to the pulmonary interstitium (increased potential for bioaccumulation unclear). Larger-sized particles (e.g. silica) may stay for a long time in the lungs and draining lymph nodes, long after cessation of exposure [TNO Occupational Health, NL].

Potential exists for some nanoparticles to be non-immunogenic (p 8) and thus avoid the action of the adaptive immune system altogether as well as posing a challenge to the immune system in some other way. Size is certainly an important factor in the immune response (Ref 4). The minimum mass for a molecule to become immunogenic is of the order of 2000D which would correspond to a size of up to 5nm depending on shape. It is however the nature of regions of the surface chemistry of a molecule/particle, (epitopes) that is the key to immunogenicity and immunorecognition (Ref 18). A nanoparticle would therefore need to possess the appropriate surface groups and arrangement [URPL, PL].

The Report's consideration of hazards states (on page 44) that due to the potential for NPs to penetrate proteins, nucleic acids and other biological molecules that there may be unique adverse effects never previously observed for chemicals in other physical forms which could occur. This may be at variance with what is written on page 51 (3.10.6), where it states that 'it is assumed that the range and type of adverse effects is likely to be similar to that identified for chemicals in other physical forms' [DEFRA,UK].

Figure 7 captures some of the known toxicological effects; are there other possible interactions that nanoparticles may have with biological systems? For example, Park et al (2003) report a K – channel blocking effect that is related to nanoparticle / nanotube size and shape [DEFRA, UK].
Vascular biocompatibility/toxicity of nanomaterials should get more attention in the document [FDA Nanotechnology Panel, USA].

2.2.3.3 Toxicity testing

General considerations

Assessment could be improved by developing new improved instrumentation and/or by developing better strategies by which the information from existing instrumentation could be improved [IOM-SNIRC, UK].

The current instruments and methods enable detailed and structured investigations of short and long term risks of NPs. The biggest shortcoming is the lack of standardised protocols that prevents comparison of results and leads to scattering of research results in NPs. To obtain substantial breakthroughs, the standardisation of these methods is of the utmost importance. The European Union has a clear task to standardise methods after consultation of the scientific community [P. Dibrell, BE]. Standardization of sampling methods and terminology required [Sopra SA, FR]. Strong support for the standardization of methods and reference materials e.g. by ISO, crucial [Henkel, DE]. Furthermore, analytical standards are necessary to allow comparison of current scientific results (reproducible aerosol generation, detection and characterisation of atmospheric anthropogenic nanoscaled particles (e.g. from combustion processes) and of nanoscaled particles in biological tissue) [ICCA, BE]. Due to uncertainty about test article characterization, it is impossible to compare results from different laboratories, or to know if published results are universally applicable to "nanoparticles" [FDA Nanotechnology Panel, USA].

When discussing tests that have proven to be capable of revealing the toxicological effects of a wide variety of materials acting by various mechanisms (many of which are largely unknown), it can be anticipated that significant toxicological effects of NPs will also be demonstrated by these existing test methods. We believe standard tests that are thoughtfully conducted and interpreted will be adequate to elucidate the potential hazards of nanomaterials [CEFIC/BE, CIA/UK].

Although the existing methodologies are appropriate to assess biological effects of nanomaterials, these methods have to be very carefully improved because there is, in most cases, a direct interference of the particles with the measurement system or analytical ingredients/chemicals. These "side-effects" lead to false positive or false negative effects [H.Krug, DE].

There is no reference at all to existing requirements and standards for risk management in the area of medical devices, while these are typically applied before such products are placed on the market. In particular, EN ISO 14971 on risk management and EN ISO 10993-17 on the establishment of allowable limits for leachable substances should be mentioned in this respect. This is an aspect which is virtually lacking from the current opinion: the only place it was found is on p 55 as a "needed development". After introducing this in section 3.10, it should also be taken up in relevant conclusion and summary sections throughout the document [RIVM, NL].

Alternative methods and animal testing

In-vitro methods have to be validated and further developed to examine and quantify the potential of nanomaterials to cross or to translocate through different types of biological membranes [CEFIC/BE, CIA/UK]. A number of methods discussed for the hazard assessment of NPs are not yet validated and their role in the risk assessment process remains to be defined (e.g. in vitro protocols for measurement of oxidative stress /inflammatory markers) [Henkel, DE].

New [in-vitro] tests should be incorporated for the toxicological evaluation of nanoparticles (p 34). These tests may be useful for hazard identification and to identify potential mechanisms of toxicity, but will these in-vitro tests be necessary to evaluate safety if in-vivo toxicity tests do not identify any increased toxicity from nanoparticles (compared to chemically similar bulk/existing materials)? [FDA Nanotechnology Panel, USA].

Hazard Considerations (p 44): In the theoretical event that nanoparticles cause unique adverse events, should the possibility that current in-vivo animal models will identify these adverse events be more clearly stated? In other words, if in-vivo animal test data is available, will that data be sufficient to evaluate the hazard (even in the absence of new in-vitro test methods)? [FDA Nanotechnology Panel, USA].

Also, how will results from any new in-vitro tests be used? If adverse results are seen in in-vitro test results for nanoparticles, but no toxicity or pathology changes are seen in in-vivo tests, how will

safety/risk decisions be affected by the results of the 'new' in-vitro tests? How applicable, in general, will the in-vitro tests be to animals or humans? Can the new tests be validated? How quickly can they be validated? Are the new tests necessary only to better characterize in-vitro toxicity or are they deemed necessary to compensate for deficiencies in how existing in-vivo toxicity tests can characterize risks from nanomaterials? [FDA Nanotechnology Panel, USA]

Vast differences in physiology and biochemistry between animals and humans is considered to exemplify why animal experimentation will not predict nanomaterial safety or nanomedicine efficacy. Therefore nanomedicine should focus on human cells and systems directly relevant to human health effects [PETA, USA].

A great deal of toxicity testing of nanomaterials needs to be done in-vitro human-based systems. Therefore institution of relevant non-animal-based standards for nanotechnology testing are needed [PETA, USA].

Dosing

A crucial difference for the testing schemes may be the dosing aspect. Apart from difficulties in analytics of nanoparticles, the mass based approach for conventional chemicals may interfere with (some classes of) nanoparticles. If nanoparticles would better be characterized on the basis of surface area per volume (rather than mass per volume) current classification and labelling cut-offs (e.g. R53 for LC50 < 1 mg/l) do not hold any longer. The same may, for example, be true for the use of assessment factors (10, 100 or 1000) that are based on classical (rule of thumb) dose-response relationships. Such assessment factors play a crucial role in the effect assessment, i.e. PNEC derivation, but will these classical relationships (e.g. acute-to-chronic ratios of 10) hold for nanoparticles? Simply referring to the TGD in Figure 8 on Hazard identification as the guidance for hazard identification may be too premature. Possibly different dose-response relationships for some categories of nanoparticles, e.g. a distinct threshold value, may implicitly also have consequences for the dosing strategy/steps in the toxicity testing [RIVM, NL].

Reference materials for toxicological studies

Toxicological testing of nanomaterials should always include <u>appropriate reference materials</u>, in order to distinguish between adverse effects produced by the chemical nature of the test substance per se and those that are due to the nano-size of the test material (e.g. nano-sized TiO2 with microfine TiO2 as a standard). The toxicity of bulk materials should be our starting point or even benchmark to so-called new nanomaterials [ECETOC, BE]. Consider how a set of standard reference materials might be arrived at for use in toxicological tests (e.g. for comparative benchmarking purposes) [DEFRA, UK]. It would be very useful to start with some examples of various types of nanoparticles, and consequently elaborate the environmental risk assessment process for these examples. Such examples could be previously assessed chemicals, e.g. zinc oxide, but also potential new ones (e.g. fullerenes) [RIVM, NL].

It will be important to pay attention to the details of experimental dose (inc. parameters like number concentration and/or surface area) and exposure characterization. Challenge in viewing the results of well conducted studies with carefully characterized nanoscaled particles with the goal of understanding the impact of particle property modifications on toxicity. The biggest benefit of carefully characterizing the dosed particles using an array of parameters will come from the subsequent ability to draw inferences about new particle types, or for the same particle type when exposure data predict that the particles will be available for exposure in substantially different form (different state of aggregation, different surface characteristics) [Degusa, DE].

Measurement methods

Routine methods are not really available. Determine relevant concentrations of nanoparticles in the exposure study (might be much lower than usual) [Business, AT]. The development of measurement technologies and parameters to be measured (mass / number / surface area / shape) and in various media are priorities [DEFRA, UK]. Development of metrology of nano-objects is mandatory [Sopra SA, FR].

Vascular biocompatibility/toxicity of nanomaterials should get more attention in the document. New methodologies (i.e. capillary electrophoresis) need to be developed for analysis of different types of nanomaterials in blood and body fluids (i.e., assessment of interactions of nanomaterials with proteins and lipids) [FDA Nanotechnology Panel, USA].

The opinion disagrees with the use of healthy animal models for toxicological testing of nanoparticle products (p 33). The issues such as potential toxicity of the plasmid DNA and transgene expression should also be included in toxicology testing, along with the nanoparticle used for the delivery. Therefore, the determination of the type of animal model to be used for toxicology testing should be made on a product-specific manner [FDA Nanotechnology Panel, USA].

Instruments such as scanning mobility particle sizers (SMPS) and scanning electron microscopes (SEM) that utilize an electron dispersive spectrometer (EDS) make NPs countable and their chemical compositions discernable [PETA, USA].

Only devices and procedures developed by nanotechnologies can yield the needed equipment for suitable exposure measurements, e.g. functionalization of AFM tip by using carbon nanotubes [S. Bellucci, IT]. Detection methods of individual particles in liquids down to around 10 nm are available for the existing air-sampling technolgy [NanoSight Ltd, UK].

2.2.4 Exposure evaluation

2.2.4.1 General approach

General Exposure Considerations (p 34, Figure 4): The text notes that Curve B represents a scenario where a small change in particle size corresponds to a large change in toxicity. This does not seem to be depicted in the figure. Only a small change in toxicity is illustrated in Curve B [FDA Nanotechnology Panel, USA].

The Opinion mainly focuses on "Workers". It is suggested to specifically note the situation of the R&D workers as they are in the frontline of the introduction of new particles [G.W. Visser, NL].

2.2.4.2 Exposure Scenarios

The use of scenarios and decision trees for exposure and hazard assessment is helpful. It is useful to keep the exposure and hazard modules (Figures 6 and 8) separate but also as a unified decision tree. DEFRA would welcome the SCENIHR views on how to answer the important questions posed under scenarios ii) and iii), which will apply where e.g. surface coatings to nanoparticles are applied [DEFRA, UK].

To effectively apply existing testing strategies, data from relevant exposure scenarios have to be collected and validated [CEFIC/BE, CIA/UK]. We support the demand for realistic exposure scenarios, which should be reflected in the toxicological test strategies. Unrealistic exposure routes (e.g. i.t.) need to be validated vs. relevant exposure scenarios (e.g. inhalation exposure) [BASF, DE].

It seems important to assess the risks related, not only to NPs themselves, but also to new processes developed for elaborating and using NPs. This is particularly the case of possible specific hazard (e.g. formation of active molecules, of radicals...) resulting from the interaction of NPs and products with which they are in contact, either during the fabrication process or when embedded for building new materials [Institute of NanoScience of Paris, FR].

2.2.4.3 Exposure monitoring methods

Very important that equipment used nowadays and practically available is not well fit for personal sampling, and cannot make a distinction based on chemical composition [TNO Occupational Health, NL]. Include ADME testing within the second tier of exposure assessment, notably for assessing e.g. in utero exposure (Figure 6). Assess whether this could be adequately covered by existing ADME testing requirements of the EU TGD [DEFRA, UK].

Analytical methods and equipment for exposure assessment need to be improved [ICCA/BE, Degusa/ DE]. Strong support is needed for the development improved analytical tools like personal sampling devices for exposure monitoring (inc. in addition to the classical mass concentration (surface area, size, size distribution, number concentration, morphology) [Henkel, DE]. The activities should concentrate on establishing analytical standards to allow comparison of current scientific results (reproducible aerosol generation, detection and characterisation of atmospheric anthropogenic nanoscaled particles (e.g. from combustion processes) and of nanoscaled particles in biological tissue). Consequently a number of activities have been launched by Degusa and other stakeholders on international level to develop such methodologies. (e.g. Kuhlbusch et al., J. of Occupational and Environmental Hygiene, 1 (660 – 671), Industry "HS&E Nanotechnology Consortium" in the US) [Degusa, DE].

Standard clinical tests (p 40) such as for example 'liver function tests' may be useful after exposure to nanoparticles, particularly after ingestion [URPL, PL].

On the subject of characterisation and quantification of nanoparticles in body tissues and in in-vitro testing (p 50 and 52); An extremely well established, in-vitro detection technology might be considered for measuring nanoparticles in solution namely that using antibodies ie. 'immunoassay', (Ref 4,5). Antibodies against nanosized and bigger particles are widespread eg vs. viruses, macromolecular complexes, bacteria, cells and tissue. It is possible to make most molecules antigenic, (as opposed to immunogenic), through a variety of techniques, even those as small as 300D, (Ref 6) [URPL, PL].

Exposure information exists on 'top down' NPs such as TiO2 and Carbon Black, but little is known about the exposure levels, control measures, and exposure scenarios. Exposure data 'bottom-up' NPs is missing completely [TNO Occupational Health, NL]. Exposure evaluation needs to be closely related to persistence and mobility/immobility of the NPs in the human body and in the environment [Henkel, DE].

The remark (p 47) on the worker safety during the manufacture of nanoparticles is questionnable. ' It is noted that typically workers are exposed to higher levels of chemicals and for more prolonged periods of time compared to the general population and this will probably be the case for nanoparticles production' on the basis of the information presently available [TNO Occupational Health, NL].

2.2.5 Risk Assessment

2.2.5.1 General approach

No general statements on hazards of nanomaterials to humans can be made and risk analysis can only be performed on a case by case basis [CEFIC/BE, Bayer/DE, ECETOC/BE, Henkel/ DE, ICCA/ BE, FDA Nanotechnology Panel, USA].

The existing toxicology and risk assessment methodologies provide a good foundation for a process to follow. As with any new material, a good risk assessment will take into account the unique hazards presented by the material and the routes of exposure. Nano-sized materials may have properties that are unique when compared to the bulk substance and these properties may contribute to or result in hazards that are not presented by the bulk material. These properties may also lead to new applications and potential exposure. Characterization of nano-sized materials will be a key activity and non-routine methods may need to be used. Consideration of these factors may lead to appropriate modifications of the risk assessment process, but existing procedures and guidelines should be the starting point [CHEMSTAR Nanotechnology Panel, USA] The need for new toxicity tests to adequately ensure the safety of nanoparticles is premature on the basis of current scientific information and may become evident only in the future [FDA Nanotechnology Panel, USA].

The complexity of the issues is not really reflected in the short summary of the Committee Opinion and it leaves too many holes to be used or missused for unqualified tests and analyses. The entire issue of appropriate methodologies needs a specific development on how to assess the risk potentially associated with the use of nanotechnologies. Strategic concepts need to be developed which have to be tailor-made to be as effective as possible and yet reduce time and cost in a most intelligent way. Otherwise I fear that staying to the rules may not provide necessary insight, assure sufficient safety and may only represent an "alibi" rather than a comprehensive analysis [W.Kreyling, DE].

The potential risks of the NPSexposure vary according to material, but also according to the life cycle. Even intrinsically non-toxic species, like carbon and silica, may be dangerous in large quantities if powders/nanopowders are inhalated. Therefore it is necessary to consider in the risk assessment 1) Production/manufacturing (reduced/annihilated risks by control of the working conditions and assessing safety procedures), 2) Consumers (devices, nanomaterials embedded in solid matrices; concerns in cosmetics as NPs diluted in liquid matrices then absorbed and possibly accumulated), 3) Medical applications (strictly controlled, in many cases the advantages may overcome the disadvantages) and 4) End of life (Mandatory and precise protocols for factories/discarded NPs, unutilized sideproducts , etc, for consumers/ discard of NPs devices (Terranova, IT). The Committee could consider which criteria would be used for assessing the likelihood of human or environmental exposure over the life cycle of the nanomaterial, from manufacture to waste disposal [DEFRA, UK].

In agreement with the SCENIHR opinion, some enterprises and their associations of the chemical industry consider existing toxicological and ecotoxicological methods appropriate to assess, in principle, the hazards associated with the products and processes involving NPs. It also agrees with the SCENIHR opinion that particular attention needs to be given in applying these methods to the mode of delivery of the NPs to the test system to ensure that it reflects the relevant exposure scenarios. Furthermore it agrees with the approach to include number concentration and/or surface area in exposure measurement, but identifies shortcomings in the analytical methods and instruments that hinder the routine methods. However, they disagree with SCENIHR opinion in regard to the statement that current risk assessment procedures require modification for NPs. According to them, the standard risk assessment procedures seem basically appropriate to assess the potential risks of NPs. This is especially the case if refined hazard and exposure assessment methods are used [CEFIC/BE, Bayer/ DE, Degusa/ DE, ECETOC/BE, Henkel/DE, ICCA/BE, Nanosys/CH, SME/DE].

2.2.5.2 Practical guidance

The algorithms and decision trees on exposure, toxicodynamics and hazard identification of the SCENIHR opinion are highly appreciated as pragmatic tools for risk assessment [DEFRA/UK, RIVM/NL]. However, the report does not provide an assessment of how fit for purpose specific existing methodologies are for nanoparticles, assessing what elements could be kept and what additional elements might be required. For example, the REACH testing regime is mentioned but the testing requirements under this Directive are neither described nor explicitly evaluated as to their appropriateness for nanoparticles. As other examples, the EU Technical Guidance Document (TGD) and Annex I of the 2001/83/EC Directive on medicinal products might both have been described and assessed more fully. Here is a need to specifically identify any deficiencies in current and proposed testing guidelines and guidance documents (extending beyond chemicals legislation) which may not allow for the detection of previously unknown effects due to size, particle number / surface area properties [DEFRA, UK].

The evaluation and prevention of potential hazards related to the use of any given 'nanomedicine' is foreseen under the pharmaceutical legislation before the application of marketing authorisation, toxicology and ecotoxicology for a specific nanomedicine, as well as the methodologies used. As for any medicinal product, nanoparticles will have to be fully characterised, their fate and toxicology will have to be established and the appropriateness of test methods will have to be demonstrated. Risks associated with the translocation of NPs in the body can be addressed using already established principles, although it is likely that new in-vivo bioanalytical methodologies may need to be developed. The pharmacovigilance system and the provisions for a risk management plan for marketed products, allows reporting of adverse events and continued monitoring of safety of patients and the environment. In accordance with Regulation (EC) No 726/2004, the evaluation of applications for the marketing authorisation for a nanomedicine via the Centralised Procedure/ EMEA will be either mandatory if the nanomedicine falls under the Annex of this Regulation (e.g. new active substances for treatment of listed diseases or manufacture using a biotechnological process) or optional, i.e. possible, if the nanomedicine is a new active substance or shows technical innovation. In view of the importance of these provisions for the risk assessment of nanomedicines, their existence should be highlighted in SCENIHR's opinion on nanotechnology [EMEA, UK].

The opinion contains a wide range of useful information, which may be helpful in the development of approaches for the assessment of medicines based on nanotechnology. However, the final section on Risk Assessment Methodologies predominantly covers the assessment of industrial chemicals and other non-medicinal applications. Generally, for human medicines the regulatory requirements for all types of products, including those based on nanotechnology or any other new technologies, are those included in the present Guidelines and Notes for Guidance (e.g. ICH and EMEA). Although these guidance documents don't specifically address possible special properties of nanotechnology based products, they are generally enough to cover them. Assessment of any product has to be done against the background of the latest scientific knowledge specific to the type of product. As soon as sufficient experience has been obtained in assessing specific categories of new types of products, if necessary, the guidance documents may be amended (or new documents may be written), in order to provide more specific guidance [RIVM, NL].

Screening and prioritisation is a useful and pragmatic option for prioritising NPs for further risk characterisation. It would be important to compare Figure 6 with existing prioritisation methodologies (e,g PBT characteristics.) [DEFRA,UK].

2.3 Further development of risk assessment methodologies

2.3.1 Characterisation

Improve the quantification of the single particle versus aggregated distributrion of particles and their dynamics; measurement of the cumulative and retained particle dose and the effective pharmaco kinetics at the target. Determine the surface chemistry of NPs and their impact on toxic response and aggregation/disaggregation behaviour in both aqueous media and biological fluids - noting that the latter may modify the surface chemistry and colloidal behaviour in the biological system of interest [University of Cambridge, UK].

Appropriate methodologies for the routine characterization of the physico-chemical properties of NPs to be developed and made available. Standard toxicity tests have to be modified and validated (for priorities, see 2.4) [Bayer, DE].

Nano-sized materials may have properties that contribute to or result in hazard or exposure which is not presented by the bulk material: It may be necessary to modify existing or develop new characterization methods to gain a more complete understanding of these properties. [CHEMSTAR Nanotechnology Panel, USA]. Many nanoparticles are not present in a nanoparticular status but form aggregates / agglomerates or are included into a matrix. Important to address the influence of coatings on safety-related properties of nanoparticles, which can be either neutral, protective or adverse, in future research on nanoparticles [Henkel, DE].

A lack in methodologies characterizing physico-chemical properties of nanoparticles was identified. Critical factors determining the toxicity of nanoparticles (e.g. shape, surface area, crystal structure, surface chemistry, etc.) must be identified and standardized. No answer provided how existing methodologies could be adapted and can be omitted. Highlight that the translocation of NPs may lead to effects in target organs that are not the primary focus of the hazard assessment of bulk chemicals (e.g. liver, kidney, spleen) thus existing methodologies need to be adapted. [Swiss Federal Office of Public Health, CH]. Use of standards and referenced methods concerning the size of nanoparticles, their shape. Valid tests are needed [Individual, BE].

Appropriate methodologies for the characterisation of physico-chemical properties of nanoparticles are needed. For an assessment of biological effects resulting from the increased surface/volume ratio and the higher surface energy of nanoparticles and nanoscale materials, the following data on nanoparticles and nanoscale substances should be obtained:
• Geometric parameters of primary particles and morphology of primary particles and aggregates and agglomerates, respectively,
• Specific surface (BET),
• Depending on use, in the individual case the catalytic activity.
Equipment and methodologies to be developed for routine exposure assessment of humans and environment. Disagree with the SCENIHR opinion that for optimization of the process of hazard evaluation including the assessment of whether nanoparticles can exacerbate pre-existing medical conditions new testing methods are required. The theoretical potential to exacerbate pre-existing medical conditions is not unique to nanoparticles and is already well taken into account in standard risk assessment processes in the form of an uncertainty factor that covers the inter-individual variability [CEFIC/BE, ICCA/BE].

2.3.2 Hazard evaluation

2.3.2.1 Toxicology of nanomaterials

Figure 6 presents a nice worked-out scheme to determine needs for exposure and kinetics data, which is much appreciated. In contrast, only limited thought seems to have been given to a strategy for the determination of data requirements for a proper hazard identification [RIVM, NL].

Not necessarily true that nano-sized materials have physical properties or hazards that are discontinuous with known properties or hazards. Small size alone does not impart new hazards, and for some biological responses, our current understanding of how size influences the response are already known [CHEMSTAR Nanotechnology Panel, USA].

Research is needed concerning particle size-specific properties (e.g. increased inflammatory properties that may be related to surface area) to distinguish between substance-specific properties and nano-size properties. The exacerbation of preexisting medical conditions is not unique to nano-sized particles and is already considered in the risk assessment by uncertainty factors for inter-individual variability [CHEMSTAR Nanotechnology Panel, USA]. Disagree that for optimization of the process of hazard evaluation including the assessment of whether nanoparticles can exacerbate pre-existing medical conditions new testing methods are required: theoretical potential to exacerbate pre-existing medical conditions is not unique to NPs and is already taken into account in standard risk assessment processes (for example in the form of an uncertainty factor that covers the inter-individual variability) [BASF/DE, Bayer/DE, CEFIC/BE, CIA/UK, ICCA/BE].

Methodologies for determining correlations between nanoparticle size/shape/surface composition/bulk composition and biological effect may not easily lend themselves to a routine testing strategy unless there is also significant mechanistic research to understand the cause of these dependencies. Therefore it is important to recognize the need for both phenomenological and mechanistic research study in this field [Scientific Liaison Advisory Services, UK].

Important to further develop methods for the detection of NPStranslocation in the human (or animal) body. Important to develop routine methods for NPs detection in food (and feed) matrices. Initiatives to develop functional assays most promising [RIKTL- Institute of Food Safety, NL].

Investigations on the interaction of e.g. NPs with materials and the environment to be considered as materials may provide a sink or even a source of nanoparticles [Individual, DE]. There is a need to emphasize the physiological state of the nano particles rather than the simple physics methods of particle sizing etc. There is also a need to emphasize modern technologies from biology and tie them in with modern physical approaches [Individual, UK].

The existing methodologies to assess the risk are based on the medical knowledge, that for instance denies the entrance of particulate matter inside the animal and human body. All the physiological barriers at all the levels (organ, tissues and cell) are inefficient, so other tests at macro, micro and nanolevel are necessary in order to analyze the real interaction [Academia, IT].

It may be too easy to fully rely on current knowledgee on dissolution protocols etc. for metal and metal oxide nanoparticles, as this refers to the free metal ion toxicity, whereas nanoparticle metals may also exert other mechanisms of toxicity. On the other hand recent advances in metal classification and labelling, e.g. the discrimination between dissolved and larger zinc particles as zinc massive and zinc powder, may be very useful for the line of reasoning for nanoparticles [RIVM, NL].

With regard to the risk assessment of nanotopographical features: on p 10, 5th par., it is stated that very little is known of the potential to induce adverse effects, however no new risks are assumed;

Although we agree with this, it would be quite helpful if you are aware of references describing this. As you know, implant technology is currently applying such topographical features [RIVM, NL].

2.3.2.2 Toxicity testing

Alternative methods and animal testing

We appreciate that in-vitro studies have been mentioned for screening purposes, but recognise that for complex tissue reactions, like inflammatory responses, these may not be completely appropriate / suitable, because these may need more than just a few cell types in a petri dish to be described in a proper way. Also, the possibilities for extrapolation from in-vitro studies to in-vivo studies remains cumbersome. At least concentrations of the test material and interactions with media components should be properly monitored in order to avoid erroneous interpretation of the quantitative aspects of such studies (the added concentration of nanoparticles may be quite different from the concentration that is seen by the cells; a problem that is also common to the already ongoing in-vitro tests with conventional chemicals) [RIVM, NL].

As indicated in the Opinion, knowledge, or absence of knowledge of the toxicological hazards associated with the nanoparticle-bulk chemical component could influence the choice of data required, but these should not be looked at separate from the physico-chemical properties of the particles as such (e.g. e.g. rate of dissolution). Overall, we feel that given the relatively new character of this new type of exposure to chemicals, it should be recognised that a certain amount of in-vivo testing cannot be avoided. In contrast to conventional chemicals comparatively little data is available to allow for cross-reading. We anticipate that this may change in future, but currently, nanotechnology seems to comprise too many uncertainties to allow for reliable health risk assessments in all cases without in-vivo testing. In this aspect we think that the scheme in figure 8 and the text on pages 51-53 is overly optimistic [RIVM, NL].

Number of in-vitro assays and human cell cultures exist and can be used to assay the effects of nanomaterials on a particular human cell type. For example, studies such as Cavalcanti et al. 2005, show the effects of each of their nanoscale matrices on the strength and speed that osteoblasts are able to organize and form new bone. In-vitro cell culture of osteoblasts was utilized in this study to test the effectiveness of the novel nanomaterials. Future clinical applications for in-vitro osteoblast organization and bone formation are foreseeable and, by utilizing human cells, this study has already proven to be human-relevant. In another pioneering study, Walsh et al. 2005, show that it is possible to direct neurite growth after trauma to the central nervous system. These studies were performed with a human cell culture system on a matrix that allowed nanoparticles to help direct the re-growth of cultured neuritis via topographic cues. This study has clear applications for people with damaged central nervous systems, and is attempting to find ways to re-direct neurite movement after trauma so that paralysis can be prevented. The field of drug discovery may be streamlined by the application of a novel method that utilizes nanotechnology to test for DNA binding proteins. Bai et al. 2005 show that a double-stranded DNA microarray is able to detect proteins that specifically bind double stranded DNA and may be a high throughput screen for drugs designed to target DNA binding proteins. Thote and Gupta, 2005, report that a system they developed using human- relevant in-vitro techniques allows the delivery of fine nanoparticles of hydrophilic sustained release drugs. They designed an in-vitro model system that mimics the pH, temperature and hydrodynamic environment of the human body in order to test the release rate of the drug. These fine details simply cannot be tested on animals [PETA, USA].

A systematic approach for the characterization of NPs as well as refinement and validation, especially for a number of in vitro methods, seem useful. A set of parameters describing NPs should be defined, including geometric/morphological properties of the particles, the specific surface, the size and size distribution and the chemical composition. Validation and further development preferably of in vitro

methods to examine and quantitate the potential of nanomaterials to cross/translocate through different types of biological membranes especially important [Henkel, DE].

Analytical methods

When considering methods to determine nanomaterials concentration, it is worth noting that other technologies used for dust measurement, particle counting, microbiological studies, etc may already have found solutions that could be applied to nanomaterials [CIA, UK].

Nanotechnologies and nanosensors are necessary to assess the nanosafety [Individual, IT]. Methodologies and terminology must be standardized, new evaluation and metrology equipments are required [Spoca SA, FR]. Given the very broad range of nanotechnologies, standardized methods are illusory [W.Passchier, NL].

2.3.3 Exposure evaluation

Available methodologies for risk assessment in principle applicable to nanoparticles. Relevant exposure scenarios have to be identified. Suitable routine measurement methodologies and models have to be modified or developed to characterize the exposure. Data from toxicological and ecotoxicological endpoints can and have to be extrapolated from non-nanosized materials to NP, whereas specific end-points may require modified or new tests. Further research areas: specific effects have their nature in the nano-size to distinguish between substance intrinsic properties and properties due to the particle size [BASF, DE].

The chemical industry agrees with the approach to include number concentration and/or surface area in exposure measurement. However, analytical methods and instruments to measure exposure with free NPs are still in a developing phase and are not available as routine methods. With regard to our efforts to develop such methods see question 3 [CEFIC/BE, Bayer/DE, ECETOC/BE, Henkel/DE, ICCA/BE, Nanosys/CH, SME/DE]

In my epidemiologically based opinion an adequate cohort study for workers (production, storage, transportation, application of nanoparticles) should be initiated and started as soon as possible. This study should be supported by a European regulation/recommendation for an occupation related documentation of possible exposures (characterisation of the nanoparticles, exposure/production/application start and duration, type of possible contact) as a proxy for the workers exposure matrix. Since the current state of knowledge (measurement of the occupational and environmental burden) is insufficient, the risks (and the duty to give a "proof" for causation in case of possible damage) are distributed one-sided. An early start of a European workers cohort study would give an opportunity to control for possible adverse effects in this "natural experiment" at least for workers - despite the fact that possible developmental effects (for the population) are not addressed in the review [Individual, DE].

2.3.4 Risk assessment

Strategic tailor-made concepts needed e.g. toxicological implications (translocation and distribution of nanomaterials to secondary target organs) to be tested interactively with the nanotechnological development and application. Pro-active risk management rather than sequential risk assessment [W. Kreyling, DE].

Several questions are not easily answered or the answer has to be elaborated carefully in Figure 6. For instance solubility: a) Physiological activity may depend on the dissolution rate and the time necessary to achieve "critical "concentrations, b) Particles are soluble: the concentration in

equilibrium with remaining particles depends on the particle size and is usually higher for smaller particles, c) "Stable" complexes may depend on env. conditions (like pH, aqueus media). Even stable particle still have a high surface area and this area may adsorb other molecules etc.[Fraunhofer institute IKTS, DE].

To improve the situation: 1. Properties relevant for the interaction of NPs with the living world and which are hazardous have to be defined in a step-by-step process. 2. Hazard to be measured at statistical relevance. Long- and short-term effects have to be taken into consideration. Appropriate methods, data bases and expert systems have to be developed. Tests should avoid animal testing where ever possible. 3. Measurement methods have to be developed that are able to measure the relevant properties in a laboratory and, if necessary, on-line [Fraunhofer IKTS, DE].

Harmonized risk management standard (EN ISO 14971:2000) and "horizontal" standards such as the EN ISO 10993 series (on toxicology and biological safety) have been judged to be effective and robust in addressing risks associated with current medical technologies [Eucomed, BE].

2.4 Information gaps

2.4.1 General considerations

International coordination

There is a need for a cross-EU organization serving as a centre for access with issues related to nanotoxicity [Individual, UK].

GIobal, anonymised database needs to be developed that contains the SHE information of well characterized particles: guidelines about factors influencing the NP safety are needed [G.W. Visser, NL].

Solving of technical questions is not enough for risk acceptance decisions [W.F.Passchier, NL].

Better use of standards and norms in order to have common references especially in Europe.
Apply Art 163 1st paragraph for using and disseminating better the results of community research in future FP7 using "Common standards and EU norms" (CEN WG 166 working on this item, the future ISO Nanotechnology Committee) [Individual, BE].

2.4.2 Characterisation

Establish whether a limit for nanoscale (p 54, p 61) in relation to (toxicological) risk, possibly depending on the type of nanoparticle/chemical, exists other than the rather arbitrary current "limit" of 100 nm [RIVM, NL].

It should be defined up from which size / up from which strength of binding forces they are not considered to be NPs any longer (e.g. through ISO norms) [Henkel, DE]. Take account of: the morphology/aspect ratio/aggregation state of NPs [Univ. of Cambridge, UK]. More data about the aggregation and agglomeration properties, especially NPs adhesion to each other and to other surfaces [BASF/ DE, Henkel/ DE, CEFIC/ BE].

Better understanding needed of the distribution of structural states and their interaction with biological systems [Univ. of Cambridge, UK]. Movement and attachment are critical for self-interacting NPs and their clusters and the resulting dose response curve [Univ.of Cambridge, UK].

Use of CAS number for NPs does not facilitate improvement of assessment procedures. OSOR approach should be followed [G.W. Visser, NL].

The question of precisely defining nanosized particles or entities and those which form the basis of reactions/interactions in biological systems remains to be clarified. Are they simply man-made entities or can they also be copies or originals of natural molecular structures? Where lies the border between chemistry and biological processes or indeed artificial or natural molecules? [URPL, PL].

The mechanisms of many biological interactions at the nanoscale are mostly very well documented. These are driven by the same physico-chemical forces which equally apply to nanoparticles, be they natural or artificial, an example of the former being the antibody-antigen binding interaction which is purely due to quantum effects. Surely the more complex a molecule then the greater is the potential for interaction with other complex molecules, many of which happen to be associated with living processes, but can for the most part be synthesised artificially if needed. Enzymes provide a good example of an elegant 'molecular motors' (Ref 7,8) as described by nanotechnology where single atoms or small groups are manipulated with a high degree of precision and efficiency to produce a chemical reaction, (ie. molecular re-arrangement), be it a synthesis (eg. an assembly), degradation or tranformation of substrate molecules ranging from the simplest to the very complex. Enzymes in common with simpler polypeptides can be synthesised artificially by chemical or recombinant means. There is therefore a great overlap in function as ascribed to nanoparticles and the biological molecules of life, be they natural or synthetic. Stricter definitions would seem to be in order. After all enzymes have remarkable properties as specialised catalysts and indeed fullfill the 'unexpected qualities' one could reasonably expect from nanoparticles, especially ones with a complex chemistry [URPL, PL].

Differences become apparent when the actual concept of nanotechnology in terms of self replicating systems is set against what practically works in the real world –namely biology. The former is envisaged as being essentially error free but purely theoretical, whereas in practice the latter works very well for the purposes of living biological systems, with a very high precision which is however insufficient for the predicted requirements of nanotechnology and engineering, (Ref 9) [URPL, PL].

The diversity of biological molecules is concerned with solving the problems of living systems for a given environment. However, nanoparticles, nanotechnology and self replicating nanosystems are intended for different aims ie. to produce perfectly tailored structures to perform various specific functions (Ref 10). Currently, nanoparticles can be rather poor fare for the envisaged nanotechnology as glaringly demonstrated by the wide polydispersity frequently observed. The production of biomolecules may be better but all living processes have an inherent capacity for error, mutation etc. , which is their strength for the perpetuation of life forms in order to meet the challenges thrust upon them by a changing environment. Nanotechnology is precision engineering resulting, it is hoped, in perfect replicating systems whose strength is reproducibility but not survival under potentially changing circumstances.

Intermolecular and surface forces embrace all forms of matter, (ie. the quantum effects as well as chemical reactions). In biological systems these are neither simple liquids nor solids but rather a myriad of dissolved solute molecules, small molecular aggregates, or nanosized-macrosized particles etc. interacting in liquid or vapour phases. It is the forces in such systems that ultimately determine the behaviour and property of everyday things; soils, milk & cheese, paints & ink, adhesives & lubricants, many technological processes, detergents & biocides, micelles, nanoparticles, biological molecules & membranes and biological organisms which can be considered in part as being a colloidal system composed of about 75% water [URPL, PL].

At the nanosize level all particles possess an inherent tendency to aggregate which increases with decreasing size due to a significant surface excess free energy, (or interfacial/surface tension), irrespective of the nature and contribution of the surface and environment to the existing forces of attraction and repulsion, (Ref 1). It is unclear to us from the text as to what precise particle behaviour

is being referred to in p 10 Smaller particles/given volume will have a much greater concentration of surface groups than aggregates, therefore the interactive behaviour can be expected to be different – certainly in the kinetics of any potential interactions with other species. Would the aggregate be itself colloidally stable? If so, it is rate of motion would be slower than for small particles, although this would be compensated by the shorter diffusion distances travelled. As well as particle solubility, colloidal stability over time in aqueous liquids is vital, being variously determined by the thermodynamics & intermolecular/particle distances, (Van Der Vaals - London forces), the surface charge, steric effects of entropic repulsion, the osmotic & hydrophobic effects and not least the environment (Refs 2,3), where for example ionic strength/species, surfactants, pH, polyelectrolytes etc. all can play a crucial role (Ref 4) [URPL, PL].

Page 7 Scientific rationale, paragraph 2, line 6 and Page 11, paragraph 2, line 5 together with others. The term 'quantum effects' or 'quantum mechanical principles' and other similar phrases referred to throughout the document we take to mean as all interactions between particles/macromolecules that are non-chemical in nature (Ref 5). Is this correct ? A classic biological example at the nanoscale level would be the antibody-antigen interaction (Ref 6), where the phenomenon of complementarity could equally be applied to a particular nanoparticle interaction with living tissue [URPL, PL].

Page 13, paragraph 4, lines 1-10 (& Page 20). We consider that the following should also be included in surface chemistry considerations, especially for particles in the liquid phase. For example the density of adsorbed or chemically bound polymers, (especially the branched types), to the surface will result in entropic repulsion, (Ref 1), due to a restriction in the number of configurations that can be adopted hence a reduction in entropy, thus an increase in free energy, (steric effect), as well as the tendency for the liquid medium to diffuse into the region between surfaces to reduce the segment concentration and so drive the surfaces apart, (osmotic effect) – (Ref 1). As well as surfactants, the presence of ampholytic surface groups, size and interfacial tension, issues of solvation, the hydrophobic effect, polyelectrolytes, the ionic species present and type vis a vis the diffuse layer and zeta potential, pH, temperature, concentration (ie. interparticle distances within a given volume as well as activity), are all important in controlling interactions of particles/solutes in the liquid phase, (Ref 2) [URPL, PL].

Page 16, paragraph 3, lines 12-14; The sentence 'nanoparticles show increased diffusivity with decreasing size therefore delayed sedimentation' is a little unclear. For a given volume a decrease in size would hasten Brownian motion, however, this would increase the distance particles must diffuse to contact one another. As sedimentation would be the result of the number of collisions/interactions between particles the net effect would be unchanged, (Ref 2 –Smoluchowski-Einstein-Stokes law) [URPL, PL].

Page 21, paragraph 3, lines 7-12. Relatively stable suspensions of particles/nanoparticles in solution, (ie. colloids) as well as those solubilised would also have the potential for interaction with biological ensembles/macromolecules, through well established non-chemical interactions , (ie. quantum effects) . In fact the degradability of colloids may be substantially different to solubilised particles –due to the tendency of the human body to excrete harmful/waste product molecules of metabolism by transformation into very polar molecules, therefore less soluble nanoparticles may be expected to be more difficult to clear [URPL, PL].

Page 34, paragraph 1, lines 6-10 & page 44 bullet point 2. It may also be worth mentioning that an increase in surface area to volume ratio will result in a much greater concentration of surface groups for a given nanoparticle population. For any potential interaction with another molecular species this would result in faster kinetics of reaction/interaction, (law of mass action). If given nanoparticles are inherently toxic, a relatively faster response might therefore be expected [URPL, PL].

2.4.3 Hazard evaluation

2.4.3.1 Toxicology of nanomaterials

Greater understanding of the toxicology of NPs needed to inform endpoint selection and that it will take some time for such fundamental work to be done [DEFRA. UK]. Priority list of actions needed: Not all the listed points are needed with the same degree of urgency. Our aim is to develop agreed methods for the measurement and toxicological assessment of NPs. It is also necessary to learn more about the aggregation and agglomeration properties of very small particles [Bayer, DE]. Agrees that safety evaluation of NPs cannot rely solely on the toxicological profile of the equivalent substance (p 59) [ECETOC, BE].

There are data gaps regarding processes like distribution and metabolism. However, in our opinion the overall conclusion should be that it is of great importance that on all kinetic processes, thus absorption, distribution, metabolism and excretion (ADME) information should be gathered. On the basis of this information insight will also be gained on accumulation, interspecies differences and intraspecies differences [RIVM, NL]. Mechanisms of the nanoparticle dissemination necessary both in the air and inside the human and animal body for understanding of their further interaction with other nano-components of the biological environment [Individual, IT]. The potential of developing and using Structure Activity Relationships (SARs) should be investigated for nanoparticles. In addition, research on the modes of action from various types of nanoparticles for environmental organisms may help in further understanding its potential harms [RIVM, NL]. The authors state (p 48) that nanoscale features of larger objects are not considered to pose an additional risk: some clarification on aggregates/ agglomerates would be helpful [ECETOC, BE].

We disagree that toxicokinetic data are mandatory for risk assessment. It will be important to support certain risk assessment assumptions [Degusa/DE, ECETOC/ BE]. Toxicokinetic data will be important to support certain risk assessment assumptions. For example, if the range of potential target organs examined in a toxicological study are truncated based on toxicokinetic considerations then it will be important to know where the particles distribute and if inferences concerning dose/exposure duration/effect are made for the purposes of extrapolating from short term exposures to lifetime exposures, then data on deposition and clearance will be important etc. While an understanding of the subcellular distribution and mechanism of toxicity may be of investigational interest, or may help to explain species differences, this information is not necessarily required to fully characterize the hazard of the materials. In fact, this situation is not different than that for many other xenobiotics for which the mechanism of action is not known but still a full hazard and risk assessment can be conducted [Degusa, DE].

Similar remarks could be made on the environmental exposure estimation, incl. bioaccumulation. For example, the well-accepted predictive power of the octanol-water coefficient (Kow) for both the bioaccumulation of chemicals in biota and the distribution over the various environmental compartments, may be of low significance for (inorganic) nanoparticles. Furthermore, many nanoparticles may turn out to be persistent and then the bioavailability aspect becomes very important. The estimation of the fate and behaviour, including bioavailability, are probably the most important challenges for the environmental risk assessment of nanoparticles [RIVM, NL].

Knowledge gaps directly relevant to food and food contact use of nanotechnology and the need to consider ingestion as well as inhalation of nanoparticles from these sources. Assessments needed on kinetics of free NPs dissolving and not dissolving in ingestion [VJ Morris, UK].

Kinetics of dissolution: ingestion of free NPs from food, cosmetics or pharmaceuticals may allow their absorption into cells in a particulate form which might affect both their metabolism and the ultimate response to these materials [VJ Morris, UK]. Throughout the text (p21, 45, 46, 51, 52, 56, and 61) the term "toxicokinetic" is applied. Unfortunately this term is defined in various ways

throughout different fields of expertise. In some areas 'toxico' refers to 'kinetics of (potentially) toxic substances' whereas in e.g. areas of drug research 'toxico' refers to kinetics for high doses. We therefore recommend to either describe the term 'toxicokinetic' on a case by case basis in the text, or to give a clear definition of toxicokinetics [RIVM, NL].

Disagrees that every endpoint mentioned is necessary to perform a risk assessment for NPs [BASF DE, ICCA BE]. Toxicokinetic data (e.g. distribution, deposition and clearance) will be important to support certain risk assessment assumptions. Understanding of the subcellular distribution and mechanism of toxicity may be of investigational interest, But is not necessarily required to fully characterize the hazard of the materials [ICCA, BE]. A special focus should be, to which extent extrapolation from the toxicology of non-nanosized particles to NPs is possible. Basic toxicology and environmental research is to be promoted with the primary focus on inhalation toxicology. Better knowledge on characterization of the hazard profile and certain exposure scenarios: risk assessment should be reviewed whenever new information becomes available [BASF, DE].

2.4.3.2 Toxicity testing

Alternative methods to animal testing

Repercussions of chemical metabolites or how well a substance is targeted to a particular organ, a microfluidic device (HuREL) can answer questions regarding how nanomaterials will interact with human tissues. Put modern, high-tech in-vitro assays at the top of the list as you set standards : relevant alternatives to animal-based tests are being utilized [PETA, USA]. Test battery of in-vitro and chemical tests (p 52): no appropriate tests are currently available [ECETOC, BE]

Strongly disagrees with "in-vitro tests could in principle play an important role in this screening process"(p 52): in-vitro tests are useful for addressing questions of mechanisms but have not been validated as toxicity or hazard screening tools (Ref: Seagrave et al. 2005). In-vitro relative toxicity screening of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. J. Toxicol. Environ. Health A. 27; 66 (12): 1113-32. Seagrave et al. 2002. Mutagenicity and in-vivo toxicity of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. Toxicol. Sci. 70 (2): 212-26.) [ECETOC, BE].

Partially agrees with the statement (p 53, 2nd para) – in-vitro studies cannot rank nanoparticles with respect to hazard potential – but only ability to generate ROS species. We particularly agree with the statement– "However, characterization of the uptake …..." [ECETOC, BE].

Analytical methods

Much effort needed to make the measuring methods comparable: prequisite & fundamental for any further analysis of exposure data [Individual, DE]. Portable sensors and filters for the nanopollution are necessary, especially in places where high temperature combustion processes occur [Univ.of Milano, A. Gatti, IT]: The EC project "NANOPATHOLOGY" (QOL-2002-147) has already set up a system to identify nanoparticles in the environmental pollution, in the food, and in the pathological human and animal tissues. Some mechanisms of interaction with the human body have already been identified and verified.

2.4.4 Exposure evaluation

2.4.4.1 Environmental exposure

Long-term effects on soils/water/plants/fish might be of equal relevance or more important than air concentration in the workplace. Not many results are published on long-term effects of nanoparticles

on bones/genomes /hyper-reactions etc. and need to be studied. Predictions methods from short-term measurements have to be developed [Fraunhofer IKTS, DE].

Long-term fate of the environment and the potential for bioaccumulation to be investigated. Public perception of hazard and risk in this area, role of education [Univ. Cambridge, UK]. What is meant by the statement "Free nanoparticle may occur naturally" (p 58), does this refer to ambient, combustion-derived ultrafine particles? [ECETOC, BE]. Investigate the "background burden" of NPs from natural and technical processes [Henkel, DE].

Other than technological sources to be considered: soot from combustion, automobiles; cigarette smoke; military actions as they may be more hazardous ones [Terranova, IT].

2.4.4.2 Human exposure

Strive for an international agreement about assessment procedures, after that the level of exposure needs to be inventorized [G.W. Visser, NL]. Exposure to engineered and natural NPs to be assessed with adequate analytical methodologies [BASF, DE] Due to analytical limitations, it is impractical to assess the exposure of different particle sizes (p 49, Fig 6) [ECETOC, BE].

To assess the exposure of engineered NPs, more detailed studies on inhalation exposure and NPs incorporation into body cells [CEFIC, BE] .

Determine the differential susceptibility of individuals (eg via genomics or proteomics profiling) and tissues to any potential bioeffects. Evaluate and, in future, regulate, the actual physical form of nanomaterial-containing products, to ensure realistic risk assessments not over or under-estimating the actual risk [Scientific Liaison Advisory Services, UK].

Most synthetic NPs are in use for decades. There is no release of NPs by a very wide range of processes, products or applications [Bayer, DE]. In our view anthropogenic nanoscaled particles stemming from combustion processes or natural sources are the major source of public exposure. Thus we would emphasise the need for a proper source, application and exposure analysis of nanoscaled particles to set priorities for toxicological and environmental research and risk assessment programmes in order to avoid rushing for regulatory activities [Degusa, DE]. Disagrees that a perceived high exposure of the public from a wide range of production processes, formulations and uses really exists. Besides large volume products (e.g. SiO2, TiO2, carbon black) only a small number of more recent materials (e. g. nanotubes, fullerenes) have come into play. These are produced in very low volumes, are embedded in matrices or are normally in the R&D status and thus would not represent a major risk to the public. We support the idea to investigate the "background burden" of these materials to assess the potential additional burden generated by engineered nanoparticles [ECETOC, BE].

In the cases where exposure to free NPs may occur specific investigations have been or are being conducted [Bayer, DE]. The evaluation of human external and, possibly, systemic exposure should be closely related to the question of persistence and mobility/ immobility of NPs [ECETOC, BE].

As already stated by Holsapple et al. (Holsapple MP et al. Research strategies for safety evaluations of nanomaterials, Part II: toxicological and safety evaluation of nanomaterials, current challenges and data needs. Toxicological Sciences 88(1), 12-17 (2005)), we think that the behaviour of nanoparticles in the human body or the environment should especially be studied at exposure levels relevant to human and environmental exposures [RIVM, NL].

We also disagree that a perceived high exposure of the public from a wide range of production processes, formulations and uses is correct. This may be a total overestimation of the actual situation.

Some large volume products (e.g. Silicas, Titanias, Carbon Black) which are in the market for many years, only a small number of more recent materials (e. g. nanotubes, fullerenes) have come into play and these are produced in very low volumes, are embedded in matrices, or are normally R&D materials and thus would not represent a major exposure to the public [Degusa/ DE, ICCA/BE]. There is a need for a proper source, application and exposure analysis of nanoscaled particles to set priorities for toxicological and environmental research and risk assessment programmes in order to avoid rushing into regulatory activities [ICCA, BE].

How does one perform a risk assessment (p 58) if one cannot accurately measure nanoparticle exposures: whether by mass, surface area or particle number? P. 59 complement with assessments of particle number and surface area. Portable-type technology is not available to accurately measure nanoparticle exposures in the workplace? [ECETOC, BE].

It can be noted that available information on exposure levels (p 56) generally differs between medicines and other chemicals. Extensive exposure/kinetic information is available for new medicines, and areas in most need of attention in regulatory guidance may differ from those compiled in the SCENIHR opinion [RIVM, NL].

Methods to measure particle number and size already exist, albeit not routinely. Measurements in the workplace can be done. Development of improved routine analytical tools in order to determine occupational exposure in a practical manner already under development [ECETOC, BE].

Determine the effect of exposure to nanoparticles (p 56 and 60) via exposure routes (ingestion, dermal, injection, implantation) relevant to medical applications and subsequent distribution mechanisms including blood brain barrier and transplacental passage [RIVM, NL].

No mention of workers exposed in the carbon black industry and welders exposed to welding fume (3.5.4). There is evidence that zinc and vanadium are associated with toxic effects (3.8.1). There are models which have developed an idea of where nanoparticles might deposit on inhalation, there are no data on understanding how these particles deposit in real life (3.8.2) [RIVM, NL].

The Committee rightly points out that in many exposures to ultrafine particles, there is also exposure to particles greater than 100 nanometres. However, where there have been exposures of this type, the results have been conflicting (3.10.8) [RIVM, NL].

2.4.5 Risk assessment

General approach

The Committee has clearly recognised the critical gaps in knowledge as well as the prioritisation needs on .56 [J. Ayres, UK]. All items listed are very important. Add the strategic approach and pro-active risk management as a conceptual base to approach the potential risks of nanotechnologies [W.Kreyling, DE]. ECETOC agrees with the section on Critical gaps in knowledge required for risk assessment priorities (p 54) and that a number of aforementioned knowledge gaps should be filled to improve our information base and ability to conduct proper risk assessments [ECETOC, BE]. CEFIC disagrees that "… there is insufficient knowledge and data … to allow for satisfactory risk assessments …" [CEFIC, BE]. Support the general conclusions, the omission of sound data on levels and occurrence of NPs in environmental bodies and food commodities is the first prerequisite followed by information of the behaviour (ADME) and toxicology in man and environment [RIKILT-Institute of Food Safety, NL].

We agree that a number of knowledge gaps such as the actual range of exposure to man and the environment, toxicological extrapolation from non-nanoscaled to nanoscaled materials and

information on effects, fate and distribution of nanoparticles in the environment should be filled to improve our information base and ability to conduct proper risk assessments. [Degusa, DE]. Therefore a vast number of research activities have been started by governments, academia and industry which address most of the information gaps. A comprehensive list of research activities can be made available by DECHEMA [Degusa/ DE, ECETOC/BE, ICCA/BE].

What is meant by "precautionary approach"(p 54) – this is too loose a term [ECETOC, BE]. What is meant (p 52) by "Similarly, classification and labelling for human health and the environment may need to be reconsidered? [ECETOC, BE].

How will a specific risk assessment (p 55) be conducted in the absence of exposure data? [ECETOC, BE].

For non-pharmaceutical medical technologies incorporating nanotechnology, the same regulatory approach and use of voluntary harmonized standards will remain generally appropriate. Relevant standards need to be reviewed and revised for the new types of risks arising from the application of nanotechnology. Review and revision of existing well-accepted tools and methodologies (this could be accomplished within an acceptable timeframe, probably under mandate from the European Commission to carry out such review and revision insofar as it relates to medical devices. A new Regulation on Avanced Medical Therapies: nanotechnological risks in human tissue-engineered products can be addressed adequately by the risk assessment and supporting technical requirements. Important not to apply "prescriptive" controls as the range of potential products is extremely high and diverse, and also so as not to inhibit innovation [EUCOMED, BE].

A satisfactory risk assessment can be achieved by incorporating the precautionary principle. Priority areas for toxicology: exposure route relevant to the use of the final product, e.g. inhalation, dermal contact, ingestion [CIA, UK].

Due to knowledge gaps, the specific gaps do not categorically obviate that a sound risk assessment can be performed. Where information is missing, reasonable assumptions may be made and decisions based on these assumptions e.g. mechanisms of release. With new information, existing risk assessments may need to be refined [CHEMSTAR Nanotechnology Panel, USA].

Existing epidemiological data should be handled with care because of too many confounders and not appropriate studied collectives [CEFIC/BE, Henkel/DE]. Current epidemiological studies focus on non-intentionally produced ultra fine particles. It is not legitimate to draw conclusions for intentionally produced nanoparticles. Production and handling of nanomaterials within the chemical industry is performed in closed systems and nanoparticles in final products are firmly embedded [CEFIC, BE].

2.5 Responses from the SCENIHR

2.5.1 Scope of the opinion

The aim of the SCENIHR opinion is, in accordance with its mandate and specialisation area, to provide a common approach to the risk assessment of nanotechnology products, including medicinal products, cosmetics, etc. The SCENIHR recognises that shortcomings in scientific data on the nanotechnology applications in the food sector (both ingredients as well as packaging materials) have impeded their evaluation in the current opinion. However, the SCENIHR considers this field important for future work. As regards the industrial safety, the SCENIHR opinion mentions potential threat for explosion (on p 17 and 43). However, as abnormal events such as an explosion, spillage or equipment malfunction were not included in the questions asked of SCENIHR, they were not considered in the opinion. The SCENIHR recognises the importance of risk-benefit analysis, but it was not included in the current mandate.

2.5.2 Characterisation

The SCENIHR opinion recognises the need for a case-by-case approach in the characterisation of the engineered NPs. However, prioritisation of size, surface and shape together with the traditional mass measurement strives for speeded developments in these key aspects that will be necessary for the development of the routine exposure monitoring methods. The SCENIHR recognises the importance to further emphasize the characterisation of the aggregates and agglomerates that may maintain the novel properties of NPs even in an aggregated form.

2.5.3 Hazard evaluation

The SCENIHR opinion highlights that the paradigm for nanotoxicology does not exist and there is a need for a case-by-case assessment of environmental and health impacts of nanotechnology products.

The SCENIHR opinion emphasises the importance of standardized methodologies in the risk assessment of nanotechnology products and the need for further development of instrumentation for the routine measurements, especially in the monitoring of occupational and environmental exposures. The SCENIHR also acknowledges the relevancy of the specific methodologies mentioned by stakeholders, but considers them too specific to be addressed in the current opinion.

The SCENIHR opinion underlines the need for appropriate testing methods for the novel properties of nanotechnology products. This includes both alternative and animal testing due to their complementary nature in the assessment of mechanisms, toxico-kinetics and relevant endpoints of nanotechnology products. The European Centre for Validated Alternative Methods (ECVAM) in the Joint Research Centre in Ispra, Italy, which is responsible for the validation of alternative methods, including those for the testing of nanotechnology products, has been established. However, SCENIHR also wants to call attention to the fact that

alternative methods can only be used in risk assessment when the assays have been validated to provide information of equal quality to conventional assays.

The SCENIHR opinion considers it helpful to bench mark studies, including reference materials with very well understood toxicology in man, such as quartz and asbestos.

2.5.4 Exposure evaluation

The SCENIHR has modified the opinion in light of the proposals on exposure evaluation. As regards the nanoparticles, the SCENIHR considers that risks are mainly associated with free nanoparticles, and fixed nanoparticles are of less (or no immediate) concern. However, during use and at the end of the product lifetime, fixed particles may be released. So, this should be considered in the risk assessment of the product.

2.5.5 Risk Assessment

The SCENIHR opinion explicitly states (p. 60) that 'Although the existing toxicological and ecotoxicological methods are appropriate to assess many of the hazards associated with the products and processes involving NPs, they may not be sufficient to address all the hazards. Specifically, particular attention needs to be given to the mode of delivery of the NPSto the test system to ensure that it reflects the relevant exposure scenarios. The assays may need to be supplemented by additional tests, or replaced by modified tests, as it cannot be assumed that current scientific knowledge has elucidated all the potential adverse effects of NPs. For exposure, the use of mass concentration data alone for the expression of dose is insufficient, and the number concentration and/or surface area need to be included. Equipment that enables routine measurements in various media for representative exposure to free NPs is not yet available. The existing methods used for environmental exposure assessment are not necessarily appropriate for determining the distribution, partitioning and persistence of NPs in the various environmental compartments. Given the above uncertainties, the current risk assessment procedures require modification for NPs.'

The SCENIHR opinion provides a comprehensive insight in the health and safety issues of nanotechnology products at a general level and recommends a life cycle approach to the risk assessment of NPs. After the 'framework' scientific opinion of the SCENIHR, the Commission may consider it relevant to ask the SCENIHR and other Scientific Committees for special assessment of Guidelines (ICH), Notes of Guidance (EMEA, SCCP) and Technical Guidance Documents (ECB) in regard to nanomaterials.

2.5.6 Further development of risk assessment methodologies

The SCENIHR acknowledges that the existing methodologies also provide the basis for the hazard evaluation of nanoparticles. However, it is also recognised that very presumably modified and/or additional testing methods will be necessary for the evaluation of the potential hazards of NPs to the environment and human health. As no toxicological paradigm yet exists for nanotoxicology, no strategy for the determination of data requirements for a proper hazard identification has been proposed. Instead, the evaluation needs to take place on a case-by-case basis.