Answers formulated by SCENIHR to French comments

Comments on overall document:

Title. Not changed. The mandate specifically asked for a guidance document similar to the guidances published by EFSA on nanomaterials in food, and the SCCS guidance on nanomaterials in cosmetics.

ISO standards. Both ISO 10993-1:2009 and ISO 14971:2007 are frequently cited in the Opinion.

Tracebility. Tracebility is not an issue in the SCENIHR mandate for the Opinion. This is an issue for the EU regulators.

3.3.1

P 14 1-29.

Similarity: This text is to raise awareness that the same or largely similar materials should be investigated also considering changes in time or changes induced by use, or release of embedded nanomaterials. It is intended to indicate that other nanomaterials (or data of other nanomaterials) can only be used if sufficient similarity is demonstrated (in analogy with the read across principle).

Impurity: this is already part of the general material characterization of a medical device and nanomaterial itself. For clarification this is added to the text on page 14.

P15

Impurities is now included in 3.3.1. The focus of the guidance is on medical devices, so manufacturing is not included. Describing the manufacturing process of nanomaterials is outside the mandate of the Opinion.

3.3.2

P17

The empty boxes in Table 2 have now been filled.

3.4

P18

Text referring to ISO 10993-1:2009 is added.

“For the examples, the categorization described in ISO 1099301:2009 is largely used.”

The categorization follows ISO 10993-1:2009. SCENIHR disagrees that the examples should be grouped differently.

For coatings some examples are presented of the use on some types of medical devices. It is beyond the scope of the Opinion to give an exhaustive list of possible coating applications.

P19

The category of injectable medical devices was specifically highlighted as these obviously show a direct systemic availability, and thus possible systemic toxicity.

MAgforce. SCENIHR agrees with the comment at the referral to Magforce has been deleted.

Medical devices under development. SCENIHR agrees with the comment. However, the listing is time dependent, so it is indeed possible that some products are now on the market.

Catheters and silver coatings. Although catheters can also be coated with silver as antibacterial agent, catheters are a different category compared to implanted devices

especially in relation to time of contact. So, they are presented in two different categories.
Diagnostic example. On page 11 the in vitro diagnostic medical devices are excluded. In the examples a possible combination of diagnostic and therapeutic imaging agent is indicated.

3.5

P22

Title 3.5.2. SCENIHR agrees with the comment. Title of 3.5.2 is changed.
"Exposure routes to nanomaterials released from medical devices".

P 22

Regarding the use of the word "short". SCENIHR disagrees with the comment. Dental practice is a relatively short activity. In ISO 10993-1:2009 the shortest time to consider is 24hrs. It is common knowledge that dental procedures are much shorter (within 1 to a few hrs).

P23

ISO 14971. The citation has been added to the text on page 23.

P24

Table 3 is a tool to support the estimation of potential exposure, without the aim to be quantitative. So, the indications High, Medium, Negligible are general indicators of exposure.

3.6.1

P25

The biological barriers are discussed in detail in 3.6.3 and 3.6.4 in which specific types of possible exposures are discussed.
Metabolism. The text on page 25 has been changed to indicate that for some nanomaterials metabolism occurs. In addition, the text does not indicate that metabolism should not be considered.

3.6.2

P26

The text indicates that the OECD guidelines are for chemical and not particles. Some explanation and examples including references to relevant literature is presented to give some possible approaches for evaluation of the toxicokinetics of nanomaterials.

3.6.4

P28

Migration into the brain. The text has been changed to indicate that not all nanomaterials migrate into the brain after inhalation.

3.6.5

P29

Translocation and macrophage rich organs. Text has been added to indicate these aspects.
"When release of nanomaterials is likely also possibilities for translocation and uptake/persistence in organs rich in phagocytic cells (e.g. liver, spleen, bone marrow) should be considered."
Impurities. The text as it is in lines 34-36 indicates that also the impurities should be characterized. They are an integral part of the device.
In vitro reference Basketter et al., 2013. Indeed the paper does not deal with nanomaterials specifically, however, the paper deals with a general overview of possibilities for in vitro alternative methods.

3.7.1

In vivo methods. Text has been added.

“When performing in vivo studies these should be performed using an administration route which is relevant to the route of human exposure to the medical device and/or nanomaterials.”

P30

Omics. SCENIHR agrees to add “omics” techniques as possibility for toxicological screening. Indeed a lot of research is dedicated to “omics” (genomics. Proteomics, metabolo-omics). Omics techniques can indeed indicate certain modes of action (MOA) and adverse outcome pathways (AOP). However, the relevance for safety evaluation and risk assessment is not yet firmly established.

Text added.

“In addition, also “omics” techniques seem promising to contribute in the future to an alternative approach for the safety testing of medical devices and/or nanomaterials.”

3.7.2

P30

Add in vitro cellular conditions. SCENIHR disagrees with this comment. 3.7.1 is a general introduction. Section 3.7.3 gives information on cellular cytotoxicity assays for evaluating general toxicity as indicator for potential hazards.

High concentration dosing. Text has been adapted.

“In certain assays it should be noted that high concentrations *in vitro* or excessive doses *in vivo* can lead to a false interpretation of results (e.g. overload dosing in inhalation studies (Valberg et al., 2009)).”

3.7.3

P32

Exposure. Statement on route of exposure has been included on page 29. You cannot determine the target organ beforehand in a toxicity study. You look for adverse effects on any organ, but you do take the organ system in which the medical devices is applied into specific consideration.

In vitro assays. On page 32 specific attention is asked for possible nanospecific reactions. The possibility for nanomaterials to induce ROS is mentioned on page 32 already. Uptake by cells is not necessary for some specific mechanisms of toxicity.

Whether the OECD must adapt its guidelines is outside the SCENIHR mandate.

Nervous system. In the safety evaluation of medical devices all organs need to be evaluated including the nervous system. ISO 10993-6 Implantation is currently under revision in which an implantation test in the brain is now included as implantation tests (to be published in the near future by ISO, Geneva. Switzerland).

Interference. Interference of nanomaterials in test systems has been addressed in 3.7.2 Potential pitfalls in toxicity testing of nanomaterials. Lupu and Popescu 2013 has been added as additional reference in 3.7.2.

Interference NR. The issue of interference is addressed in 3.7.2 including the reference of Monteiro- Riviere et al. 2009.

ECVAM. The reference to the ECVAM report has been included in the text on page 32.

Acute toxicity. SCENIHR disagrees with the comment. The tests described follow ISO 10993-1 in which an acute toxicity test is one of the tests to consider. In addition, the test can give information on the relative toxicity of various (nano)materials used for the production of a medical device.

Relevance for NM. So far, no tests have been described that are relevant to nanomaterials as the whole area of nanotoxicology is still under development. It might also be doubtful if there is a nanospecific toxicity as organ and cell reaction follow established patterns.

Irritation test. The Opinion addresses assays that have to be performed for medical devices whether a nanomaterial is present or not, highlighting that there are no validated assays for nanomaterials. Therefore this Opinion wants to indicate what the possibilities are for the safety evaluation of medical devices manufactures using nanomaterials. The issue of cytokine release is not addressed in the irritation assay although for the *in vitro* alternative testing sometime cytokines can be used as read out system. The reference of Val et al., 2009 is included in the section on artifacts in the assays section 3.7.2.

P33

Solutions for sensitization testing. Sensitization testing is one of the areas that needs further development and research for nanomaterials. So, the text as provided gives the current situation.

P34

Adjuvant activity. The potential adjuvant activity of nanomaterials has been indicated on page 33 lines 38-40.

3.7.3

P34

Genotoxicity. The statement is only applicable when non-genotoxic components are used for the manufacturing of a medical device (page 34). So, if the genotoxicity is unknown this should be addressed. Text has been adapted to indicate this.

“However, if the genotoxicity of the ingredients including nanomaterials is unknown genotoxicity testing is necessary. “

Assays. These assays are described in ISO 10993-3 standard and should be mentioned. So far, a negative outcome in a genotoxicity assay has no meaning if exposure is not demonstrated. This is also true for the in vivo genotoxicity tests.

The text has been amended according to the comment. “recommended” is replaced by ”seems to be”. Text has been added to warn for other effects occurring in a genotoxicity assay like cytotoxicity inducing DNA damage due to high dosages.

“A recent review on genotoxicity testing concluded that genotoxicity testing should also consider other potential toxic effects in the assays (Magdolenova et al., 2014). “Many studies, both in vitro and in vivo, show positive effects most likely due to the use of concentrations that are not relevant to possible environmental exposure. In many studies a demonstration of genotoxicity simply reflects cytotoxicity, as excessively high concentrations are used. Thus, cytotoxicity should be an integral part of genotoxicity testing to avoid false-positive results” (Magdolenova et al., 2014).”

Text on comet assay is added on page 34.

“In addition also an in vitro Comet assay may be considered.”

Internalization. SCENIHR agrees with the comment. This aspect has been indicated several times in the text p34L29-34 for the Ames test. P34 L47-49.

P35

In vivo assays. SCENIHR agrees with the comment that hematopoietic cells of the bone marrow is a difficult target for genotoxicity by nanomaterials especially under normal exposure conditions to a medical device. However, these assays are used to identify potential genotoxicity as hazard. So, also intravenous administration may be considered in which the exposure of the bone marrow is much more likely.

Transgenic rodent mutation test. SCENIHR agrees with the comment. Additional text is included.

“In addition, the transgenic rodent mutation assay was identified as adequate to detect chemically induced gene mutations some limitations in all tissues but there may be practical limitations when performing the assay (ECHA 2012).”

Chronic inflammation. SCENIHR agrees with the comment that chronic inflammation can induce secondary genotoxicity resulting in tumors. However, there are no assays to investigate such mechanism other than a chronic carcinogenicity study. Some text raising the awareness for chronic inflammation and cancer is included on page 34 line 18.
"It should be noted that besides direct genotoxicity also other mechanisms of toxicity may result in DNA damage and induce indirectly genotoxicity, notably chronic inflammation (Kundu and Surh 2008. Donaldson et al., 2011)."
P39
Reproductive testing. Text has been adapted according to comment for further clarification.
"If additional tests are considered necessary, in view of the outcome of the screening test, they shall be performed…."
Suitability of tests. SCENIHR disagrees with the comment. All tests are based on the safety evaluation of medical devices. Regarding nanomaterials there are, with some exceptions, no specific standards or guidelines available that describe assays specifically suitable for nanomaterials. So, considerations are presented how existing assays for chemicals might be used for nanomaterials or, more specifically, medical devices including nanomaterials. The consideration of the suitability of these tests for nanomaterials is indicated after describing the assays that need to be considered.
Effects on development. The assays are described in the order of the comment.

3.8

P40
Phys: chem data. SCENIHR agrees with the comment. Explanation is added to the table.
"Phys:chem, indicates data from physical chemical characterization of the nanomaterials. "
High//medium/low exposure. Indeed the categories high/medium/low are not further quantified. This information is presented to give some suggestion on exposure, without the aim to be quantitative. All testing should also be considered in the context of ISO 10993-1:2009.

3.8.5

P42
Specific medical devices. SCENIHR disagrees with the comment. These types of medical devices are indicated as they may pose an increased risk in view of the use of nanomaterials.

3.8.4 and 3.8.6

Section 3.8.6 indicates that distribution should be considered by evaluating target organs. In addition, a whole section is dedicated to possible release of nanomaterials from medical devices (3.5.1) and toxicokinetic studies (3.6).

4

P44
Impurities. SCENIHR disagrees with the comment. The determination of impurities is part of the characterization of a nanomaterial as indicated in section 3.3.1.
The risk due to the release of particles is independent whether the particles are already present in the medical device as nanoparticles or whether they are generated by wear and tear. This is covered by the Figure 2.
Table 5
SCENIHR agrees with the comment. The assays to be considered are indicated in ISO 10993-1:2009, but not all the assays have to be performed for every medical device. This also has an impact on the testing of the nanomaterials in such medical devices. So,

the framework presents some suggestions in terms how extensive the evaluation should be.

P44-46

SCENIHR agrees with the comment that the document provides a guidance that will give a testing strategy, supported by examples of evaluation process.