Answers formulated by SCENIHR to CEFIC comments submitted at Public Consultation

General comments.

SCENIHR partly agrees with the general comment that there is still a lot unresolved regarding the nanomaterial safety and risk evaluation. Some text has been added in the conclusions. However, there is a lot of information already available specifically on potential problems associated with the safety testing of nanomaterials. This information is included in the Guidance to raise awareness also in the area of medical devices to emphasize that the use of nanomaterials needs some specific considerations. For those risk assessors dealing with the evaluation of particle toxicity and nanomaterials specifically this guidance, similar to the guidances for food/feed products and cosmetics, provides useful information how to deal with the risk assessment of nanomaterials and to consider potential pitfalls in the safety testing/evaluation of nanomaterials when used in medical devices.

Regarding the OECD guidelines SCENIHR has indicated in which cases the guidelines may not be applicable to nanomaterials. This is not a problem of the OECD guidelines but is embedded in the properties of the nanomaterials. The OECD guidelines are written with the aim to evaluate chemical solutions, and in a few instances particles. Also within OECD discussions are ongoing whether and how the OECD guidelines would need to be adapted specifically for nanomaterials or whether nanospecific guidelines should be prepared. The OECD has launched already in 2007 a specific working party for these discussions (Working Party on Manufactured Nanomaterials, WPMN). The WPMN has published several documents dealing with testing and safety evaluation of nanomaterials. In this Guidance on the use of nanomaterials in medical devices more specifically the combination of nanomaterials and medical devices is discussed and guidance is presented on aspects that need specific consideration.

Characterization is a critical point for the use of nanomaterials also in relation to identification of the nanomaterial tested in the various safety assays. It cannot be that a manufacturer just uses an ingredient without knowing what he is using or without knowing that it is safe to use. For medical devices the final product not just the ingredients shall be evaluated in the risk assessment although the safety evaluation/testing of ingredients may help in the final evaluation of the medical device itself.

The wear-and-tear of medical devices such as implants is a common phenomenon and shall be considered in the risk assessment of a medical device, since these wear particles may have a size in the nanorange. There are tests available to evaluate the wear and degradation of materials used for the manufacturing of medical devices and medical devices themselves.

Regarding ADME studies. Toxicokinetics is an important part of the safety evaluation of nanomaterials. As some nanomaterials do not dissolve the kinetics is ruled by the particulate nature of the nanomaterial and not by the concentration in the blood as is the case for chemicals. (Nano)particles are actively removed from the blood by cells of the mononuclear phagocytic system (MPS) that is mainly present in organs of the immune system (spleen, bone marrow) and liver. This is clearly explained in the Guidance.

So far, in vitro assays are, with a few exceptions, not suitable for risk assessment purposes but are very useful for screening to compare toxicity between nanomaterials (thus choices can be made for a low toxicity ingredient), and studies to the toxicity mechanism when toxicity is observed (as indicated on page 29). Some in vitro assays can be used for hazard identification (irritation test with reconstructured human epidermis), but are not suited for quantitative risk assessment as no or limited

information becomes available regarding a dose response effect that could be extrapolated to human exposure.

For in vitro tests a number of cell lines have been proposed. Indeed in view of the fact that most nanomaterials end up in phagocytic cells it is advisable to use both a phagocytic cell line (like a macrophage cell line) and a non-phagocytic cell line.

Specific comments.

Page 11 Line 23. The example is indeed an example. With proper justification grouping and read across might be possible. However, so far, grouping has only been applied successfully within one nanomaterial but produced by different manufacturers like ZnO and TiO_2 as indicated in the Opinions of the Scientific Committee on Consumers Safety.

Page 13 Figure 1.

1. The rationale to direct the risk evaluation to the general safety assessment principles is based on the issue that when there is no exposure to a nanosized material, the nanomaterial specific properties (or particle properties) do not need to be considered anymore. However, it cannot be assumed that the medical device and the materials used are safe. So, a proper safety evaluation and risk assessment needs to be performed as it has to be performed for any medical device.
2. Third box invasive versus non-invasive. This box categorizes the medical devices in which the nanomaterials are used. Not the nanomaterials themselves. Risk assessment of medical devices is based on the potential exposure to a medical devices. On page 10 the use of nanomaterials and how these are applied is described. The other pages describe mainly the classification of medical devices as being invasive and non invasive.
3. The characterization is needed to gain insight in possible changes that may occur in the nanomaterial, after it has been applied in a medical device. Ultimately the risk assessment needs to be done on the nanomaterial as it is present in the medical device.

Page 25 line 15.
ADME needs to be considered and if deemed necessary studies have to be performed. These studies should take into consideration the potential route of exposure likely to occur for the specific medical device in which the nanomaterial is used. So, not all routes of exposure have to be investigated, only the route which is associated with the medical device under investigation. The text addresses the issue based on the fact that all routes of exposure are possible in view of the numerous types of medical devices. Before doing extensive toxicokinetic studies, it should first be evaluated whether indeed release of particles can occur from the medical device. Some in vitro systems like in vitro penetration studies for skin may be suitable for nanomaterials as well. Some practical problems in terms of quantification of the amount of nanomaterial penetrating the skin do remain.
Page 26 line 25.
The text gives an example that TiO2 nanoparticles can persist for a long period in the body. Repeat dose toxicokinetic studies should be considered when multiple exposures to a nanomaterial through the use of a medical device is foreseen. This might be when there is a continuous release of nanomaterials from a medical device. Whether a single or repeated exposure needs to be evaluated has to be considered in relation to the use of the medical device (e.g. a single short use versus a permanent implant with continuous exposure).
Page 28 3rd paragraph.
The oral route is a common route of exposure to investigate the toxicity of chemicals as most chemicals show systemic bioavailability after oral administration. The text explains that this may not be true for particulate materials like nanomaterials. General oral

toxicity studies of nanomaterials may have little relevance for systemic toxicity if there is no uptake from the GI-tract in the body. Only local toxicity in the gut may then be evaluated. The safety evaluation and risk assessment of medical devices needs to take into consideration the site of use i.e. application of the device. So, for medical devices with applications in the GI-tract or dental material such oral studies are indicated.

Page 34 line 23.
The doubt for the suitability of the Ames test is based on the observation that the bacteria used in the Ames test do not ingest the nanomaterials. A negative outcome in the Ames test cannot be interpreted that the nanomaterial is negative for genotoxicity. It may be that the DNA was not exposed to the nanomaterial. Only when exposure of the bacterial DNA to the nanomaterial can be established, a negative outcome of the Ames test has some meaning. The issue of possible false negative results is explained in the paragraph on page 34 line 23 and onwards.

Page 34 line 37.
SCENIHR agrees with the comment. The CHO/HGPRT mutation assay is also indicated in OECD 476 and is added to the text as a possibility for testing of gene mutations. These assays are considered more suitable than the Ames test as most mammalian cells were demonstrated to take up nanomaterials in in vitro test systems. This is also addressed at the end of the in vitro genotoxicity section page 34 line 47-49.

Page 34 line 47.
As the OECD guideline is meant for chemicals the uptake is generally based on a concentration equilibrium. The importance of demonstrating nanoparticle uptake by the cells is that without uptake there may be considerable doubt on the exposure of the cellular DNA to the nanomaterials. The testing is not waived; a negative outcome has no meaning as it cannot be stated that the tested nanomaterial has no genotoxic potential. This is indeed explained in line 47 – 49.

Page 35 line 1.
A positive in vitro test does not necessarily mean that an in vivo test is needed. For example, when a product is only used on the skin and skin penetration can be excluded (e.g. like the use of nanomaterials in sunscreens) then an in vivo test is not needed as there is no or negligible uptake and thus no or negligible systemic exposure.

Page 35 line 10.
The evaluation of the target tissues as indicated by the OECD guidelines is based on chemicals. A positive result in an in vivo genotox test can be ascribed to the chemical and/or nanomaterial treatment. A negative outcome does not necessarily indicate that the nanomaterial is negative regarding in vivo genotoxic activity. If the organ investigated was not exposed to the nanomaterial the negative outcome has no meaning. The possibilities for testing are mentioned including the in vivo comet assay. No preference is presented.

Page 35 line 19.
Thank you for the comment. The OECD TG 489 is added to the text.

Page 35 line 21.
When investigating the genotoxicity potential of nanomaterials themselves the routes of exposure as indicated in the OECD guidelines may be used. However, exposure of the target organs that are evaluated for genotoxicity should be demonstrated. The recommended route of exposure for in vivo tests of medical devices is dependent on the application route/exposure site of the medical device in which the nanomaterial is used.

Page 35 paragraph 4 line 25.

SCENIHR agrees with the comment. The paragraph has been moved to the in vitro section.

Page 35 paragraph 5 line 32.
SCENIHR agrees with the comment. The paragraph has been moved to the in vitro section.

Page 40 Table 4.
SCENIHR agrees with the comment. The text has been modified. It now states "*In vitro* and *in vivo* (repeated dose) genotoxicity testing".

Comment on testing strategy.
SCENIHR agrees with the comment for a tiered approach to the nanomaterial testing. However, this guidance deals with medical devices for which the testing strategy is described in ISO 10993-1:2009, in which testing considerations are based on the type of medical device, the exposure time to the medical device, and the location of use of the medical device. Section 3.8 specifically describes the testing of various types of medical devices. Page 40 line 10 indicates that for the testing of the nanomaterials used in medical devices a similar testing strategy can be used as for the medical device in which the nanomaterial is applied.

Editorial comments.
The typo's are corrected.