Cefic comments - SCENIHR opinion on potential health effects of nanomaterials used in medical devices

26 September 2014

In the context of the recent published SCENIHR opinion on nanomaterials used in medical devices, Cefic would like to empahsise the following aspects:

General comments

The SCENIHR preliminary opinion on the *Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices* is well written and comprehensive, and attempts to meet the need of guidance documentation. It captures the current situation well. However, it can be argued that one of its weaknesses is that it is authored during a time when so many of the most elementary issues around nanomaterial safety and risk assessment are still unresolved, including considerations about the definition, characterization and categorization of nanomaterials. On the other hand there are several projects and efforts focusing on "all aspects nano", which raises hopes for scientific breakthroughs and data –driven consensus statements in a few years' time. Would it be worth to hold the issuance of this guidance document for some time instead of issuing it in a rather "raw" form with limited practical applicability?

The document correctly recommends the use of the **OECD Test Guidelines (TGs)** for safety assessment, as is currently done for medical devises representing different chemical spaces and categories. The obvious reason is that there is no suitable set of nanospecific TGs to follow.

The use of the OECD TGs has been challenged technically and methodologically, which is captured in the document. However, few solutions are given; instead of clarifying the testing recommendations the guidance document enforces the uncertainty about the acceptance of the results from the OECD TG compliant tests by stating that the TGs have not been "specifically evaluated for testing of nanomaterials", or in some instances that the tests are unsuitable for validation of nanomaterials. What impact will this have, particularly, on results indicating no adverse effects associated with the nanomaterial that is being tested? There is a danger that there will be reluctance to accept that the (biological) endpoint has been satisfied, with requests for additional testing? Therefore the guidance document would need to address the fundamental question: Are the current OECD TGs appropriate and relevant also for the assessment of nanomaterials? If they are not appropriate, or require adaptation, then the guidance document should provide explanation and justification for this.

For example, are commonly used cell types and animal models suitable for testing of nanomaterials? Can the same solvents and vehicles be used as for other types of materials? Are the OECD TG – recommended limit doses and dose-range regimens appropriate? Are the commonly used endpoints and organs suitable? How should toxicity be assessed? Would there need to be inclusion of nanomaterial – specific positive controls – and are there any?

The guidance document correctly states the relevance of a characterization of the nanomaterial that is to be tested. However, this information is hard to provide (as noted on

page 17, Line 10) and a through characterization will be both timely and costly, and still not deemed accurate. Since the definition of what constitutes a nanomaterial is still under debate, there is little guidance for how to decide if the characterization analyses provided the necessary information. Even if characterized, it is not clear what form of the nanomaterial should be tested, or should one material be tested in many forms; as particulate, agglomerate, and aggregate, and should dispersibility be included in the testing protocols – and how should it be assessed in that case?

A special concern may rise around the requirement to test potential nanomaterials from chemical breakdown of "wear-and-tear" of implanted devices. There is a need to provide Guidance on how to design such studies including acceptance criteria for nanomaterials from chemical breakdown of "wear-and-tear" of implanted devices.

Another concern is the ability to meet the requirement of **ADME assessments** and measurements. Are there reliable methods available? What instrumentation is needed, and how will sensitivity levels and exposure routes be taken into account, and how should time points be determined – according to the nanomaterial specific physicochemical properties (if known) or the time points determined by the biological system that is being used? Will toxicokinetics work need to be completed prior to initiating testing, in order to use the correct target organs on the TG studies? What impact will the toxicokinetics work have on the required testing if systemic exposure and uptake cannot be shown? Will the testing be waived? What is the justification for including toxicokinetics in the guidance document and simultaneously recognize the lack of methods for nanomaterials (see chapters 3.6.2. and 3.6.3)

Similar and also additional questions pertain for ***in vitro* tests**, including cytotoxicity testing, which are largely considered not to be suitable for nanomaterial testing (see Chapter 3.7.3). Still, *in vitro* methods are included for genotoxicity assessment. What is the difference between these and other *in vitro* tests, that make the genetoxicity approach acceptable?

Should the routinely used cell lines be used even for nanomaterials, or should a cell type capable of phagocytosis be applied? For this, extensive validation work is needed before it can be used as guidance. As noted on page 36, there are 'no standards currently available for the evaluation of particle and especially nanoparticle interaction with phagocytic cells".

Specific comments

Page 11, Line 23: It is considered that extrapolation from one nanomaterial to another is not possible, with a reference to Park et al, 2011 and nanosilver. However, this is too broad as a general statement, and the extrapolation might be possible for other nanomaterials. The statement is also conflicting with current efforts for grouping of nanomaterials, and the desire to develop QSARs for nanomaterials. This statement should be softened not to completely exclude extrapolation (read-across), when justified.

Page 13, Figure 1:

1. The five first boxes in the figure are logical and easy to follow, but the flow beyond the "Is systemic exposure possible" (5th box) seems illogical. What is the explanation for directing a material with no local or systemic effects, or if a material is no longer in the nanoscale, to the "general safety assessment principles"?

2. The third box categorises the nanomaterials into non-invasive and invasive. However, this is contrasting to the categorization on p. 10, 22, 29, and 45.

3. The physicochemical categorization is done twice, once in the beginning of the assessment and once towards the end. This will not be easy to achieve in practice. Also, although there is a clear benefit with this the consequences are hard to predict. Will any data generated with a nanomaterial that changes scale be considered invalid or unreliable? How can one be certain about the actual characteristics of the material tested and the biological effects associated with one scale and/or another?

Page 25, Line 15: The requirements for the ADME are extensive, particularly when the easiest accessible organ (blood) is considered less relevant than other tissues. Combined with the statement on **Line 45**, that potentially all exposure routes are possible, it can become a daunting, if not impossible, task to fulfil the ADME requirement. The complexity of the task and situation is indeed verified in the following chapter 3.6.2., describing the methods for toxicokinetic evaluation. However, to include this implies that it should be followed. Did the authors consider the associated difficulties and costs, even if the material would be characterized and a method detected? Since the work is conducted *in vivo* this will also increase the animal use numbers in conflict with the 3R principles and community expectations. Alternative ADME methods are apparently not going to be available in the near future, considering that the current in vitro assessments suffer from the same problems as the *in vivo* (as stated on **page 27, Line 21**).

Page 26, Line 25: Guidance for how to determine when repeat dose exposure followed by extended follow up periods should be used.

Page 28, 3rd paragraph: This paragraph addresses the uptake after oral exposure, which is a relevant exposure route both for nanomaterials and OECD TG. The paragraph is based on individual studies with partly conflicting results, and adds little in terms of guidance. Could some general advice be added for when uptake after oral exposure can or should be expected, or when the oral route should be chosen even without demonstrated uptake?

Page 34, Line 23: It might be too early to state that the Ames test (OECD 471) is not suitable for testing any nanomaterial. However, since it is not recommended in the guidance, can it be assumed that an Ames test is not needed for any of the implied testing, and can be omitted? However, on **Line 45** it is stated that, in certain instances, an Ames test might still be informative. Can this be resolved in any other way than by doing the test? – **need for clarification**

Page 34, Line 37: Please explain why the mouse lymphoma (MLA) test is recommended and not also the CHO/HPRGT test. Both tests measures mammalian cell gene mutations, and since the MLA test is conducted in suspension it might be impossible to remove the nanomaterials, especially if they have formed aggregates and will be spun down with the cells. Both assays are sensitive for precipitation, which actually could render them unsuitable for nanomaterial testing and potentially lead to false results. Why these tests are considered more suitable than the Ames test?

Page 34, Line 47: Demonstration of the uptake of the test material is not required by the OECD TGs for genotoxicity testing, and concurrent toxicity to the test system is usually the only indication of internal cellular exposure. How is the uptake to be demonstrated for the nanomaterials, and is the testing invalidated / waived if there is no uptake? On the other hand, there is very little experience with the use of, for example, phagocytic cells – **need for clarification**

Page 35, Line 1: How can it be adequately demonstrated that the positive in vitro findings with a nanomaterial are not relevant for the in vivo situation? Will a positive finding in practice trigger in vivo testing? – **need for clarification**

Page 35, Line 10: The target tissue for a nanomaterial is often not the same as the target tissues validated in the OECD TGs for *in vivo* genotoxicity, or the tissue that the laboratory is qualified for. Please explain how the requirement for assessing the genetic effects in the target tissue should be taken into account when selecting an *in vivo* test. Is the recommendation always to do a Comet assay or OECD 488, which has a wider range of tissues to sample for analyses than for example, OECD 474 or 475?

Page 35, Line 19: The OECD TG for the *in vivo* comet assay has been finalized and is expected to be adopted in the early fall of 2014. It will be TG 489.

Page 35, Line 21: Which is the recommended route of exposure for the *in vivo* tests?

Page 35, Paragraph 4, beginning at Line 25: This paragraph pertains to the *in vitro* micronucleus assay, OECD 487 - please move to the previous section.

Page 35, Paragraph 5, beginning at Line 32: The first part, based on Corradi et al., 2012, also pertains to OECD 487. Please check the correct location for the references Madolenova et al., 2012 and 2014, since these papers discuss both *in vitro* and *in vivo* assays -

Page 40, Table 4: What is meant with *"Full genotoxicity testing"*, and how is this different from "Genotoxicity *in vitro* and *in vivo*"?

Furthermore, in light of amending the REACH Annexes for nanomaterials and thus to seek harmonization across different European legislations, we suggest that nanomaterials should be characterized by means of a stepwise tiered approach as proposed by the NanoSafetyCluster working group and presented at the ECHA level within the REACH Annexes adaptation to nanomaterials process[1].

[1] Oomen, A.G., Bos, P.M.J., Fernandes, T.F., Hund-Rinke, K., Boraschi, D., Byrne, H.J., Aschberger, K., Gottardo, S., van der Kammer, F., Kühnel, D., Hristozov, D., Marcomini, A., Migliore, L., Scott-Fordsmand, J., Wick, P., Landsiedel, R., 2014a. Concern-driven integrated approaches to nanomaterial testing and assessment - Report of the NanoSafety Cluster Working Group 10. Nanotoxicol. 8, 334-348.

Oomen, A., Bos, P.M.J., Landsiedel, R., 2014b. Concern-driven safety assessment of nanomaterials: An integrated approach using material properties, hazard, biokinetic and exposure data and considerations on grouping and read-across. Ch. 16. In: Safety of Nanomaterials along their lifecycle: Release, exposure and human hazards. Eds. W. Wohlleben, T. Kuhlbusch, C.-M. Lehr, J. Schnekenburger, Taylor & Francis 2014, ISBN 978-1-46-656786-3, 380 pp.; available as pre-print-pdf.

Stone, V., Pozzi-Mucelli, S., Tran, L., Aschberger, K., Sabella, S., Vogel, U.B., Poland, C., Balharry, D., Fernandes, T., Gottardo, S., Hankin, S., Hartl, M., Hartmann, N., Hristozov, D., Hund-Rinke, K., Johnston, H., Marcomini, A., Panzer, O., Roncato, D., Saber, A.T., Wallin, H., Scott-Fordsmann, J.J., 2013. Research prioritisation to deliver an intelligent testing strategy for the human and environmental safety of nanomaterials ITS-NANO consortium, 128 pp.; available at: http://www.its-nano.eu/wp-content/uploads/2013/12/ITS-NANO.pdf.

Stone, V., Pozzi-Mucelli, S., Tran, L., Aschberger, K., Sabella, S., Vogel, U.B., Poland, C., Balharry, D., Fernandes, T., Gottardo, S., Hankin, S., Hartl, M.G., Hartmann, N., Hristozov, D., Hund-Rinke, K., Johnston, H., Marcomini, A., Panzer, O., Roncato, D., Saber, A.T., Wallin, H., Scott-Fordsmand, J.J., 2014. ITS-NANO - Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy. Part. Fibre Toxicol. 11, 9.

Typos and small edits noted

Page 30, Line 35: Delete the citation mark after the first word and period.
Page 30, Line 48: Delete the second period after “testing”.
Page 51: The definition of OECD is incorrect. It is supposed to be “Organisation for Economic-Co-operation and Development”, not just “Organisation for Economic Co-operation”. Please correct.
Page 59, Line 1: The word “health” is misspelled “helath” - please correct.
Page 61, Line 1: The title is written with uppercase letters - please use same style as for rest of citations.
Page 62, Line 15: Right margin adjustment adds long spaces between the words - please edit.
Page 63, Line 18: Please delete second period after citation.
Page 64, Line 6: Please correct line spacing between lines 5 and 6.