RECOMMENDATIONS TO MANUFACTURERS ON THE BIOLOGICAL ASSESSMENT OF MEDICAL DEVICES CONTAINING NANOMATERIALS

The following recommendations must be read along with the related scientific report, entitled "Biological assessment of medical devices containing nanomaterials" and published by the French Health Products Safety Agency (Afssaps).

Overall, the current existing regulatory and guidance documents (harmonized directives, guidelines, standards…) are considered to provide a suitable framework for the biological risk assessment of medical devices containing nanomaterials, which are likely to come in contact with the patient/user's body. Nevertheless, there is a need to formulate and clarify some nano-specific recommendations and guidelines.

● *Assessment of the benefit/risk ratio:*

The toxicological profile must be contrasted to the expected benefits resulting from the inclusion of nanomaterials in the medical device. Then, this benefit/risk ratio has to be weighed against those of available alternatives. The use of nanomaterials seems to be justified only when the comprehensive analysis provides sound evidence for a favorable balance.

● *Information disclosure and transparency:*

In order to ensure information transparency on the presence of nanomaterials, it is mandatory to explicitly mention in the "Instructions For Use" document the use of nanomaterials in the medical device, which are likely to come in contact with the patient/user's body.

● *Identification and characterization of the materials used:*

As a rule, similarly to any medical device, the responsible manufacturer has to make sure that its raw materials are properly characterized and authorized by the prevailing REACH European regulations. Special attention shall be given to the characterization of nanomaterials (nano-objects and nanostructured materials), whose physico-chemical properties may change over time and during the product life cycle.

For this very reason, the physico-chemical characterization of the final product containing nanomaterials must be performed before any biological risk assessment. Likewise, final product batch

to batch consistency and reproducibility are crucial, in order to ensure the validity of the biological risk assessment performed.

Since medical devices containing nanomaterials, which are likely to come in contact with the patient/user's body, may wear out, deteriorate over time and release nanosized particles, biodegradation must be properly addressed in the risk analysis of the medical device, in its intended use conditions. Should its assessment be required, comprehensive determination and characterization of the released nanoparticles will be necessary in the physiological conditions similar to the standard conditions of use. The release kinetics, quantity and fate of the free nanoparticles in biological media have to be evaluated.

The most relevant physico-chemical parameters to assess the biological risks of a nanomaterial are the following: size and size distribution, morphology, aggregation/agglomeration state, solubility/dispersability, specific surface area, composition (including chemical composition and crystalline structure, amongst others), surface charge, surface chemistry. Those parameters considered for the biological risk assessment have to be carefully contemplated with regard to the medical device containing nanomaterials and its intended use, because the toxicological profile can greatly differ according to its physico-chemical characteristics. It is recommended to indicate the method and the measurement uncertainty for each parameter measured. Moreover, measures should be performed on an appropriate number of samples.

A qualitative and quantitative evaluation of impurities has to be carried out, especially their physico-chemical, biological and toxicological characterization must be provided. In case of unavailability, this should be justified. Since nanomaterials are prone to adsorb impurities, it is highly recommended to routinely check for their absence before batch release (pyrogenicity, etc.).

- ***Caveats in the biological risk assessment:***

Generally, toxicity is specific to the tested nanomaterial and cannot be generalized or extrapolated, even within the same chemical family. Furthermore, *a priori* the concept of equivalence is not acceptable, because difficult to prove.

According to the prevailing regulations, biological risk assessment is performed on the final product. This approach stays applicable to medical devices containing nanomaterials. However, there might be situations where biological risk assessment on the final product seems satisfactory, while the biological evaluation on the nanomaterials alone is not. Therefore, and in case the risk analysis reveals a likelihood of contact between nanomaterials and the patient/user's body, it may be required

to carry out a separate biological assessment on the nanomaterials alone, especially to perform tests related to major risks such as genotoxicity and carcinogenicity.

Relevant toxico-kinetic studies on free nanomaterials and/or nanosized degradation particles are highly recommended. The methodology can be adapted from testing protocols of drugs (ADME type– Absorption, Distribution, Metabolism, Excretion). Biodistribution studies should be designed with an appropriate labelling (e.g. radioactive or fluorescent), which should not modify the physico-chemical and biological properties of free nanomaterials and which should stay firmly attached to nanomaterials during the whole time of study.

The conventional dose metrics, namely mass and surface, may not be the most appropriate metrics for the biological evaluation of medical devices containing nanomaterials. If other dose metrics (specific surface area, number of particles…) seem to establish more informative results, closer to reality, then these adaptations are recommended and should be documented in the risk assessment analysis. Likewise, it might be more suitable to perform the extractions according to the specific surface area instead of mass, when preparing medical device samples for biological assessment.

Experimental conditions for the biological risk assessment should be as close as possible to the clinical conditions, for example regarding exposition route, quantity and frequency of exposure or aggregation/agglomeration state.

Special attention should be given to the reproducibility, reliability and sensitivity of the *in vitro* toxicological tests selected, before drawing any hasty conclusion. Particularly, caution should be taken because of potential interferences of nanomaterials with test protocols relying on colorimetric or fluorescent agents, such as those in cytotoxicity testing. In such cases, corroboration of several test results coming from different methodologies is required for a scientifically sound interpretation.

Evaluation of haemocompatibility must be performed on medical devices containing nanomaterials in direct or indirect contact with blood. Moreover, if the toxico-kinetic study reveals a potential translocation of free nanosized particles originated from the medical device into the systemic blood circulation, then haemocompatibility should also be evaluated.

As for any medical device, it is essential to carry out several tests to evaluate the genotoxicity of the device, namely at least two different *in vitro* tests and an *in vivo* test. The relevance of the testing protocols with the tested nanomaterials should be ensured (risk of false-negative results from the Ames test, test duration…), adapting them or switching to more appropriate tests if necessary.

Since some scientific studies suggest that nanomaterials may affect the immune system, the risk of delayed-type hypersensitivity and more generally of sensitization must be addressed. Risk analysis has to evaluate the need to carry out immunotoxicological tests.

It is recommended to design systemic toxicity studies on medical devices containing nanomaterials as comprehensive as possible, including the evaluation of clinical, biological and anatomo-pathological parameters. According to the nature of the nanomaterial, additional histological investigations should be performed.

Given the very limited clinical data, the risk of carcinogenicity must be addressed in the risk analysis of the medical device, according to the intended use of the medical device and the results of the toxico-kinetic studies.

Similarly, if there is a potential accumulation of free nanomaterials in some specific biological tissues (reproduction organs, central nervous system…) and/or a potential physiological membrane crossing (placenta, blood brain barrier…), then toxicological effects on reproduction, teratogenicity and neurotoxicity have to be investigated.

To conclude, the current toxicity testing approaches provide an appropriate framework and starting point to address the biological risk assessment of medical devices containing nanomaterials, with adaptations on a case-by-case basis if required. Although more appropriate analytical tools and experimental methods for nanomaterials still have to be adjusted and developed, data on the properties of nanomaterials should be generated and gathered in order to fill the significant knowledge gaps. Safety issues arising from the use of nanotechnologies in the medical device field should be addressed in a cautious and step-wise way, whilst keeping in mind the risk to benefit balance.

As part of its market surveillance activity, the French Health Products Safety Agency (Afssaps) pays specific attention to medical devices containing nanomaterials which have recently obtained the CE marking and launched on the French market. Upstream of CE marking, a close follow-up for the development of such devices may be given within Afssaps' comprehensive approach of innovation support, in order to facilitate rapid patient access to medical innovations whilst providing a framework for the risks induced by these new technologies.