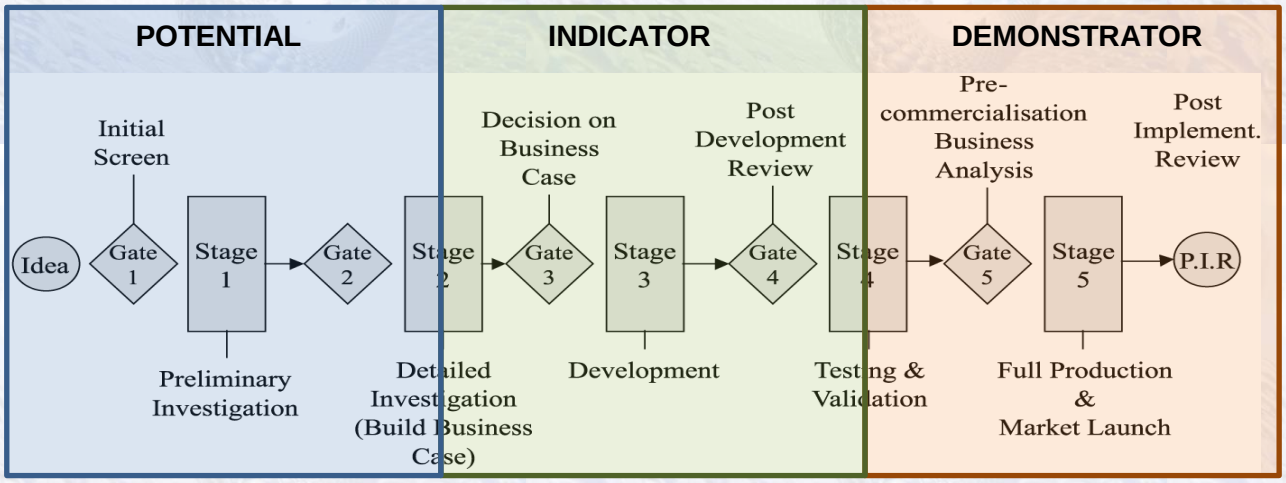
# Inventory of existing regulatory accepted toxicity tests applicable for safety screening of MNMs
## Deliverable 6.04

## Introduction

Safe-by-design has to take place in early stages of innovation. In present everyday practice, innovation is focused on technology readiness rather than investigating potential for health risks. In this deliverable (D6.4), an innovative and efficient screening strategy is proposed to identify potential risks of MNMs at early stages of innovation. The basis for the screening strategy are the six key risk potentials: solubility/dissolution rate, stability of coating, accumulation, genotoxicity, inflammation and ecotoxicity. The second step of the screening strategy addresses the testing strategy: which parameters are key to describe the risk potentials at the first stages of the innovation process. The final step of the screening strategy addresses the tools: which available tests are applicable to measure the parameters?

The safety screening strategy focusses on the first two stages where the potential of a MNM to give rise to human or environmental health risks can be deduced on the basis of a limited set of information (Figure below, blue part). The aim of this screening strategy is to bring information on potential health risks in line with the stage of innovation. This screening strategy is a concrete method within the Safe by Design concept, useful for both industry and regulators at the first stages of the innovation process.




## Main results

### Selection of risk potentials

The following aspects were considered when selecting nano specific potentials for health risks:

- Aspects specific for MNMs like solubility (if a particle readily dissolves into its molecular or dissociated chemically form, then it no longer needs to be treated as a MNM) or stability of the coating of a particle (if the coating readily dissolves of the core then one needs to know to consider whether the coated particle and the uncoated particle may exhibit the same kinetics and toxicity)

- Markers for increased probability of health risks like accumulation in tissue and organs or in environmental compartments

- Unacceptable toxicity like genotoxicity as a first marker for carcinogenicity or inflammation as a general marker for many (chronic) pathological conditions

The argumentation behind the selection of each of the six risk potentials is described below.

| Risk potential | Argumentation |
|---|---|
| Solubility: | Solubility and dissolution rate influence the properties of the MNM and determine its fate in organisms and the environment. By the time a MNM is fully dissolved, it need no longer be regarded for as an MNM, but it its molecular form needs to be regarded. Where his is measured (in water, in cell medium, etc.), final form (molecular form or ions) and speed of this process. Solubility should be described very precisely, as it is sometimes confused with dissolution or dissolution rate. |
| Stability | The stability of the coating of a MNM is very important for the behavior and effects in humans and the environment. This risk potential should answer the question if the surface coating or modification of a MNM will maintain or will be removed from the MNMs in its different life cycle stages. Kinetics and toxicity depend on the stability of the coating of a MNM and this may differ from the core. Therefore, information is needed about the form and coating of a MNM in order to gather the relevant kinetic and toxicity data. |
| Accumulation | Accumulation of MNMs in the human body or environment is a marker for an increased likelihood of long term effects. Therefore, accumulation is included as a risk potential. MNMs will probably only seldom cause acute toxicity. However, MNMs tend to accumulate, which may cause long term effects after chronic exposure. |
| Inflammation / immune toxicity | Inflammation or immune toxicity is an important marker for chronic pathological conditions, such as lung cancer, cardiovascular disease or neurological diseases. MNMs are likely to trigger the immune response due to a high degree of surface reactivity and the cell membrane permeability. Depending on the material, size, and ligands, particles themselves can induce the immune response or lead to other health effects. |
| Genotoxicity | Genotoxicity is the ability of substances to damage DNA within organisms. Genotoxic agents can give rise to mutations. Because mutations can lead to cancer, genotoxicity evaluation has been utilized widely to evaluate the carcinogenic potential of chemical and physical exposures. Although both positive and negative results have been reported on the genotoxicity of MNMs in various cell and animal test models, some data indicate that MNMs may be genotoxic. Since MNMs may be genotoxic, genotoxicity is included as a risk potential. |
| Ecotoxicity | In parallel to immunotoxicity and genotoxicity for human health, toxicity for the environment should be regarded. The potential for ecotoxicity is described here as a kind of bulk potential but is explored for further refinement in this report. Up till now several publications have indicated there is a ground for assuming ecotoxicity caused by MNM (Wang et al., 2012, Van Hoecke et al., 2009, Tong et al., 2015, Garner et al., 2015). |

**From potentials to a screening strategy**

The arguments for the suggested potentials are based on a logical reasoning thereby presuming a certain order of addressing the potentials. The figure below visualizes this order and demonstrates that it is important to first address the question that relate to whether a material is to be considered as a MNM. This is the starting point of the flow chart, followed by addressing the risk potentials in case of a MNM.

Arguments for concluding “no nanospecific potential for health risk” can roughly be divided into two types:

1) the material is not to be regarded as a (nano) particle, or
2) the material is regarded as a (nano) particle but no indications for causing toxicity were observed.
The first types of arguments can be addressed by physical-chemical properties, whereas the second types need to be addressed by simple toxicity tests.

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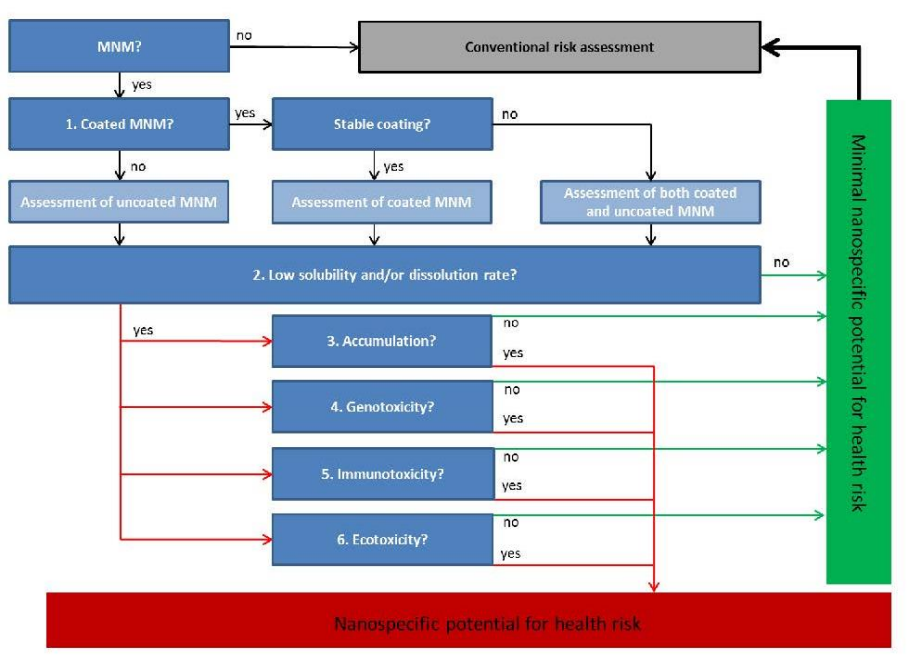


## Key parameters

For each of the steps in this flow chart the deliverable describes the key parameters and the methods to determine these key parameters. The table below presents the key parameters per risk potential for safety screening of MNMs.

| RISK POTENTIAL<br>KEY PARAMETERS | MNM characteristics | Stability of coating | Solubility<br>Dissolution | Accumulation | Genotoxicity | Inflammation | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Particle size (distribution) | X | X | X | X | X | X | X |
| Composition and impurities | X | X | X | X | X | X | X |
| Shape | X | X | X | X | X | X | X |
| Surface area | X | X | X | X | X | X | X |
| Surface charge | X | X | X | X | X | X | X |
| Agglomeration<br>Aggregation | X | X | X | X | X | X | X |
| Surface coating | | X | X | X | X | X | X |
| Degree of coating | | X | X | X | X | X | X |
| Coating stability | | X | X | X | X | X | X |
| Solubility/dissolution | | | X | X | X | X | X |
| Hydrolytic stability | | | X | X | X | X | X |
| Acid dissociation | | | X | X | X | X | X |
| Exposure route | | | | X | | | |
| Dose (external and internal) | | | | X | | | |
| Protein binding | | | | X | | | |
| Excretion (half life) | | | | X | | | |
| Cell uptake | | | | | X | | |
| Cytotoxicity | | | | | X | | |
| ROS generation | | | | | X | X | |
| Hydrophobicity<br>Lipophilicity | | | | | | X | |
| Cytokine induction | | | | | | X | |
| Test medium | | | | | | | X |
| Crystalline structure | | | | | | | X |
| Reactivity | | | | | | | X |
| Photoreactivity | | | | | | | X |

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