Nanomaterials and REACH – What Do I Have to Do Differently?

Part 3: Adjustments to toxicological, eco-toxicological and phsyico-chemical testing for nanomaterials

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No toxicological mechanisms that are specific to nanomaterials as a whole have been identified as yet. This has led to the conclusion that the testing requirements applicable to bulk substances under REACH can also be applied to nanomaterials. However, the unique properties of nanomaterials must be taken into account when designing a study. Ongoing work by the OECD towards developing standardised testing protocols is yielding new methods, but whilst this is still to be completed, ECHA has produced a number of Annexes to their guidance documents detailing recommended adjustments to testing strategies that should be employed when testing substances in their nanoforms. The advice given in these documents can be simplified into two overall conclusions:

- Ensure that you know exactly which nanoform is being tested.
- Ensure that the target of the testing is being exposed to the nanomaterial in the form you require.

In silico and read across methods

One of the key aims to REACH is to minimise animal testing. Research has shown that different nanoforms of the same substance can display different toxicology. In order to avoid animal intensive and expensive in vivo testing on each nanoform, it is clear that in silico, in vitro and read-across methods will be a key tool in hazard and risk assessment of nanomaterials. Much ongoing research is directed towards finding the physico-chemical parameters that have a correlation with toxicology thus allowing for grouping of nanoforms. Currently these are relatively basic but are still useful points from where to start a testing strategy.

- Insoluble, high aspect ratio particles cause concern for inhalation toxicity.
- Very water soluble nanomaterials can be treated as the bulk form for ecotoxicology.

More detailed guidance is available at:
https://echa.europa.eu/documents/10162/13564/appendix_r_6-1_nano_caracal_en.pdf/a07d989c-99ec-d87f-0f4d-b198022f4744

Physico-chemical testing

Testing methods for the physico-chemical properties must be designed to measure the endpoints they are targeted towards. For example, the standard water solubility tests involve the filtration of an aqueous solution and the analysis of the filtrate. Unfortunately, nanomaterials can form colloidal suspensions of particles small enough to pass through filters and then be detected by analysis. This would give an artificially high solubility for the substance. In addition, it would lead to inaccurate assumptions regarding the form of the substance the test creatures are exposed to during eco-toxicological studies. Therefore, ultrafiltration or centrifugation are recommended for separation of solids from the solution in addition to further analysis of the supernatant for nanosized particles. Other physico-chemical endpoints are not applicable to suspended solids, such as partition co-efficient, so the alternative measures that most closely mimic the original endpoint should be used.

More detailed discussion can be found at:
https://echa.europa.eu/documents/10162/13564/appendix_4_nano_registration_committees_en.pdf/1abb12d1-88a2-b386-0907-c67d05105378

Toxicological testing

One of the failures of testing in the infancy of nanotoxicology was poor characterisation of the nanomaterial being tested. This was not only of the nanomaterial being used as the input to the study but also of how the nanoform might change during the study because of interactions with biological fluids or changes in pH. Extensive knowledge of particle characterisation is now a pre-requisite to receiving meaningful results from testing. It is also important to choose the correct nanomaterial to perform the studies. Although characterisation to meet the definition of a nanomaterial would require modifications to try to stabilise suspensions of primary particles, the substance that should be tested under

REACH should be the substance as it is placed on the market as far as possible, which could be a highly agglomerated form. Some studies required under REACH will always give negative results because the target can never be exposed to the test substance, so consideration to waiving the study and using a better test method should be given. For example, solids cannot cross the bacterial cell wall, meaning the Ames test required in Annex VII might give a false negative conclusion for mutagenicity (it must be noted that this applies to all solids, not just nanomaterials).

More details can be found at:
https://echa.europa.eu/documents/10162/22334053/draft_for_committees_app_r7-1_r7-2_en.pdf/e0efc82b-fed8-f80e-692b-408b75fbae2d

Ecotoxicological testing

As with toxicological studies, the key point to ensure for eco-toxicological studies is that the nanomaterial being tested is fully characterised both before and during testing. The partitioning of substances in solution is largely dependent on their partition co-efficient (Kow), which is a thermodynamic equilibrium. This does not apply to suspensions of nanomaterials, whose partitioning in the environment is a kinetic phenomenon. This must be accounted for in any studies. The toxicology of many metallic nanomaterials is believed to arise from ions released from the nanomaterial (e.g. nano-silver), meaning that a full understanding of the dissolution behaviour (solubility and rate of dissolution) is essential to draw meaningful conclusions. Many nanomaterial form agglomerate with natural organic matter and can accumulate in sediments, so it is recommended to choose the most appropriate target organism for toxicology testing.

More detailed discussion can be found at:

https://echa.europa.eu/documents/10162/22334053/draft_appendix_r7a_caracal_en.pdf/1233776b-a684-c8e3-a5fd-7f279e7200b2

https://echa.europa.eu/documents/10162/22334053/draft_appendix_r7b_caracal_en.pdf/ca2de51a-3068-2d4c-0eeb-a449df41bd2d

https://echa.europa.eu/documents/10162/22334053/draft_appendix_r7c_caracal_en.pdf/f6af9bb9-2d23-9ba9-abc3-430d10ddba98

The hazard assessment of nanomaterials should be done across the whole lifecycle, so the choice of the sample tested is crucial to interpreting the results. The current testing requirements for a bulk substance are deemed adequate for nanomaterials under REACH with some modifications. These are being continuously developed and are likely to appear in guidance documents as they are revised.