

Workpackage WP H: Harmonization

Lead: BfR

The aim of the WP is support harmonization between laboratories with a focus on harmonization on a European and US level. The harmonization will be conducted by means of three techniques applied within this project: cytotoxicity assay, sp-ICP-MS and comparison between PDA and Laser diffraction.

Cell-based assays are widely used to determine if chemical compounds have an effect on cellular proliferation or have a cytotoxic response leading to cell death. From the various potential assays susceptible to be used in this project, the MTS assay has been chosen because it is a one-step assay and therefore introduces fewer variations than other available protocols when used by different partners and, because it is also being used in various interlaboratory efforts at the European Level (NANoREG). The MTS test is based on the capability of a soluble tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-cyrboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) to form a formazan product in the presence of phenazine methosulfate. This product has an absorbance at 490-500 nm in phosphate buffered saline. Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell, therefore, reduction of MTT and other tetrazolium dyes depends on cellular metabolic activity due to NAD(P)H flux. The harmonization work package will carry out a throughout study of a viability test of high impact (widely used and easily implemented) to assess the causes and effects of every step of the assay susceptible to introducing variability among different laboratories. This exercise, also being carried out in other EU initiatives such as NANoREG, will complement on-going studies and contribute to the implementation of an in vitro strategy for MNM safety assessment. MNM to be used in this study are also part of the core group of MNM within the NANoREG initiative, increasing the impact of this harmonization exercise. Harmonization efforts towards nanosafety assessment will provide useful data to the international harmonization exercise carried out under the umbrella of the Nanosafety cluster and other national and international initiatives.

Based on the ISO draft ISO-NP TS 19590 "Nanoparticles - Detection and characterization using single-particle ICP-MS" (new project approved) – further in depth assessment will be evaluated to create the foundation for the inclusion of metal oxides, considering complex matrices, into the ISO guide.