The Ultimate Goal – Achieving Comparability in a Nano-Cytotoxicity Assay Measurements

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Abstract:

Cytotoxicity assays are routinely used as first tier screen to identify potential hazards. They need to be adopted for assessing potential hazards in engineered nanomaterials (ENM). There is a considerable pressure for industry and regulatory bodies to have available validated nano-cytotoxicity assays. These types of assays most often lack an elaborate equation of the measurand (measurement model). Hence they are based on a lose correlation between ENM concentrations and a biological effect. Therefore it is important to develop high-quality assays that provide a strategy to ensure confidence in the measurement results and their uncertainty estimates. Such an assay will include an appropriate experimental design with a number of suitable controls and a detailed experimental procedure. An interlaboratory comparison can verify transferability of the procedure. Furthermore we obtain an idea of the comparability of the results and an estimate of the measurement uncertainty. In addition we are able to identify the size and relevance of influence quantities. This helps verifying the chosen approach.
A common assay for cell viability and therefore also usable for cytotoxicity is the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. It is an assay with just a few operator interactions and hence an ideal starting point for such prove of principles. In this talk some of the considerations and tools that brought the MTS assay to a reasonable level of reliability and reproducibility will be presented. Based on a cause and effect analysis Empa and NIST engineered an assay plate design with a number of controls. They provide measurements related to each of the steps in the measurement procedure (e.g. pipetting, cell ceding etc.). If these measurement results are within given specifications, then they provide confidence that the test results of unknown samples are valid and can be compared with other test results that have been gained with the same assay. This approach will be illustrated with the results of a recent interlaboratory comparison between 5 laboratories (Empa, KRISS, JRC, Nanotech and NIST). Our results indicate that control measurements are critical for achieving confidence in the measurement results and that comparability of nano-cytotoxicity assays results can be achieved in this way. With all theses measures the MTS-assay is now “fit for purpose”.