Comparison of Visual Inspection to CellTiter-Glo® in Evaluating Cytotoxicity in the LUMI-CELL® ER Bioassay

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There is a need to determine if a decreased response observed (for agonist and in particular antagonist testing) in an endocrine disruptor screening assay is a result of lower activity due to a chemical-receptor response or whether the compound is cytotoxic, resulting in a lower observed response. A comparison study was conducted in cooperation with NICEATM to compare cytotoxicity measured using the Visual Inspection assay with that obtained using Promega's CellTiter-Glo® assay which is a method of estimating viable cell number based on quantitation of ATP. Visual Inspection viability score codes were developed with: 1 = normal cell morphology and cell density, 2 = altered cell morphology and small gaps between cells, 3 = altered cell morphology and large gaps between cells and, 4 = few (or no) visible cells. Comparison of the Visual Inspection and CellTiter-Glo® assays demonstrate that, in general, a score of 1 corresponded to greater than 80% viability, 2 corresponded to 80 – 60% viability, 3 corresponded to 60 – 40% viability and 4 corresponded to less than 40% viability. Eight coded compounds were selected to test for estrogenic activities and eight coded substances to test antagonist activities in the LUMI-CELL® ER assay, a cell based assay in which estrogenic chemicals induce firefly luciferase. The CellTiter-Glo® and Visual Inspection assays both were able to detect cytotoxicity, with a significant correlation between the two techniques for determining cytotoxicity. Based on this limited database, either of these methods may be useful in determining the cytotoxicity of chemicals and extracts tested in the LUMI-CELL® ER assay, thereby eliminating cytotoxicity-dependent false positives. Supported by NIEHS Contract N01-ES-85424, NIEHS SBIR ES10533-03 and Superfund Basic Research Grant ES04699.