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Retrofitting an Estrogen Receptor Transactivation Assay with Metabolic Competence Using Alginate Immobilization of Metabolic Enzymes (AIME)

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The VM7Luc4E2 estrogen receptor (ER) transactivation assay is an OECD approved method (TG 457) for the detection of ER agonists and antagonists, and is also part of the Tox21 high-throughput screening (HTS) portfolio. Despite its international acceptance as a screening assay, immortalized cell lines such as VM7Luc4E2 do not express a full complement of xenobiotic metabolizing enzymes. This has led to calls for improved methods for the incorporation of metabolic competence into *in vitro* assays, particularly those used in the detection of endocrine active chemicals. The Alginate Immobilization of Metabolic Enzymes (AIME) platform is an HTS-compatible solution that retrofits existing assays with metabolic competence by attaching alginate-hepatic S9 microspheres to solid supports extending from microplate lids. To determine if the AIME platform could be coupled with the VM7Luc4E2 assay, methoxychlor (MXC) was used as a proof-of-concept reference chemical for bioactivation to an ER agonist. AIME lids were prepared using Aroclor-1254 induced rat hepatic S9, heat-inactivated S9, and alginate-only (empty) microspheres. Cells were maintained in 384-well plates with phenol-red free DMEM/1% FBS supplemented with an NADPH regeneration system and treated in 15-point concentration-response with 17β-estradiol (E2, positive control) and MXC. AIME lids were added to the microplates and incubated with the test compounds for 8 hours at 37ºC. The AIME lids were removed, the conditioned medium transferred to estrogen-stripped VM7Luc4E2 cells and further incubated for 24 hours at 37ºC after which luciferase activity was measured. The results indicate that the AIME platform produced the expected increase in MXC agonist activity with average EC_{50} values of 6.54 µM and 19.01 µM for active S9 and empty microspheres, respectively. The average EC_{50} value for MXC in the heat-inactivated control was 96.02 µM indicating that some compound may be sequestered by protein binding. A shift in the E2 activity curve from an EC_{50} of 28.92 pM (empty microsphere) to an EC50 of 1,556 pM (active S9) provided further confirmation of the robust metabolic activity conferred with the AIME method. These results demonstrate the utility of the AIME platform as a metabolic retrofit for specific HTS assays. *This abstract does not necessarily reflect the policy of the US EPA.*