SETAC HTS 2018

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 international acceptance as a screening assay, immortalized cell lines such as VM7Luc4E2, which was derived from the MCF7 human breast cancer cell line, 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 a more potent ER agonist. AIME lids were prepared using phenobarbital/β-naphthoflavone- or aroclor-1254 induced rat hepatic S9 as well as heat-inactivated S9 used as a protein binding control. Phenol-red free DMEM/1% FBS supplemented with an NADPH regeneration system was added to 96-well microplates and dosed in 8-point concentration-response with 17β-estradiol (positive control; no metabolism) and MXC. AIME lids were added to the microplates and incubated with the test compounds for 2 hours at 37°C. The AIME lids were removed, the conditioned medium transferred to estrogen-stripped VM7Luc4E2 cells, and further incubated for 24 hours at 37°C prior to measuring luciferase activity. The results demonstrate that the AIME platform produced nearly a 10-fold decrease in the EC50 value of MXC with active S9 microspheres (0.71 µM) compared to the heat-inactivated control (6.8 µM). A similar, albeit smaller, fold change was observed between the EC50 values observed with active S9 microspheres and MXC tested without the AIME lid (4.98 µM) indicating that some MXC may be sequestered by protein binding. Overall, these results demonstrate the potential 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.*