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

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# Evaluating Xenobiotic Metabolism in High-Throughput Chemical Screening

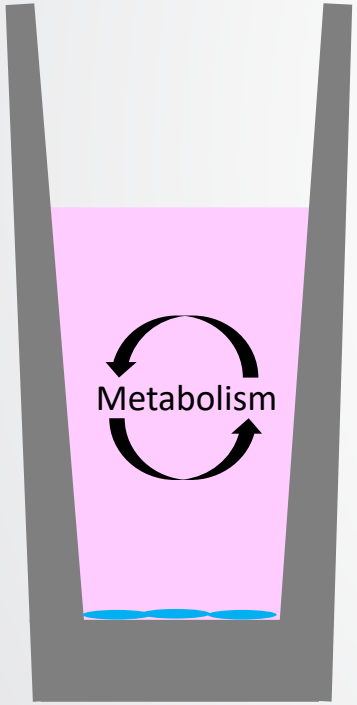
- The US EPA's ToxCast program consists of approximately **600 assay endpoints** that are comprised of both **cell-free** and **cell-based** technologies which are run in **high-throughput screening (HTS)** platforms.

- **Problem**: Uncertainty regarding the effects of liver- and tissue-specific xenobiotic metabolism on these assay endpoints.

- **Objective**: Incorporate metabolic capabilities onto existing HTS assay platforms to provide a more comprehensive evaluation of potential toxicological hazards.

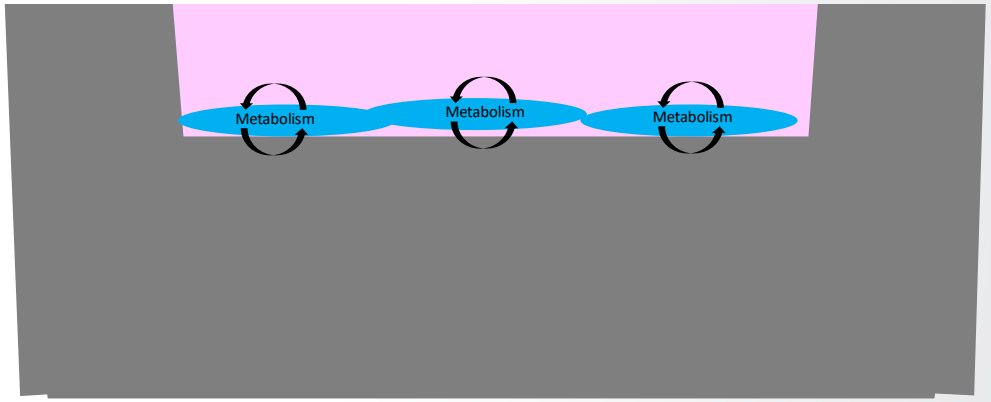
# Technical Strategies for Retrofitting High-throughput Assays with Metabolic Capabilities

## *Extracellular/Cell-free Method*




- Capable of metabolizing chemicals in the medium of both cell-based assays and cell-free assays
- More closely models hepatic metabolism and effects of circulating metabolites

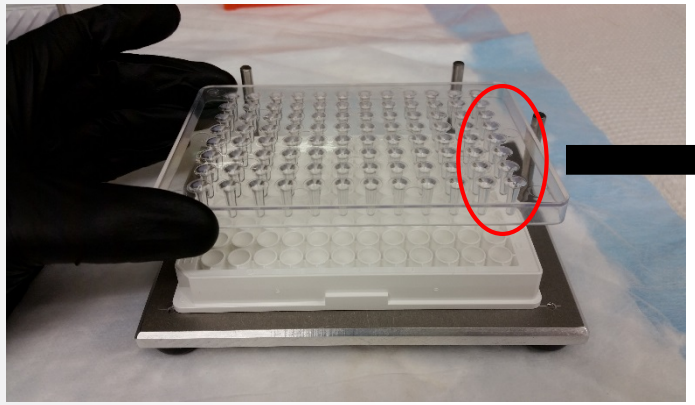
## *Cell-based Method*




- Capable of metabolizing chemicals inside the cell, but only for cell-based assays
- More closely models effects of direct-acting metabolites

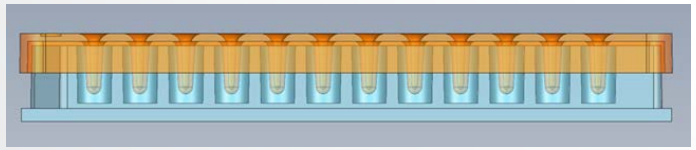
# Alginate Immobilization of Metabolic Enzymes (AIME)

***The AIME platform retrofits existing HTS assays with metabolic competence by encapsulating and attaching induced rat liver homogenate (S9) to solid supports extending from custom microplate lids.***



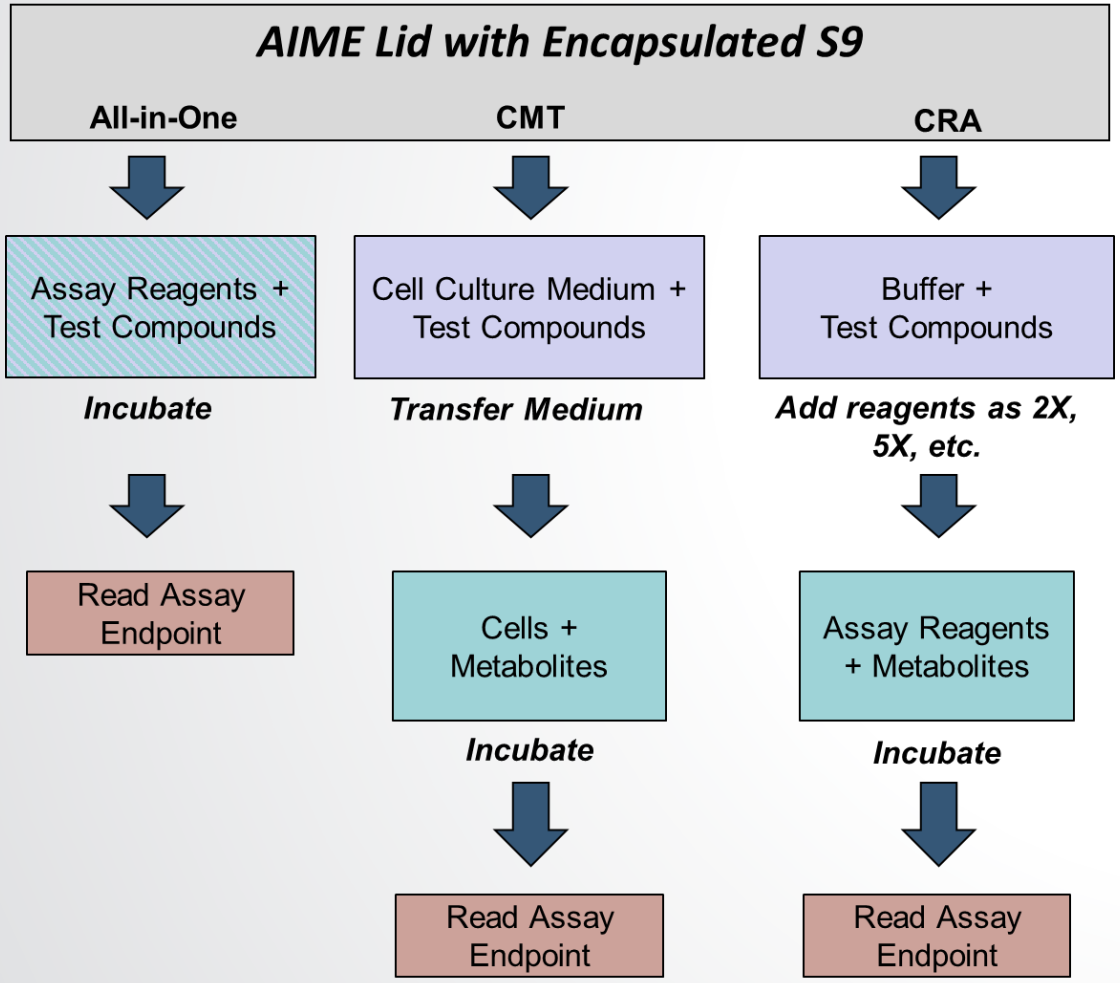
AIME Lid & 96-Well Assay Plate

Enlargement of AIME alginate/S9 microspheres



Cross section of AIME Lid & Assay Plate

# AIME Deployment Strategies for Cell-Based & Cell-Free Assays




**All-in-One Method:** Metabolism of test compounds & assay are run simultaneously.

**Conditioned Medium Transfer (CMT):** Test compound metabolism occurs separately & prior to assay. Medium containing metabolites is then transferred to the assay plate.

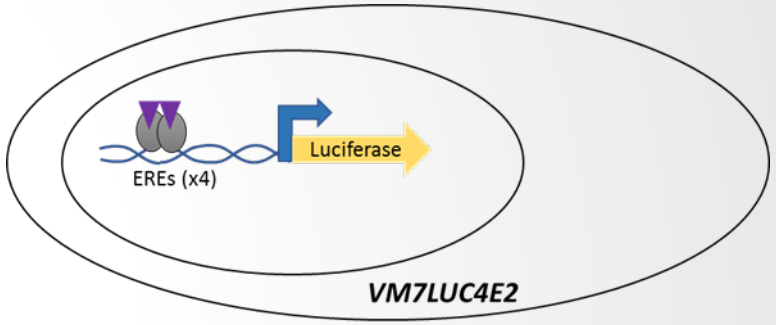
**Concentrated Reagent Addition (CRA):** Test compound metabolism occurs in the assay plate in conditions favorable to enzymatic activity. Following metabolite generation, concentrated reagents are added to initiate the assay.

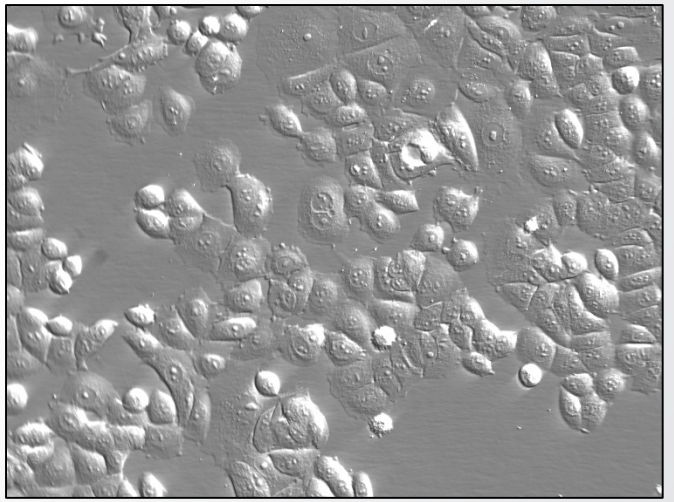
# VM7Luc4E2 Estrogen Receptor Transactivation Assay

- Developed by Jane Rogers and Michael Denison (*In Vitro & Molecular Toxicology, 2000)*
  - Human breast carcinoma cells (MCF-7 variant) containing a stably integrated ER-responsive luciferase reporter gene
  - Originally designated as BG1Luc4E2
  - Endogenously expresses ERα
  - Little to no expression of ERβ

- OECD approved method for the detection of ER agonists and antagonists (TG455/457)

- Part of theTox21 high-throughput screening portfolio



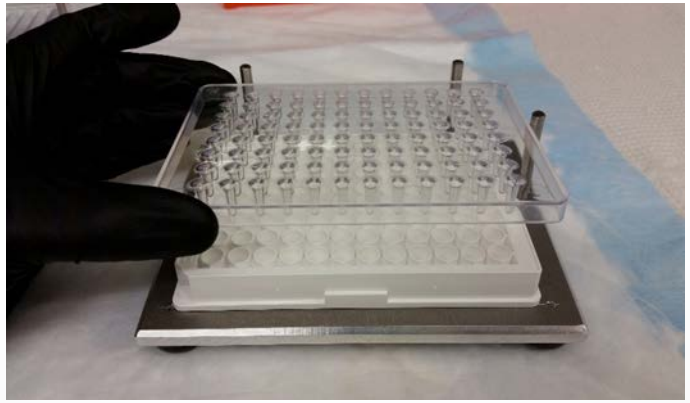
# Retrofitting the VM7Luc4E2 ER Transactivation Assay with AIME

## VM7Luc4E2 Estrogen Receptor TA

**Plate estrogen stripped cells in microplate** → **Recover 24 hours & remove medium** → **Medium containing metabolites** → **Determine luciferase activity**

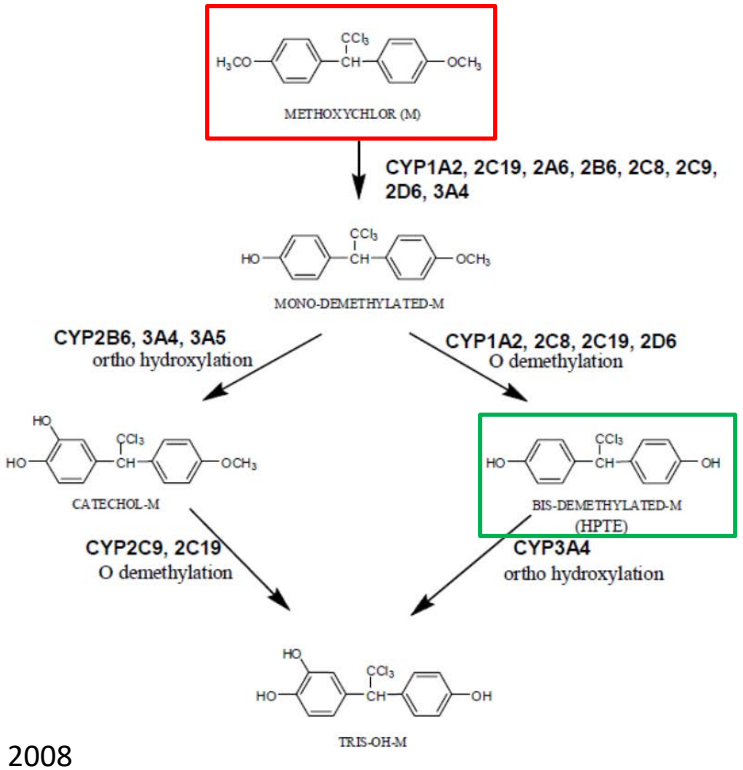
***Conditioned Medium T***


**Dose test compounds into medium**

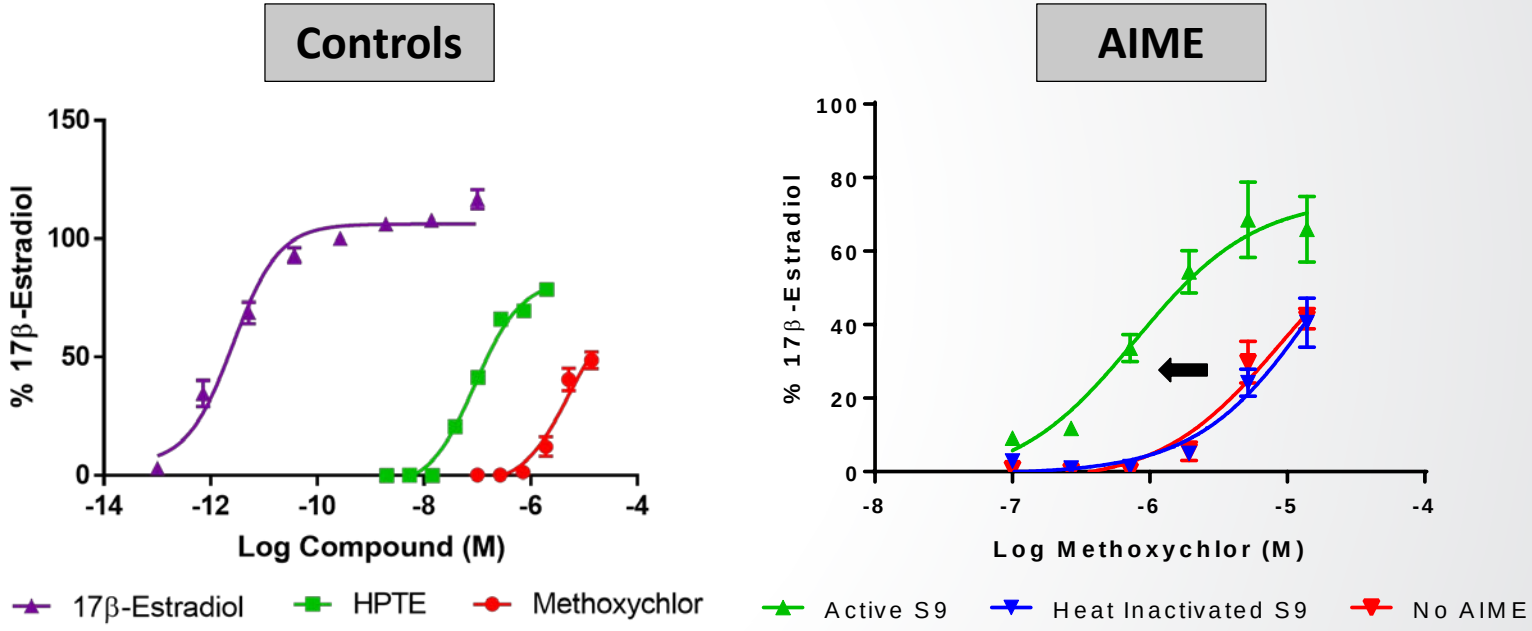


***Incubate test compounds with AIME lid***


# Methoxychlor – A Reference Chemical for the Influence of Metabolism on Estrogen Receptor Activity

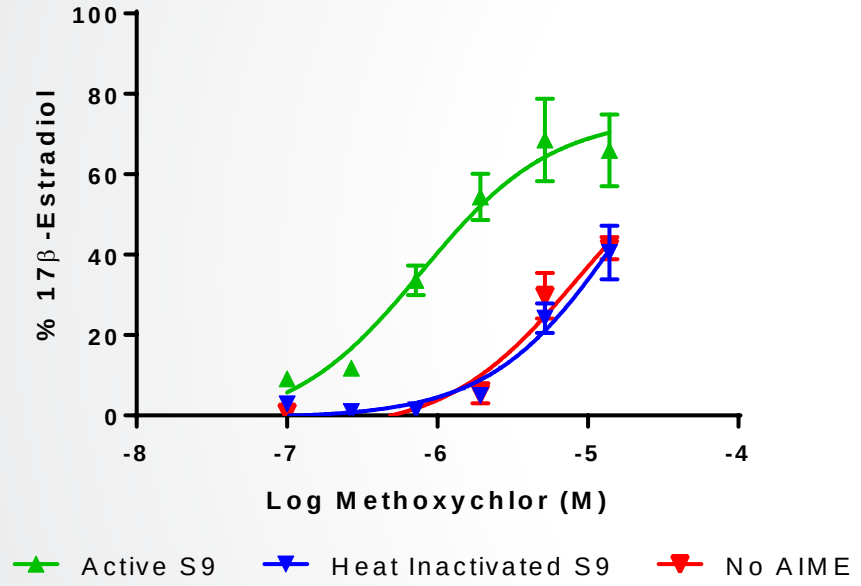


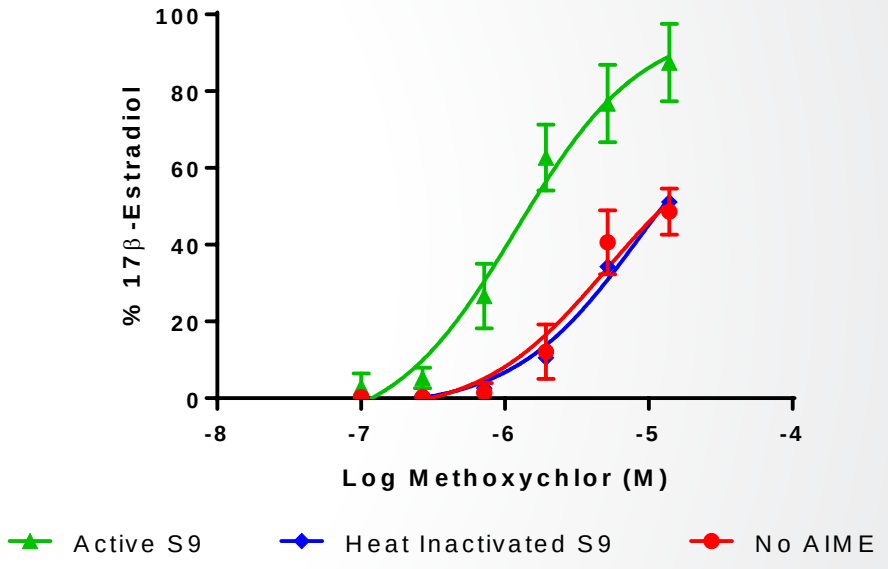

OECD 2008




***Potency shift between methoxychlor and HPTE defines assay window for estrogenic metabolites***

# The AIME Platform Can Be Successfully Scaled to a 384-Well Format

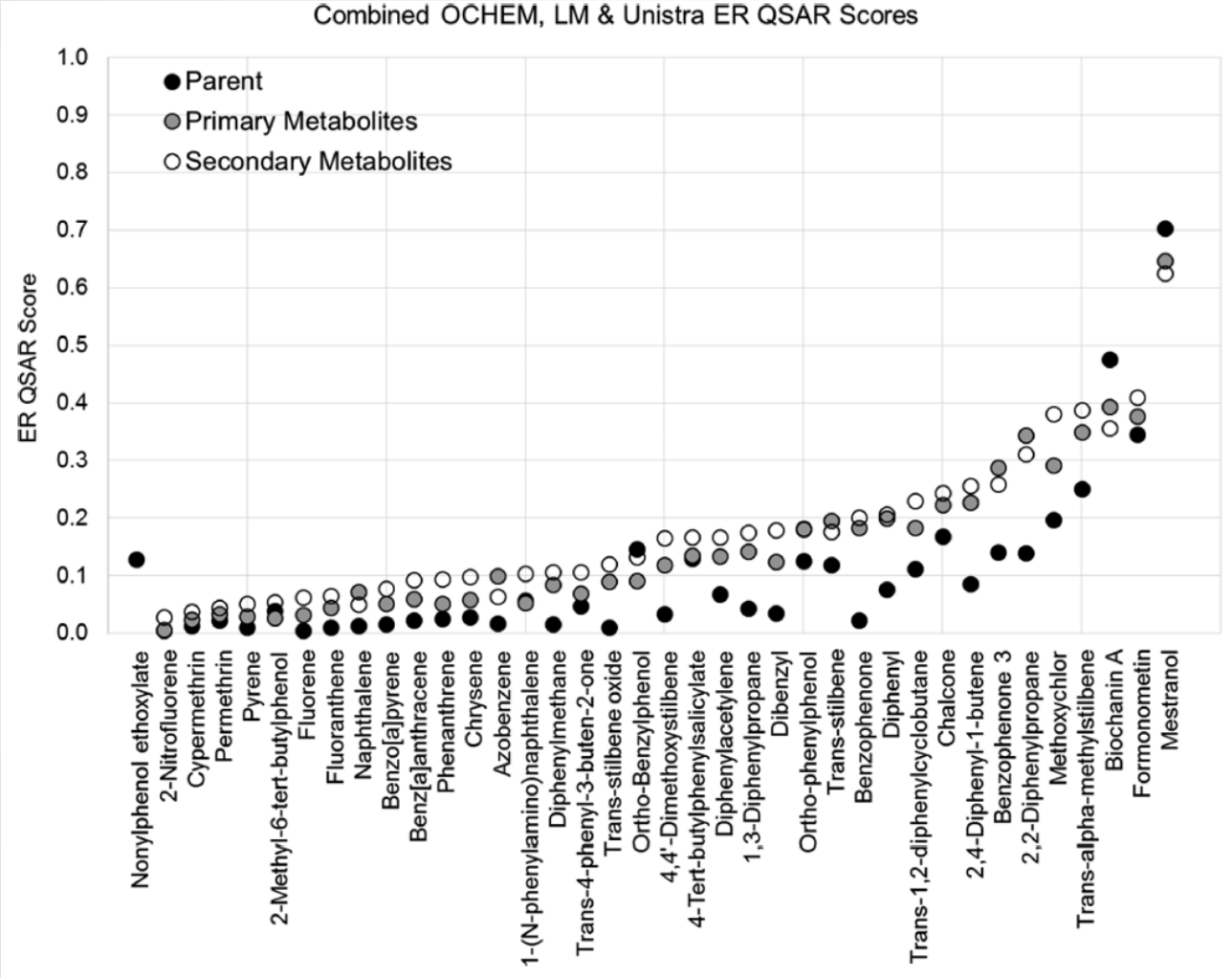



| 96-Well | EC50 (μM) | AUC |
|---|---|---|
| Active S9 | 0.78 | 88.20 |
| Heat Inactivated S9 | 16.86 | 22.82 |
| No AIME | 8.44 | 24.91 |

| 384-Well | EC50 (μM) | AUC |
|---|---|---|
| Active S9 | 1.20 | 92.87 |
| Heat Inactivated S9 | 8.40 | 37.10 |
| No AIME | 5.07 | 33.86 |

# Screening for Predicted Estrogenic Metabolites in the AIME-coupled VM7Luc4E2 Assay




***Proof-of-Concept - Screening of "Pinto Library"***

- 38 chemicals with reported estrogenic metabolites (predicted true positive)
- 12 chemicals with no predicted estrogenic metabolites (predicted true negative)
- 20 additional chemicals of interest (VM7Luc4E2 assay positive & negative controls)

Pinto *et al*. (2016). Prediction of Estrogenic Bioactivity of Environmental Chemical Metabolites. *Chemical Research in Toxicology*

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