Chemical Screening in an Estrogen Receptor Transactivation Assay with Metabolic Competence

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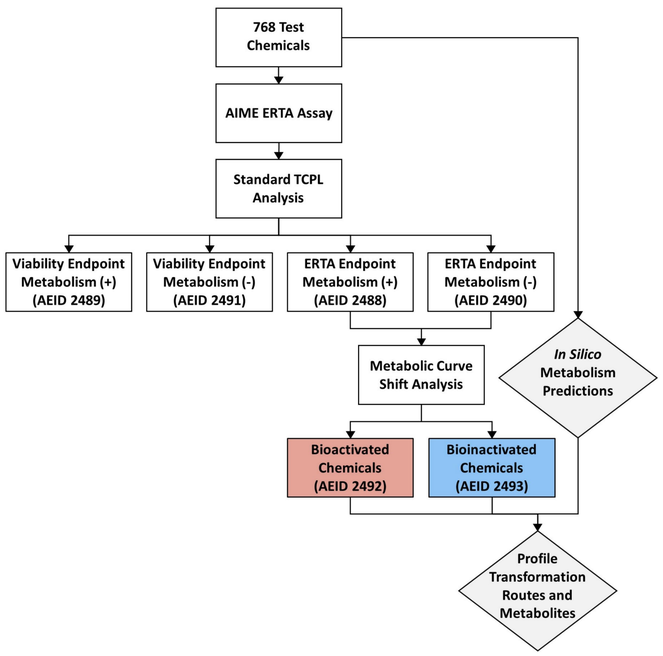
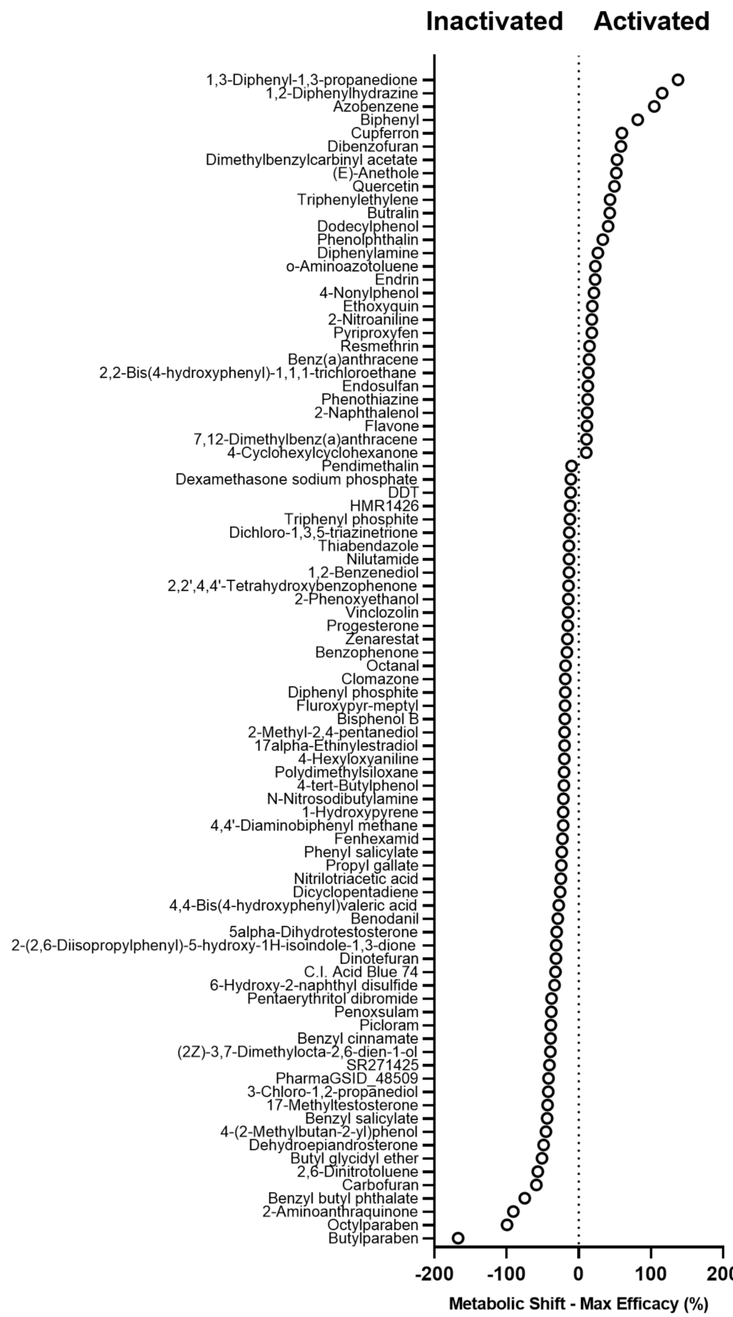
Abstract

The U.S. EPA continues to utilize high-throughput screening data to evaluate potential biological effects of endocrine active substances without the use of animal testing. Determining the scope and need for *in vitro* metabolism in high-throughput assays requires the generation of larger data sets that assess the impact of xenobiotic transformations on toxicity-related endpoints. The objective of the current study was to screen a set of 768 ToxCast chemicals in the VM7Luc estrogen receptor transactivation assay (ERTA) using the Alginate Immobilization of Metabolic Enzymes (AIME) hepatic metabolism method. Chemicals were screened with or without metabolism to identify estrogenic effects and metabolism-dependent changes in bioactivity. Based on estrogenic hit calls, 85 chemicals were active in both assay modes, 16 chemicals were only active without metabolism, and 27 chemicals were only active with metabolism. Using a novel metabolism curve shift method that evaluates the shift in concentration-response curves, 29 of these estrogenic chemicals were identified as bioactivated and 59 were bioinactivated. Human biotransformation routes and associated metabolites were predicted *in silico* across the chemicals to mechanistically characterize possible transformation-related ERTA effects. Overall, the study profiled novel chemicals associated with metabolism-dependent changes in ERTA bioactivity, and suggested routes of biotransformation and putative metabolites responsible for the observed estrogenic effects. The data demonstrate a range of metabolism-dependent effects across a diverse chemical library and highlight the need to evaluate the role of intrinsic xenobiotic metabolism for endocrine and other toxicity-related health effects. *The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

Study Design

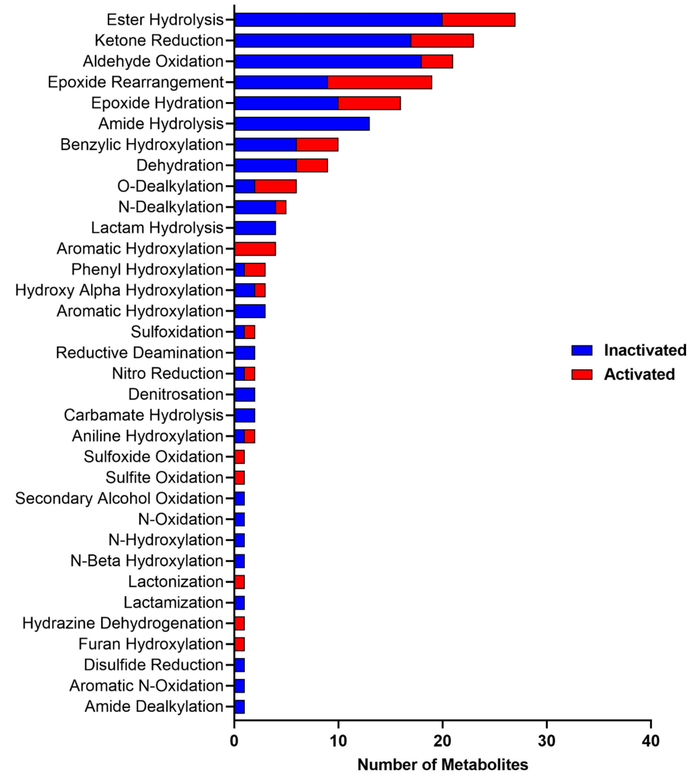


Metabolism-dependent Changes in Estrogen Bioactivity



88 chemicals exhibiting metabolism-dependent changes in ERTA bioactivity. The AIME assay was conducted with rat hepatic S9 fractions. Compounds are rank-ordered by maximum efficacy of the curves fit in the metabolic curve shift analysis. A positive directional trend indicates bioactivation (activated; n=29) and a negative trend indicates bioinactivation (inactivated; n=59). The zero-centered dashed line indicates no relative change in activity with metabolism.

In Silico Prediction of Mechanisms and Metabolites



Predicted metabolites and mechanisms of biotransformation. The number of human hepatic phase I metabolites predicted across each mechanism of biotransformation for the chemicals identified as having metabolism-dependent effects. Metabolites were predicted out to two generations by the EPA Chemical Transformation Simulator. Activation (red) and inactivation (blue) of parent chemicals corresponding to the predicted metabolites.

Conclusions

- Adaptation of the AIME method to a high-throughput 384-well format is a viable approach to retrofitting *in vitro* assays with hepatic metabolism.
- *In vitro* screening profiled novel chemicals associated with metabolism-dependent changes in ERTA bioactivity.
- *In silico* predictions suggested routes of biotransformation and putative metabolites responsible for the observed estrogenic effects.
- The data support refinement of the prioritization and prediction of estrogenic chemicals using an *in vitro* New Approach Method with metabolic competence, contributing toward a broader goal of reducing animal testing.
- **Citation**: Chemical Screening in an Estrogen Receptor Transactivation Assay with Metabolic Competence, Toxicological Sciences, 2022;, kfac019, https://doi.org/10.1093/toxsci/kfac019