United States Environmental Protection Agency

Screening ToxCast Chemicals in an Estrogen Receptor Transactivation Assay with Metabolic Competence

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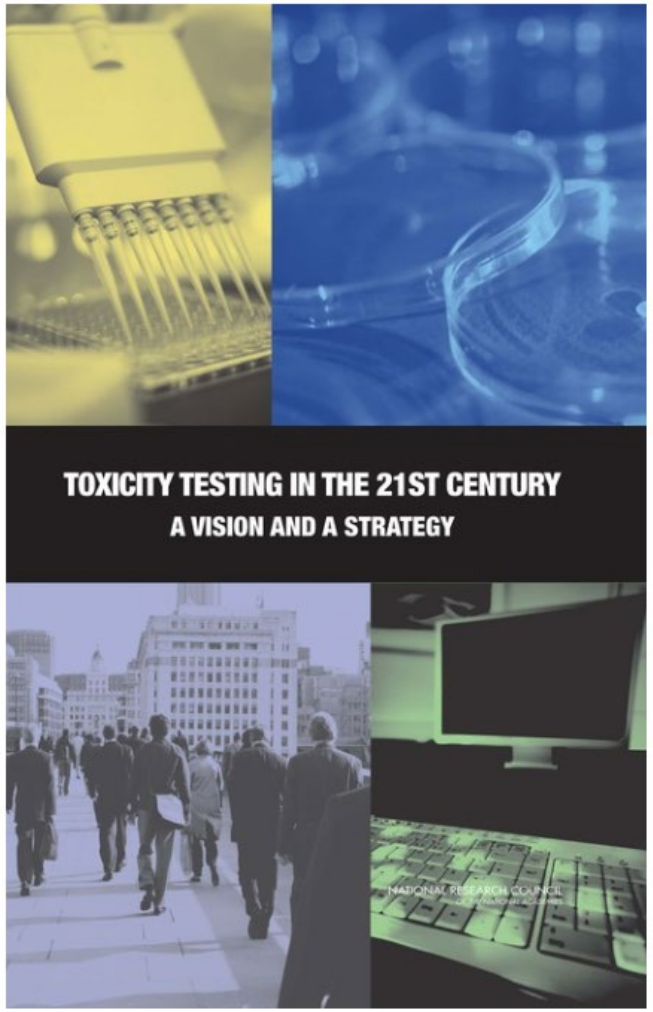
21st-Century Challenges for 21st-Century Toxicology

National Research Council 2007 report calling for a genuine commitment to the reduction, refinement, and replacement of animal testing.

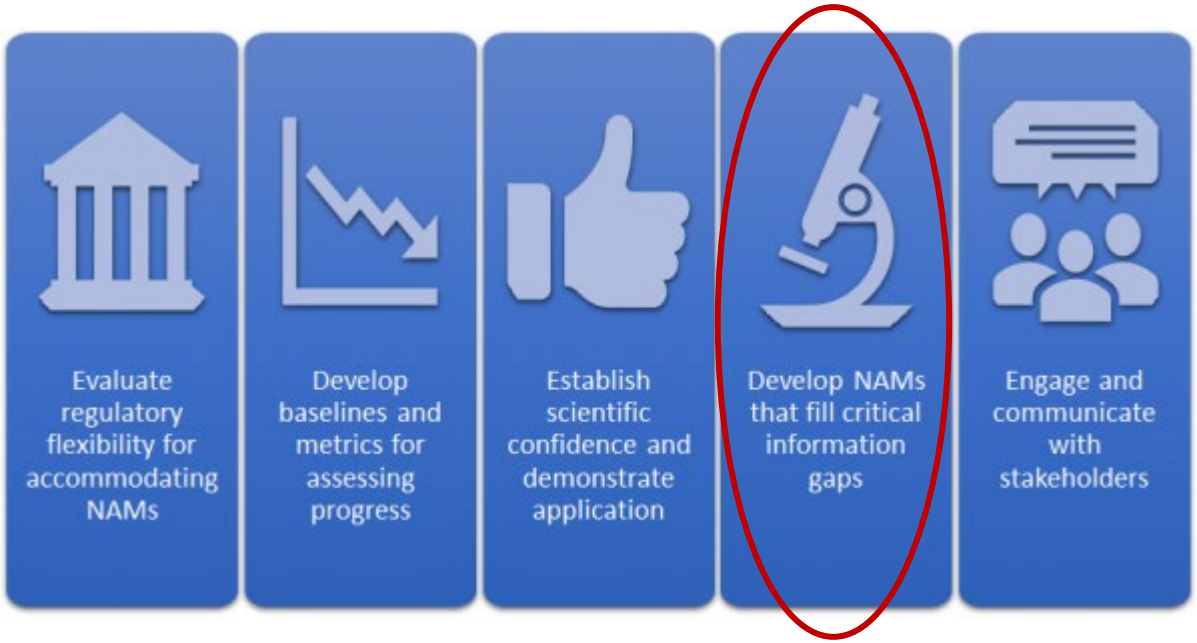
Key Questions for Implementation – Addressing Xenobiotic Metabolism

- "One of the challenges of developing an *in vitro* test system to evaluate toxicity is the current inability of cell assays to mirror metabolism in the integrated whole animal..."
- Methods to Predict Metabolism - How can adequate testing for metabolites in the high-throughput assays be ensured?
- Recommendations
  - Screening using computational approaches where possible.
  - Limited animal studies that focus on mechanism and specific metabolites.



http://nap.edu/11970. DOI 10.17226/11970

EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing



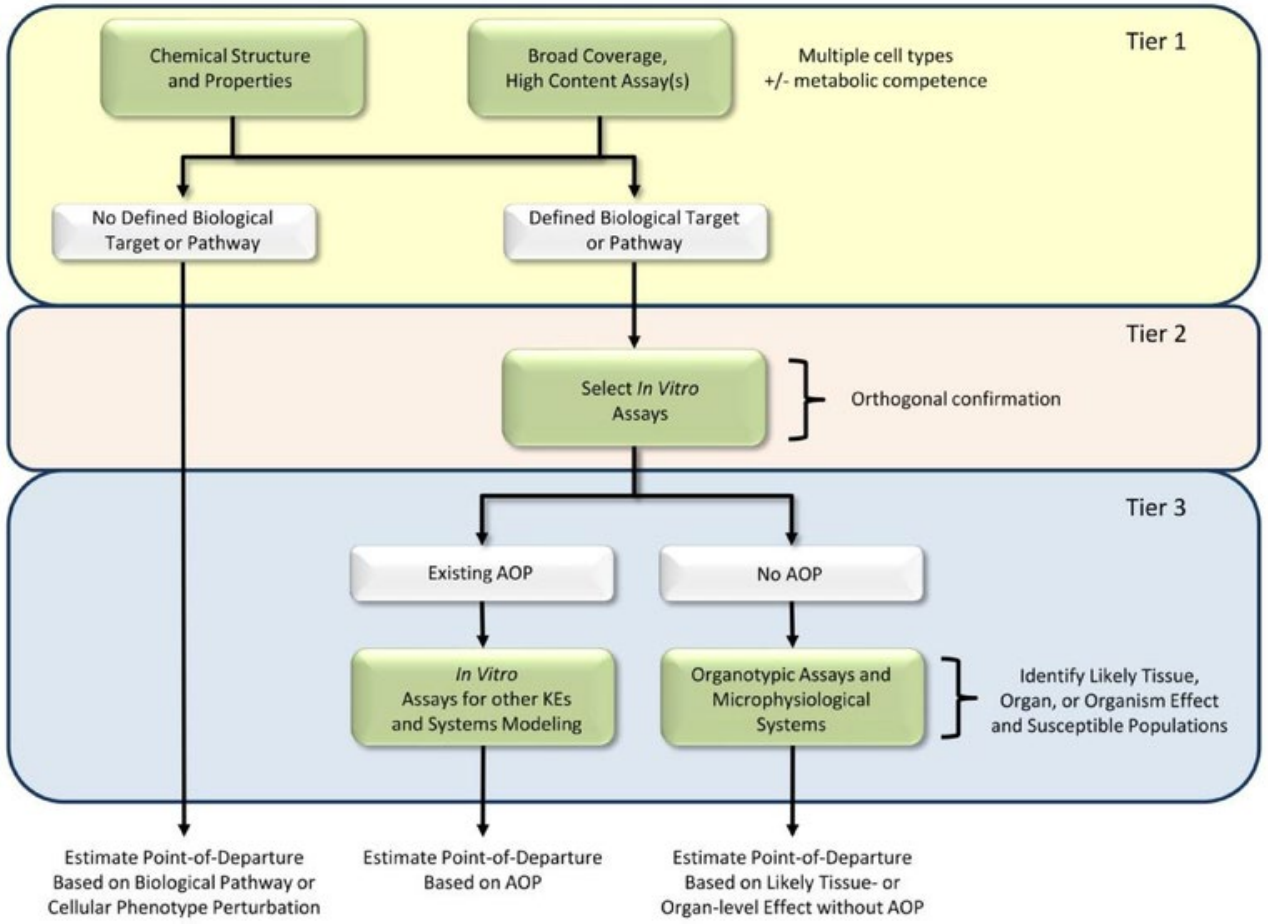
Five work plan objectives

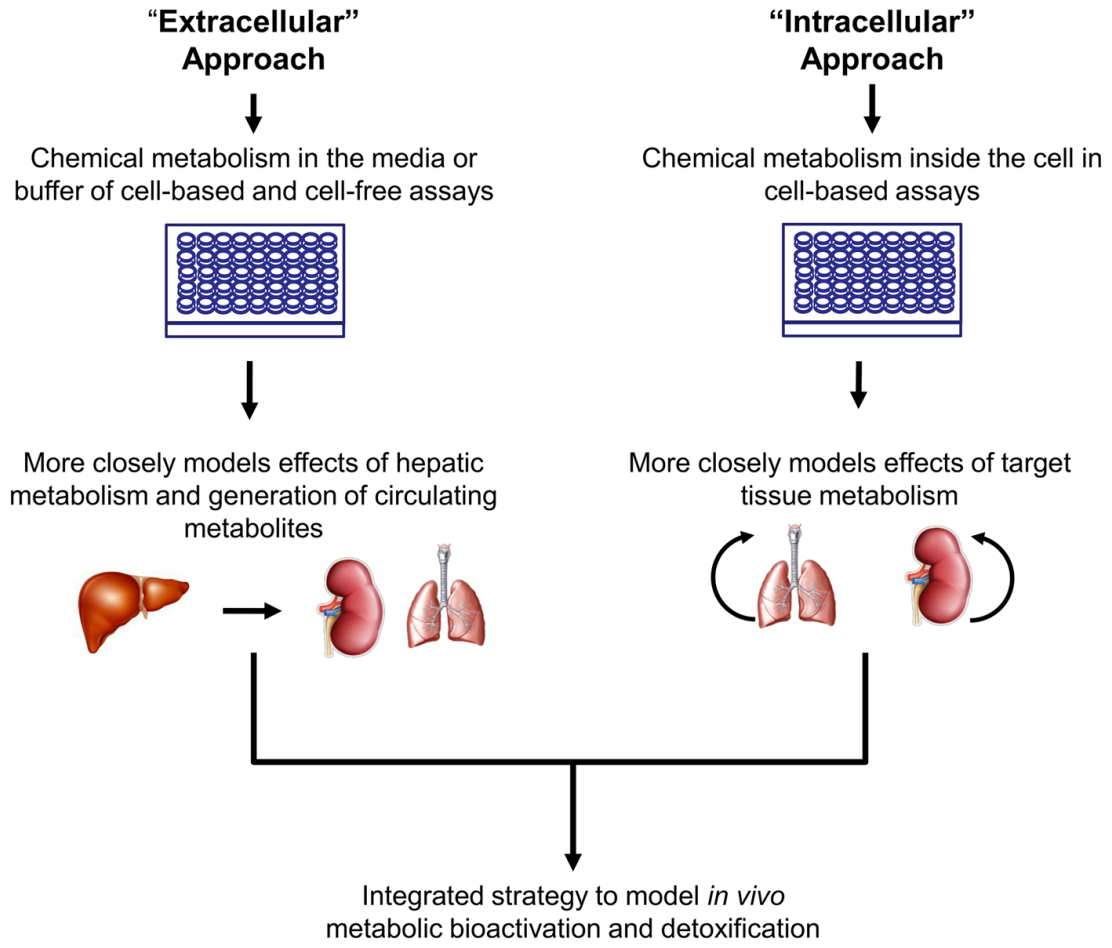
Examples of information gaps

- Inadequate coverage of biological targets.
- Limited capability to address tissue- and organ-level effects.
- Lack of robust integrated approaches to testing and assessment (IATAs).
- Minimal capability for addressing xenobiotic metabolism in *in vitro* test systems.

https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf

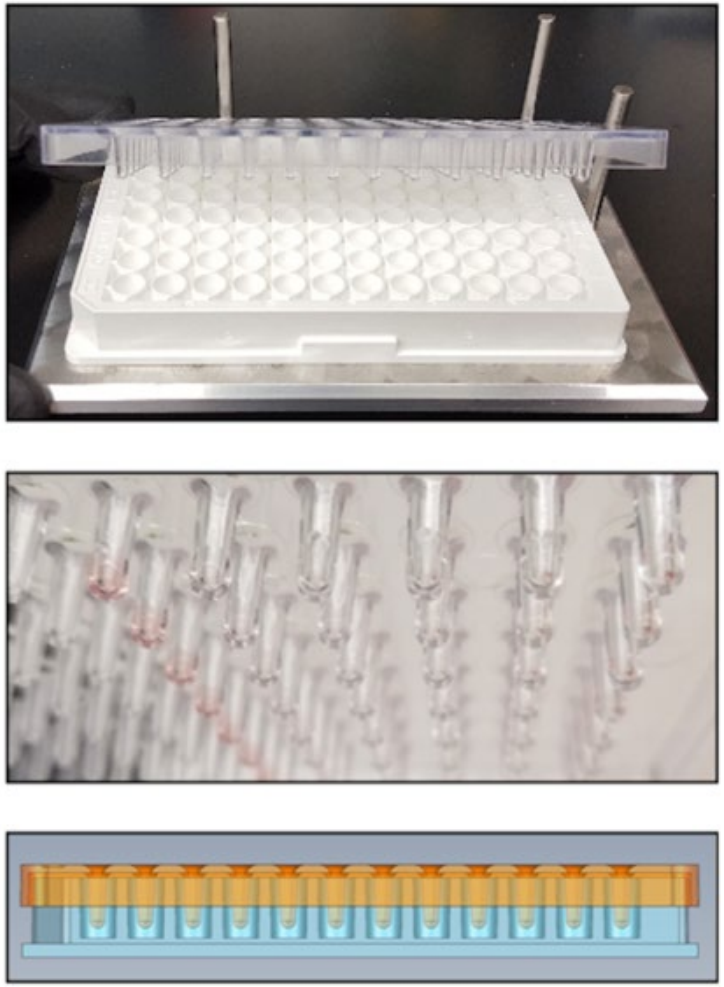
The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

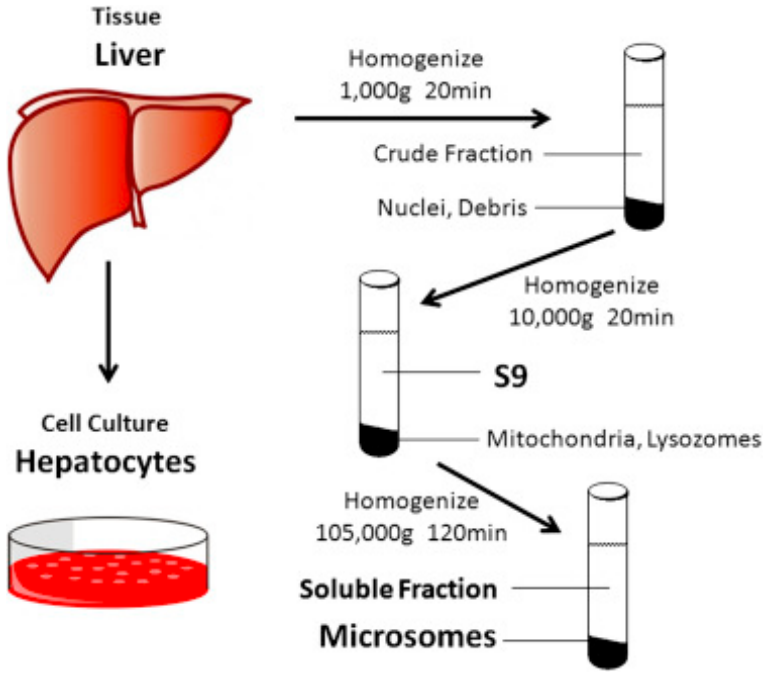




Toxicol Sci. 2019 Jun 1;169(2):317-332. doi: 10.1093/toxsci/kfz058

Extracellular Approach: The Alginate Immobilization of Metabolic Enzymes (AIME) Method





- **Liver Metabolism:** Hepatic S9 fractions derived from species of interest.
- **Alginate Hydrogel:** Widely used in a variety of pharmaceutical and biomedical applications due to high biocompatibility, low toxicity, and mild gelation by divalent cations.
- **AIME Method:** The AIME platform consists of custom 96- or 384-well microplate lids containing solid supports attached to encapsulated hepatic S9-alginate microspheres.

J. Vrbanac, R. Slauter. doi.org/10.1016/B978-0-12-803620-4.00003-7.

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Research Article

The Alginate Immobilization of Metabolic Enzymes Platform Retrofits an Estrogen Receptor Transactivation Assay With Metabolic Competence

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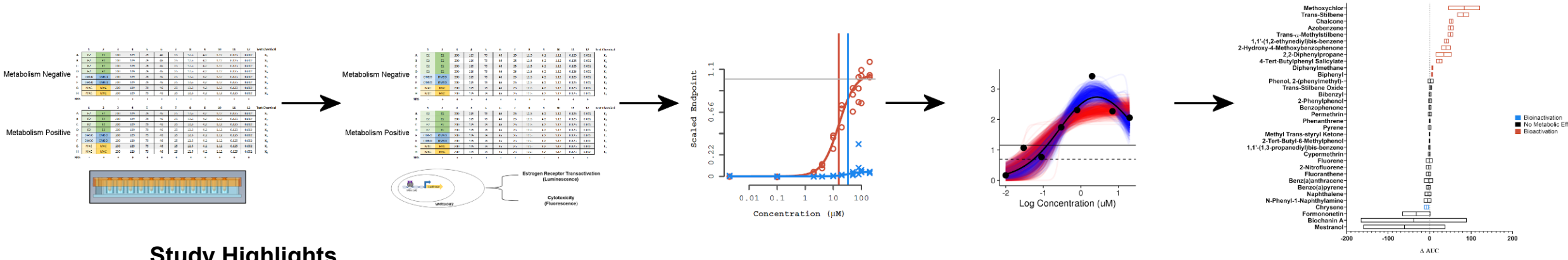
AIME Metabolism Assay → **Estrogen Receptor Transactivation Assay** → **ToxCast Pipeline** → **Toxboot Uncertainty Quantification** → **Rank Metabolism-dependent Bioactivity**



Study Highlights

- Reprioritization of hazard based on metabolism-dependent bioactivity.
- Demonstrated utility of applying the AIME method for identification of false positive and false negative target assay effects.
- Enhanced *in vivo* concordance with the rodent uterotrophic bioassay.

Screening ToxCast Chemicals in an Estrogen Receptor Transactivation Assay with Metabolic Competence

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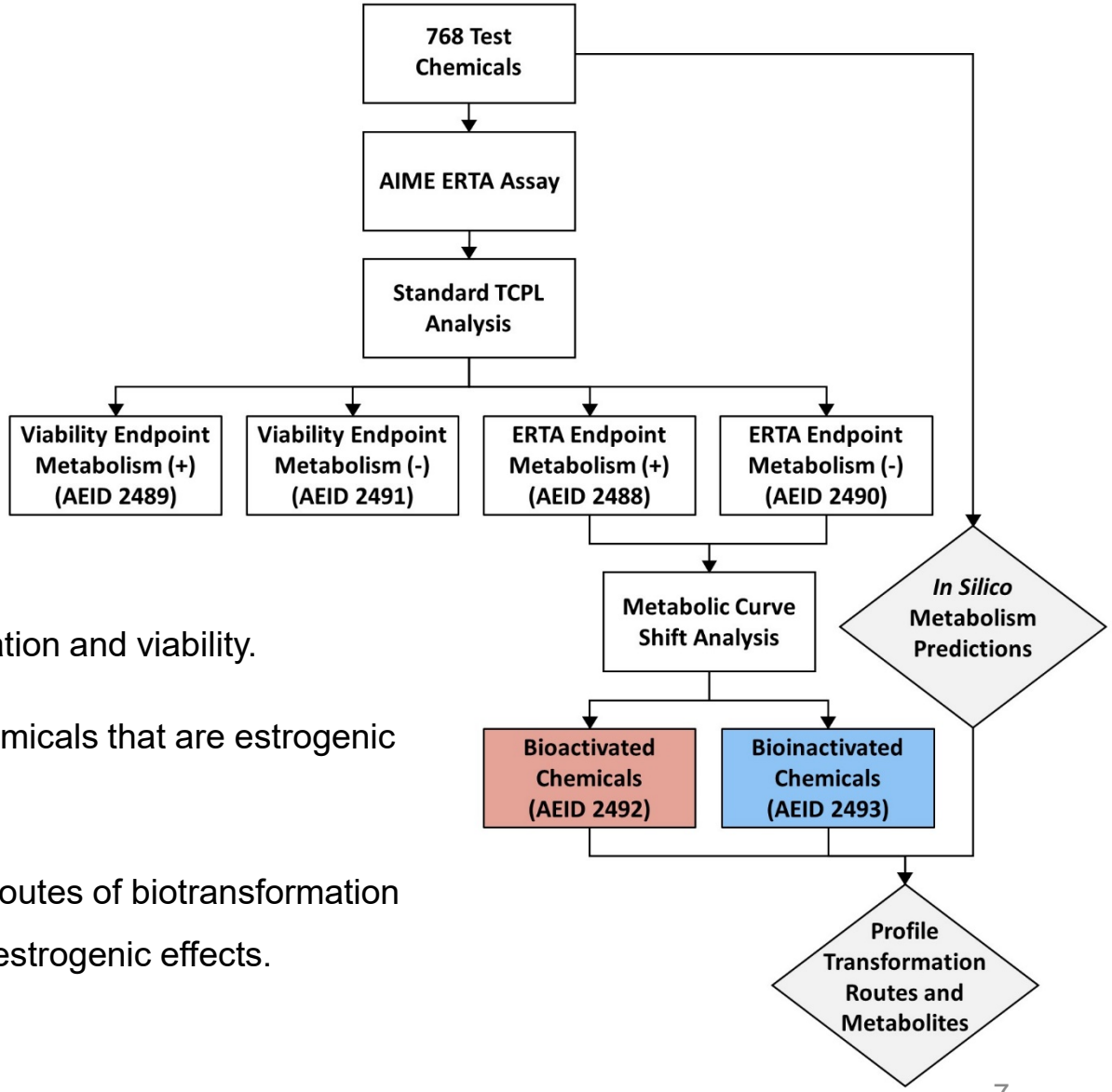
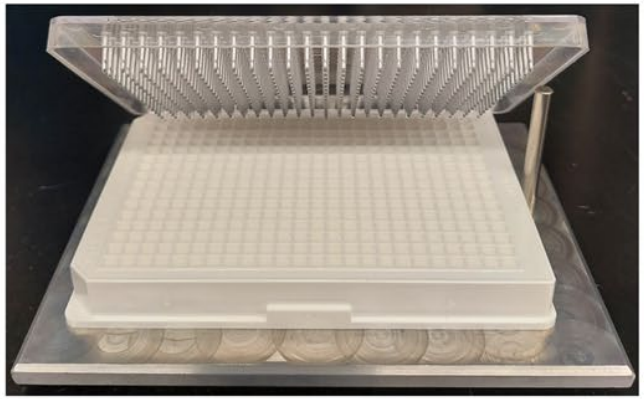


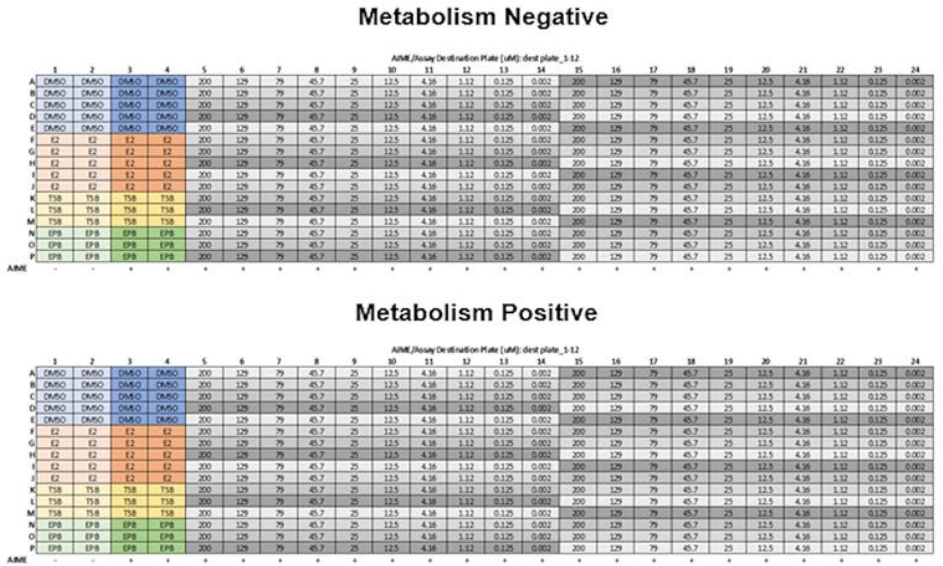
Study Highlights

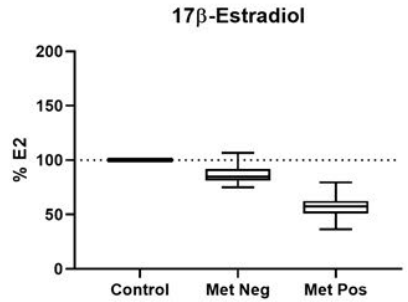
- **ToxCast Chemical Library**: Screen 768 chemicals for ER transactivation and viability.
- **Hazard Identification and Prioritization**: Identify and rank-order chemicals that are estrogenic and exhibit metabolism-dependent changes in bioactivity.
- ***In Silico* Prediction and Profiling**: Profile the common mechanistic routes of biotransformation and the identify of putative metabolites associated with the observed estrogenic effects.

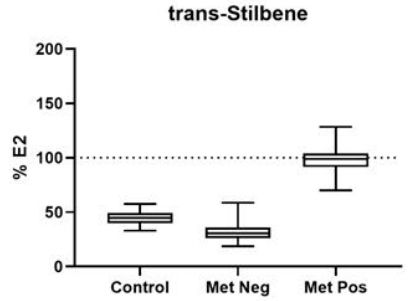
Deployment of the 384-well AIME Method to the VM7Luc Estrogen Receptor Transactivation Assay

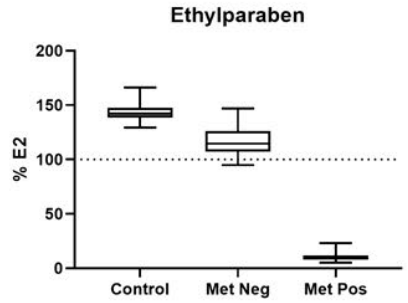
- **Target Bioassay**: OECD TG 455 - VM7Luc Estrogen Receptor Transactivation (ERTA) Assay
- **Metabolism Assay**: AIME system configured for rapid 384-well high-throughput screening.
- **Experimental Design**: Parallel evaluation of parent and metabolite effects.
- **Reference Chemicals**: Selected for ER target bioassay and the AIME metabolism assay.
  - ERTA: 17β-Estradiol
  - AIME (activated): *trans*-Stilbene
  - AIME (inactivated): Ethylparaben
- **Performance Statistics**: Determined for ERTA and AIME assay.







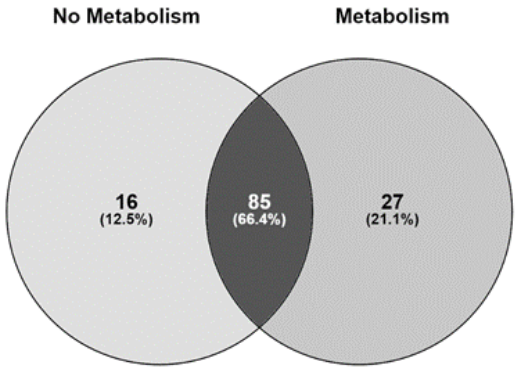


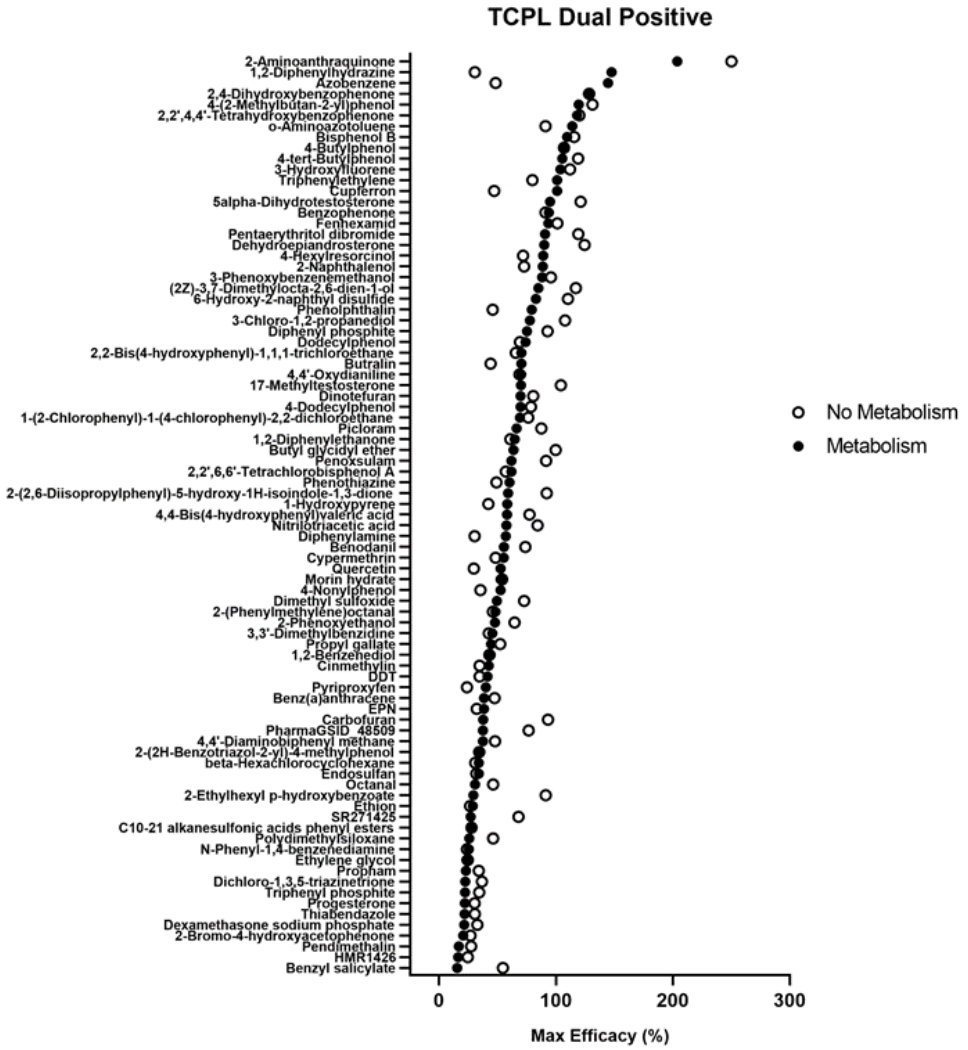


ToxCast Pipeline Analysis

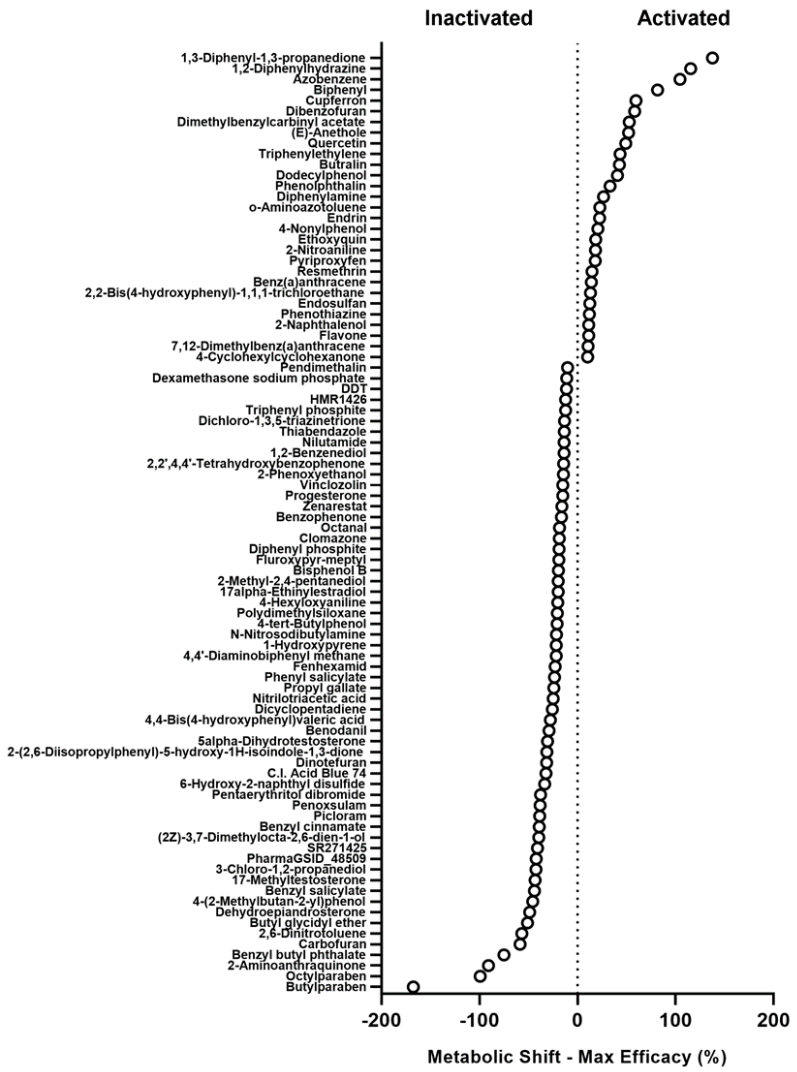
ToxCast Pipeline (TCPL) analysis run to identify estrogenic chemicals.

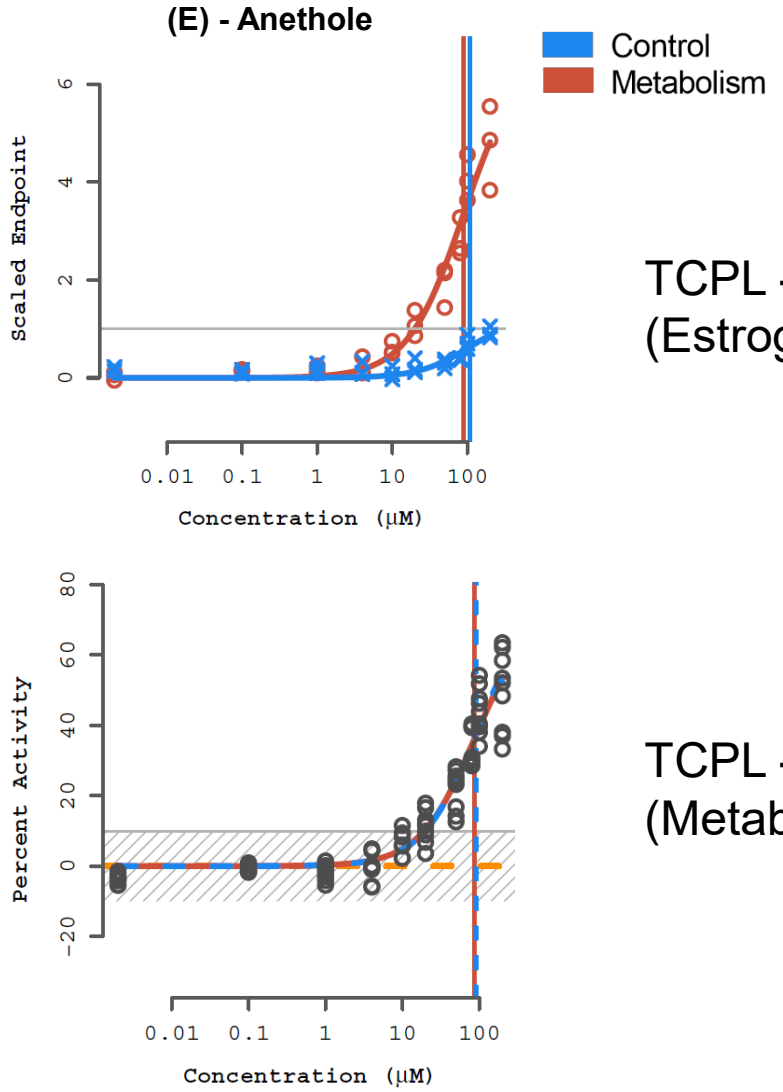
- 128/768 (17%) chemicals identified as bioactive for ER transactivation.
- The majority of chemicals (67%) are estrogenic with or without metabolism.
- A minor subset are bioactive exclusively with or without metabolism.





Metabolism Curve Shift Analysis





TCPL - Standard
(Estrogenic effect)

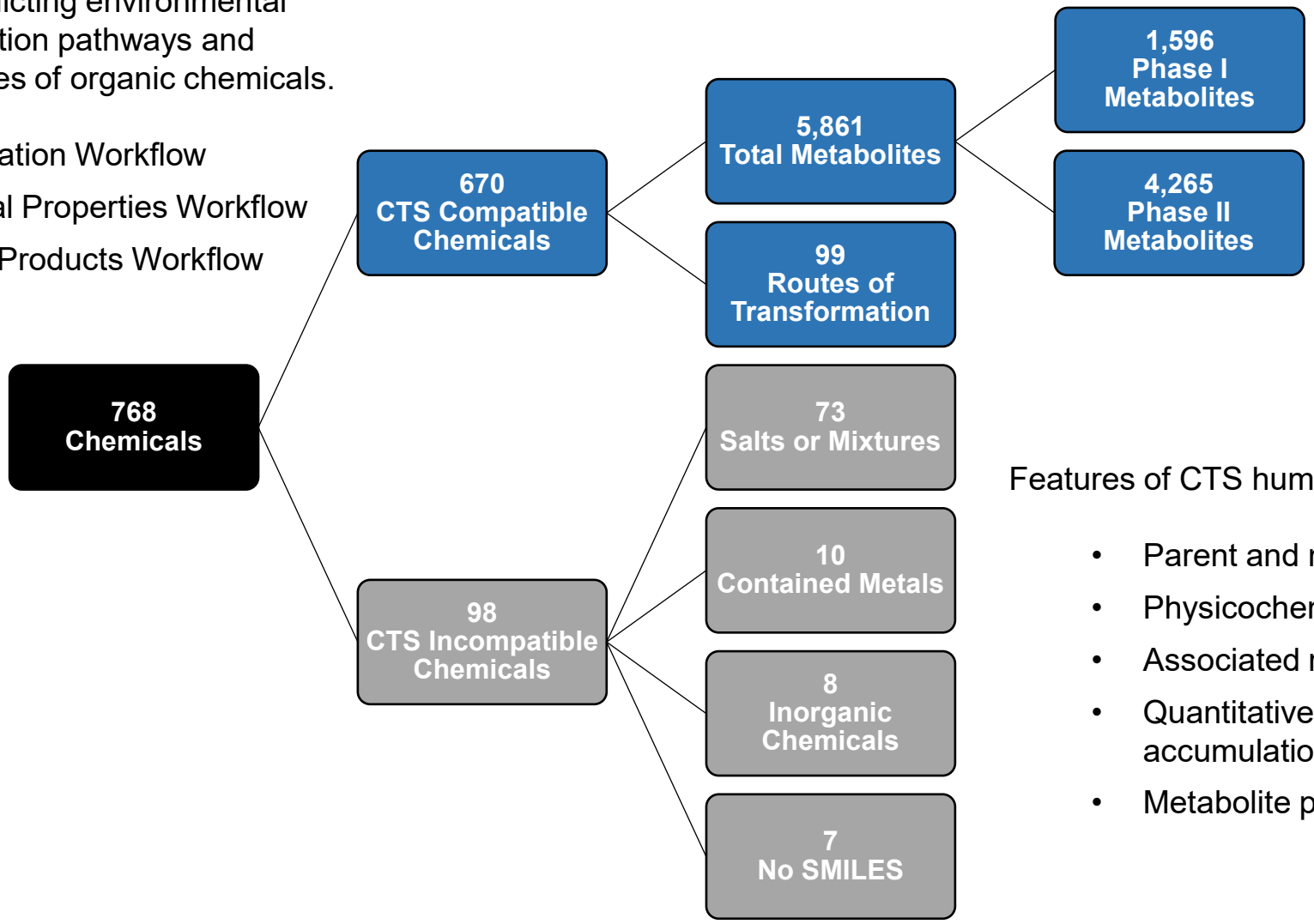
TCPL - Metabolic Curve Shift
(Metabolism effect)

- Screening-level classification of activated and inactivated chemicals.
- Reprioritization of hazard based on empirical shift in metabolism-dependent bioactivity.

In Silico Metabolite Predictions with Chemical Transformation Simulator

The Chemical Transformation Simulator (CTS) is a web-based tool for predicting environmental and biological transformation pathways and physicochemical properties of organic chemicals.

- Chemical Speciation Workflow
- Physicochemical Properties Workflow
- Transformation Products Workflow

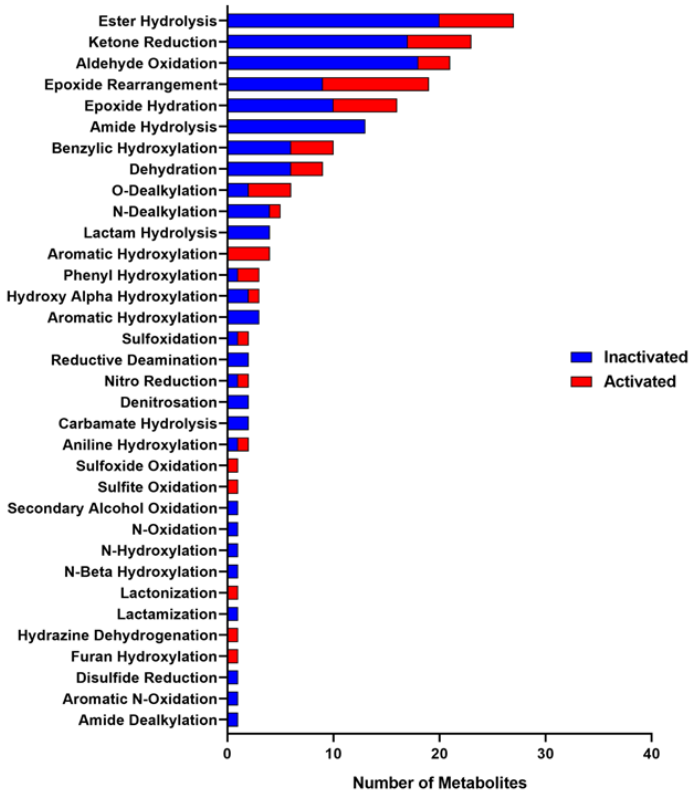


Features of CTS human phase I metabolite database:

- Parent and metabolite identifiers
- Physicochemical properties
- Associated routes of transformation
- Quantitative estimates for production and accumulation
- Metabolite probability classifications

https://qed.epa.gov/cts/

Profiling Predicted Metabolites and Common Mechanisms of Biotransformation



Profiled novel chemicals associated with metabolism-dependent changes in ERTA bioactivity:

- Common mechanistic routes of transformation.
- The identify of putative metabolites associated with the observed estrogenic effects.

Profiling Predicted Metabolites and Common Mechanisms of Biotransformation

CASRN	*Name*	*Classification*	*Metab Routes*	*Metab Global Accumulation*	*Metab Likelihood*	*Met_Neg Hitc*	*Met_Pos Hitc*	*Biotransformation*	*Metabolic Shift*
94-26-8	**Butylparaben**	Parent	NA	NA	NA	1	0	inactivated	-167.3818927
99-96-7	4-Hydroxybenzoic acid	Metabolite	EsterHydrolysis	0.4988	LIKELY	1	0	inactivated	-167.3818927
71-36-3	1-Butanol	Metabolite	EsterHydrolysis	0.4256	LIKELY	1	0	inactivated	-167.3818927
1219-38-1	**Octylparaben**	Parent	NA	NA	NA	1	0	inactivated	-99.32932278
99-96-7	4-Hydroxybenzoic acid	Metabolite	EsterHydrolysis	0.4987	LIKELY	1	0	inactivated	-99.32932278
111-87-5	1-Octanol	Metabolite	EsterHydrolysis	0.4255	LIKELY	1	0	inactivated	-99.32932278
85-68-7	**Benzyl butyl phthalate**	Parent	NA	NA	NA	1	0	inactivated	-74.98383135
88-99-3	Phthalic acid	Metabolite	EsterHydrolysis	0.2469	LIKELY	1	0	inactivated	-74.98383135
100-51-6	Benzyl alcohol	Metabolite	EsterHydrolysis	0.3104	LIKELY	1	0	inactivated	-74.98383135
71-36-3	1-Butanol	Metabolite	EsterHydrolysis	0.3177	LIKELY	1	0	inactivated	-74.98383135
118-58-1	**Benzyl salicylate**	Parent	NA	NA	NA	1	1	inactivated	-43.94470165
69-72-7	Salicylic acid	Metabolite	EsterHydrolysis	0.4944	LIKELY	1	1	inactivated	-43.94470165
100-51-6	Benzyl alcohol	Metabolite	EsterHydrolysis	0.4135	LIKELY	1	1	inactivated	-43.94470165
103-41-3	**Benzyl cinnamate**	Parent	NA	NA	NA	1	0	inactivated	-39.07342352
621-82-9	Cinnamic acid	Metabolite	EsterHydrolysis	0.4789	LIKELY	1	0	inactivated	-39.07342352
100-51-6	Benzyl alcohol	Metabolite	EsterHydrolysis	0.4092	LIKELY	1	0	inactivated	-39.07342352
121-79-9	**Propyl gallate**	Parent	NA	NA	NA	1	1	inactivated	-24.05419192
149-91-7	Gallic acid	Metabolite	EsterHydrolysis	0.4989	LIKELY	1	1	inactivated	-24.05419192
71-23-8	1-Propanol	Metabolite	EsterHydrolysis	0.4259	LIKELY	1	1	inactivated	-24.05419192
118-55-8	**Phenyl salicylate**	Parent	NA	NA	NA	1	0	inactivated	-23.42557027
69-72-7	Salicylic acid	Metabolite	EsterHydrolysis	0.4997	LIKELY	1	0	inactivated	-23.42557027
108-95-2	Phenol	Metabolite	EsterHydrolysis	0.4997	LIKELY	1	0	inactivated	-23.42557027
81406-37-3	**Fluroxypyr-meptyl**	Parent	NA	NA	NA	1	0	inactivated	-18.89353032
69377-81-7	Fluroxypyr	Metabolite	EsterHydrolysis	0.457	LIKELY	1	0	inactivated	-18.89353032
123-96-6	2-Octanol	Metabolite	EsterHydrolysis	0.4848	LIKELY	1	0	inactivated	-18.89353032

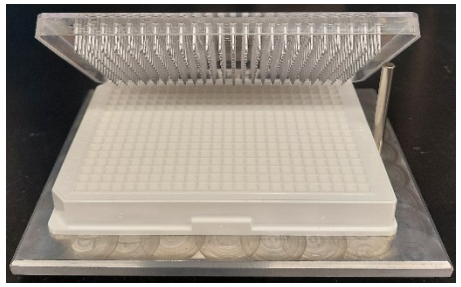
Parent and Metabolite Identifiers

Route of Transformation

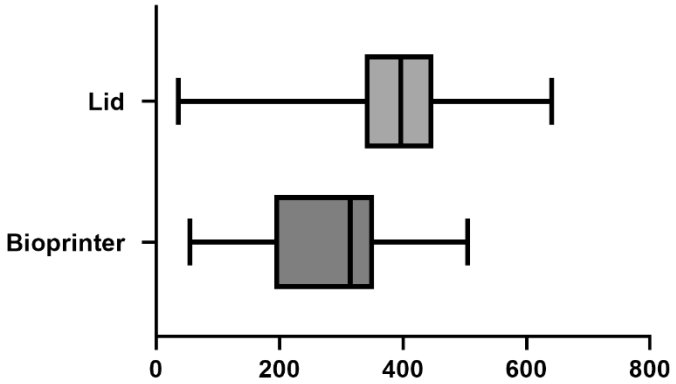
Accumulation and Probability Classification

In Vitro Metabolism Data

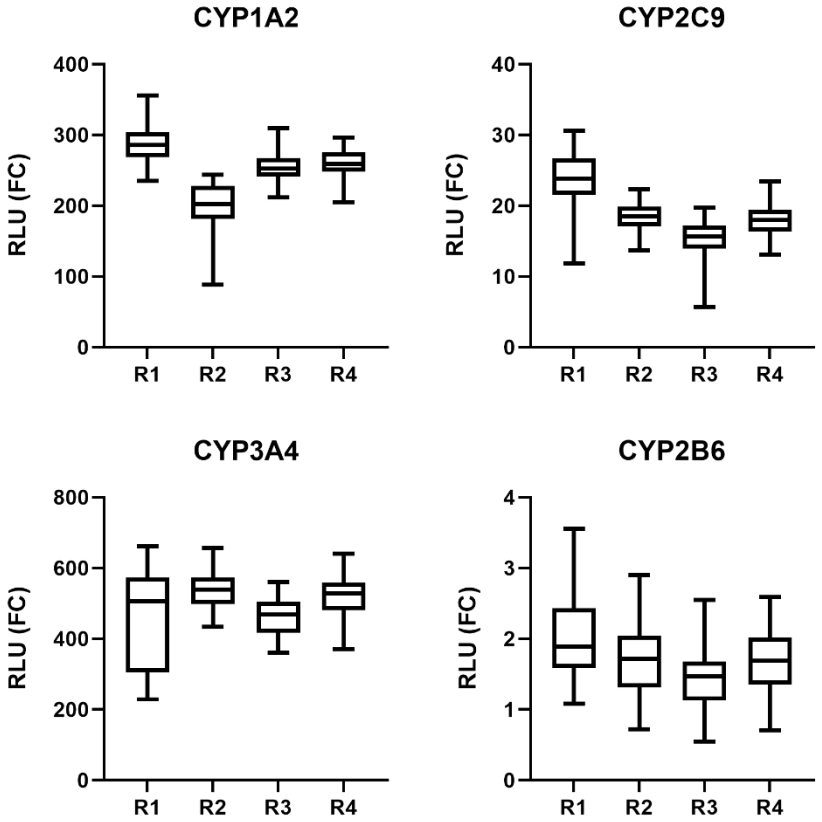
Adaptation of the Alginate Immobilization of Metabolic Enzymes Platform to a 3D Bioprinting Approach for Metabolism-based High-throughput Screening



Lid vs Bioprinter Method



Phase 1 Metabolism



Goal: Adapt the AIME method to an automated 384-well approach using bioprinting.

Kristen Hopperstad, PhD
21st Century Challenges
Flash Poster # 10

Acknowledgements

Kristen Hopperstad
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