United States Environmental Protection Agency

Retrofitting *in vitro* Systems with Metabolic Competence

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US EPA CSS-HERA BOSC Chemical Safety Subcommittee Meeting
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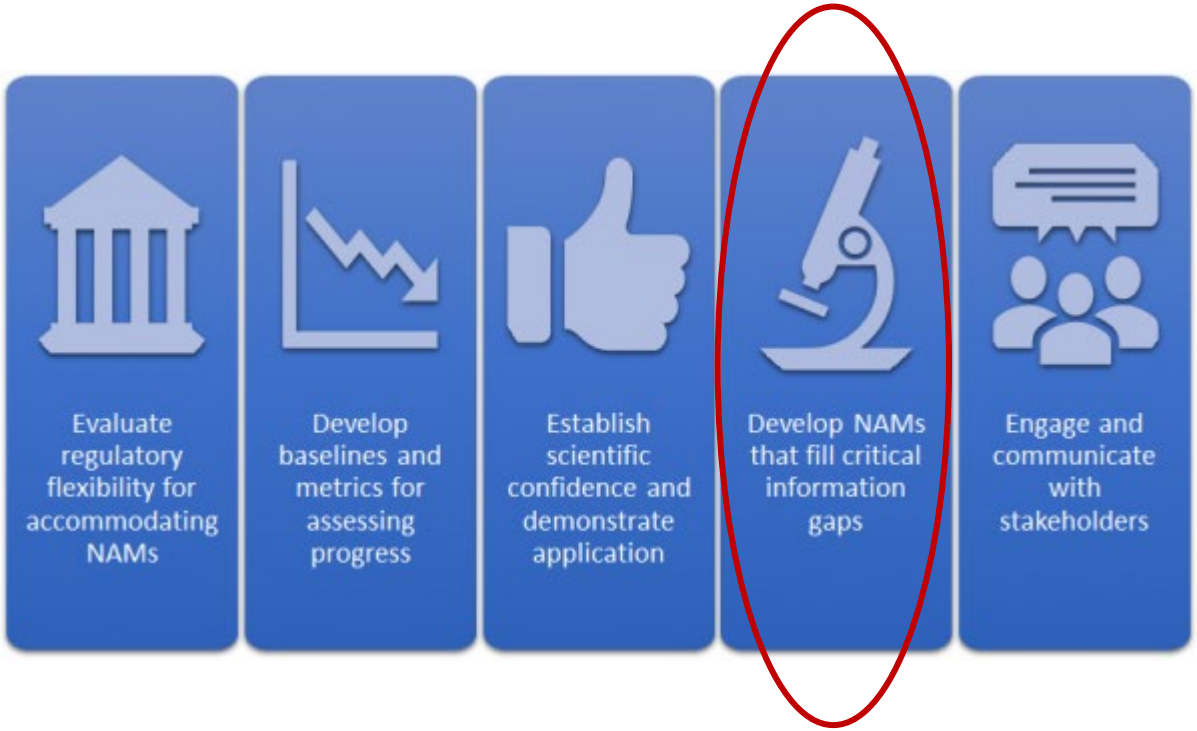
EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing



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

Outline

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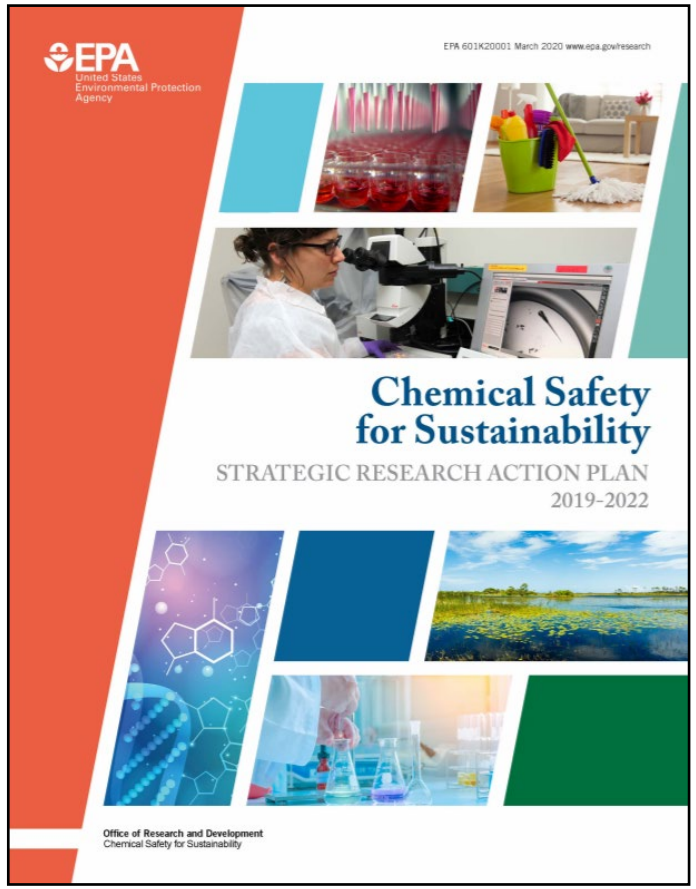


CSS.1.5 (High Throughput Toxicology): Develop and apply methods to incorporate endogenous and exogenous xenobiotic metabolism into high-throughput *in vitro* assays.

CSS.1.5.1: Application of the Alginate Immobilization of Metabolic Enzymes (AIME) method to incorporate hepatic metabolism into an Estrogen Receptor transactivation assay.

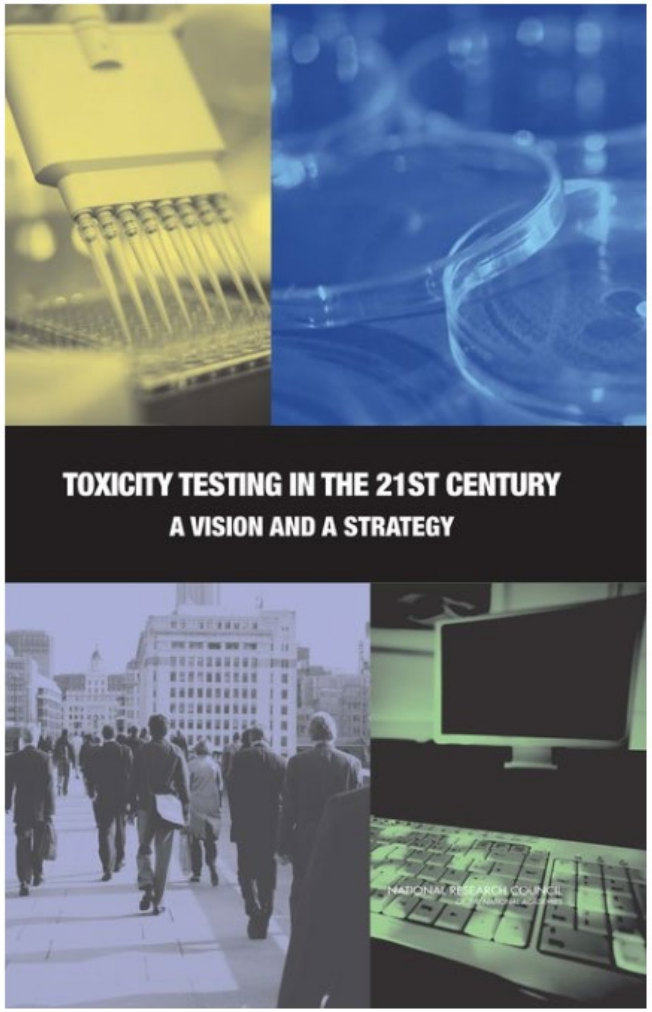
CSS.1.5.2: Development of a bioprinting approach to adapt the Alginate Immobilization of Metabolic Enzymes metabolism method for high-throughput screening applications.

Toxicity Testing in the 21st Century

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

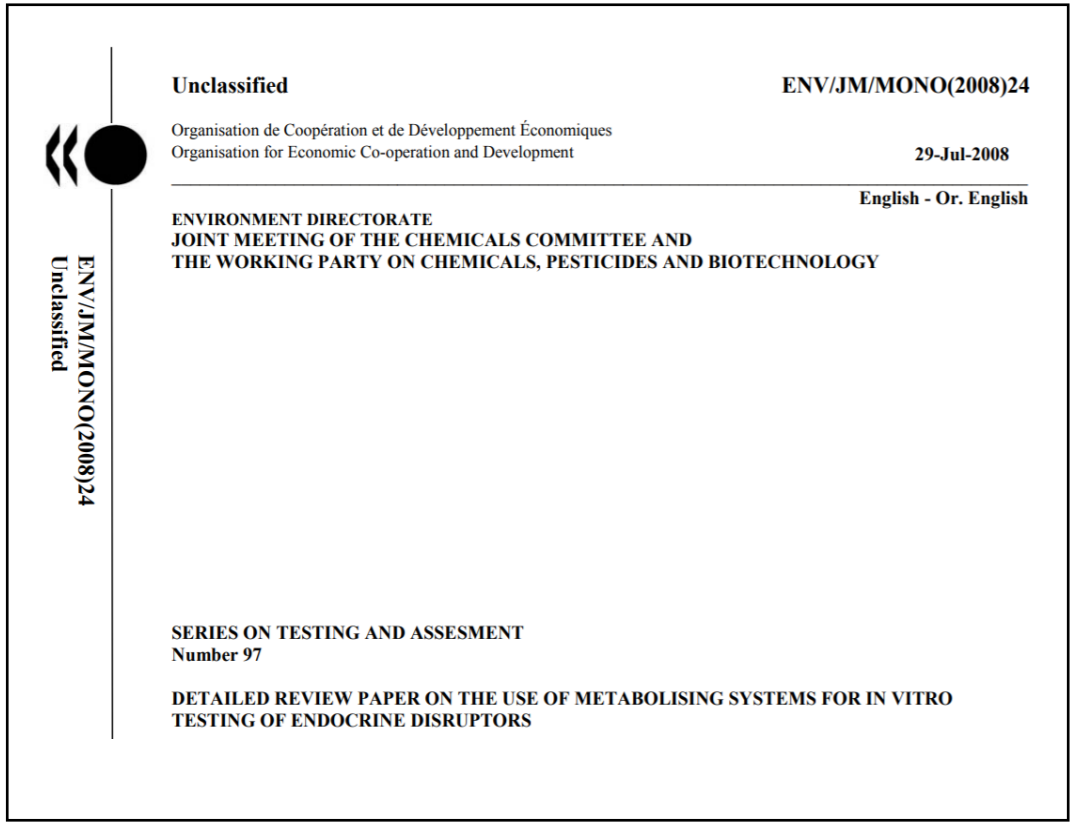
Key Questions for Implementation – Addressing Xenobiotic Metabolism

- "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 possible.
  - Limited animal studies that focus on mechanism and specific metabolites.



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

OECD Detailed Review Paper (DRP 97) (2008) - *In Vitro* Metabolism Systems for Endocrine Disruptors



Unclassified ENV/JM/MONO(2008)24

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

29-Jul-2008

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2008)24
Unclassified

SERIES ON TESTING AND ASSESMENT
Number 97

DETAILED REVIEW PAPER ON THE USE OF METABOLISING SYSTEMS FOR IN VITRO TESTING OF ENDOCRINE DISRUPTORS

The Validation Management Group for Non-animal Testing (VMG-NA) meeting (2003)

- "…it was necessary to consider and preferably incorporate metabolism of compounds when considering the development of *in vitro* tests for endocrine active substances, to reflect the real *in vivo* situation, and so reduce the risks of false positives and false negatives."
- "Tests to detect EAS and tests that can predict the influence of chemicals on metabolism of endogenous or exogenous substances, or the influence metabolism of a chemical on its ultimate effect, are still being developed."
- "…the eventual need to combine the outcome of these developments will be an important component of the development of each field."

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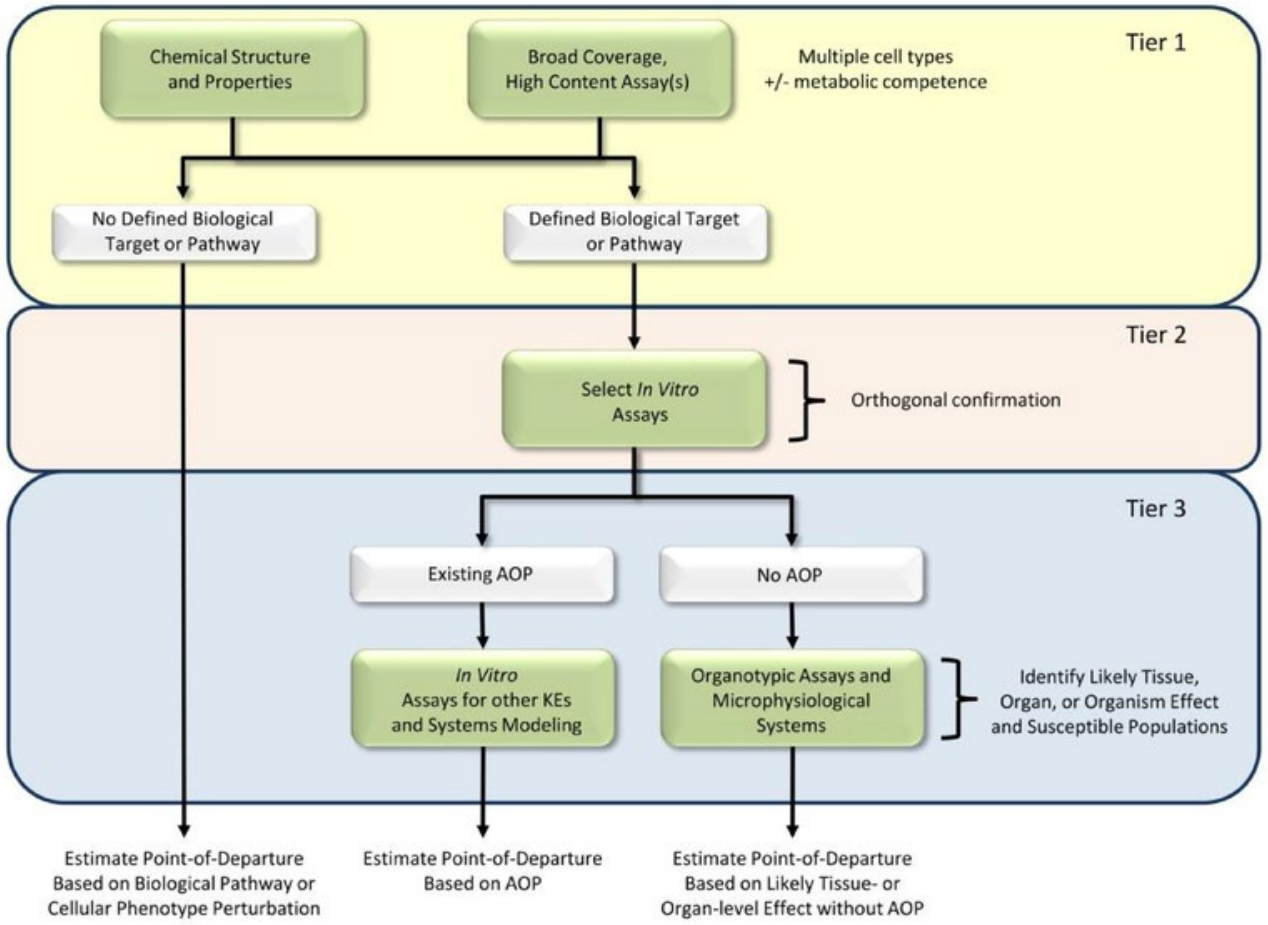
TRANSFORM TOX TESTING CHALLENGE

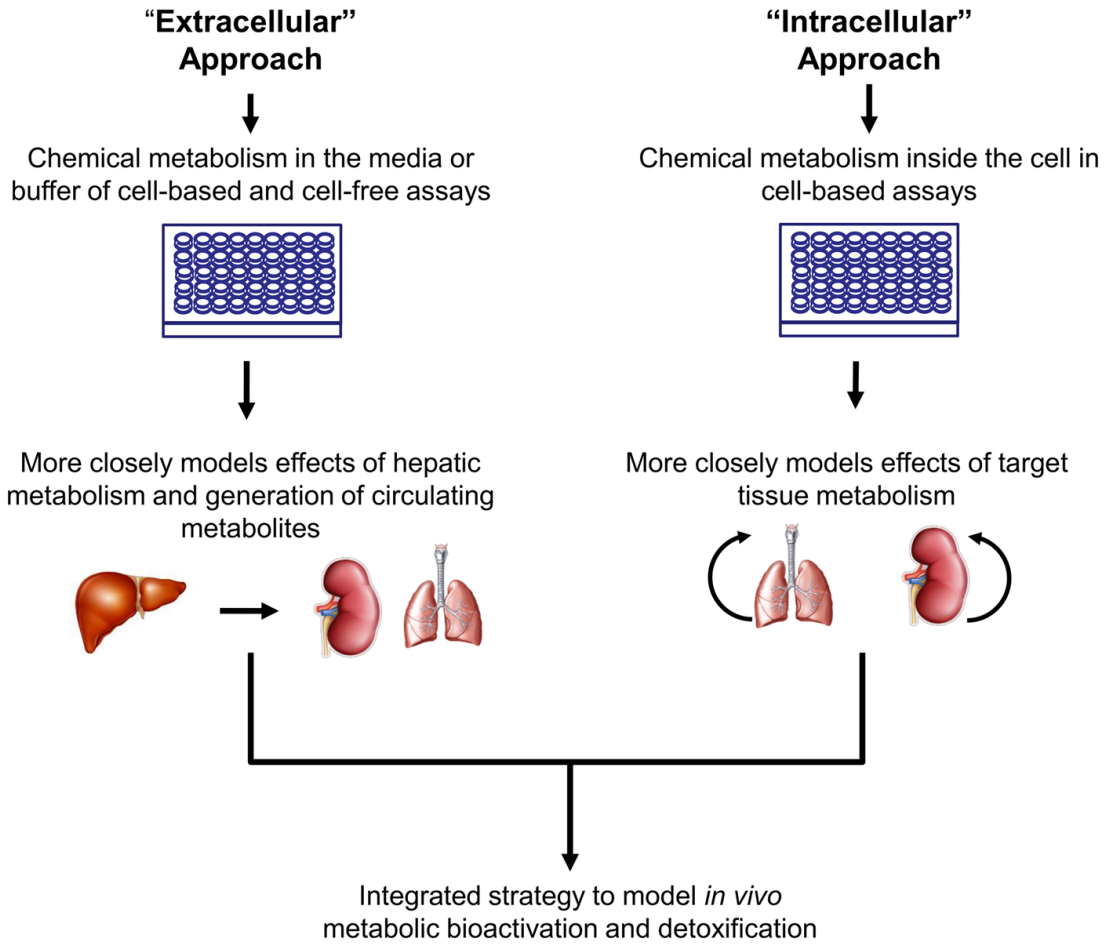
INNOVATING FOR METABOLISM

Identify innovative solutions to retrofit high-throughput assays with metabolic competence

(2016-2017) EPA, NTP, NCATS

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



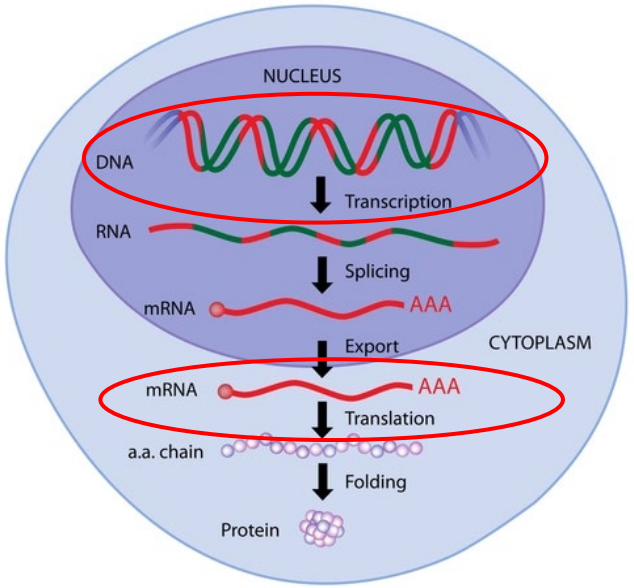


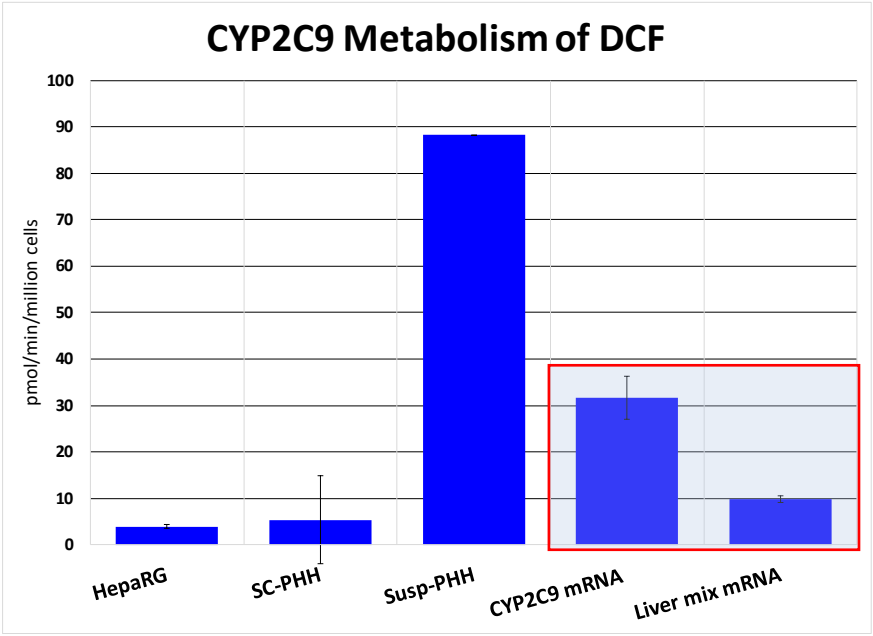
Toxicol Sci, June 2019, DOI:10.1093/toxsci/kfz058

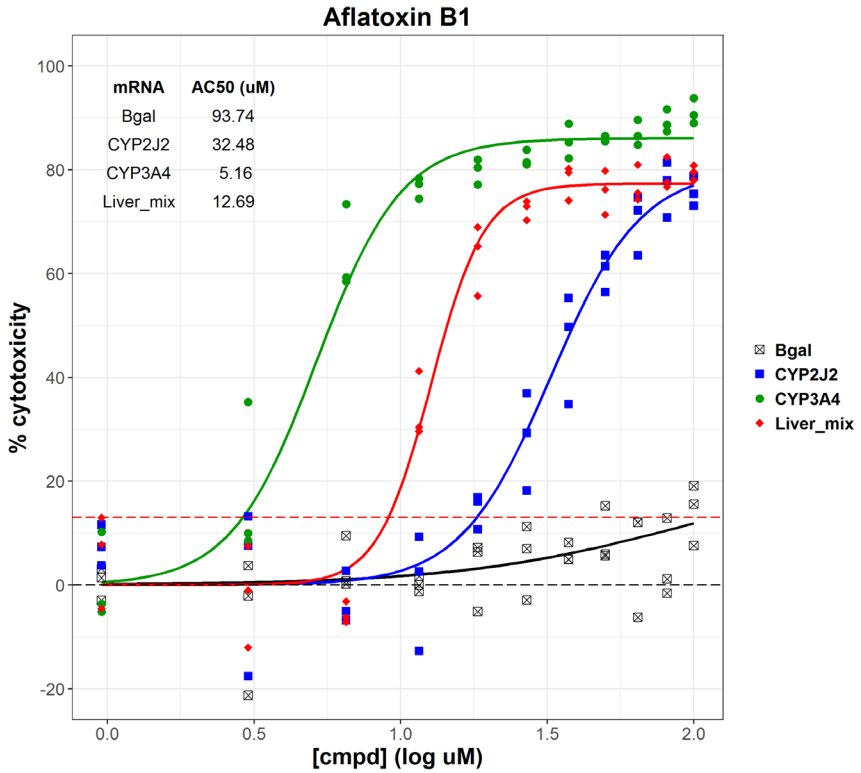
Intracellular Approach: Xenobiotic Metabolism by mRNA Transfection

Steve Simmons (EPA)

- Traditional DNA-based gene delivery methods use viral gene promoters to drive mRNA transcription.
- mRNA transfection is a novel approach that bypasses cellular DNA transcription.
- Rapid expression of metabolizing enzymes (steady state within 8-16 hours).
- User-defined composition and ratios of multiple input mRNAs.

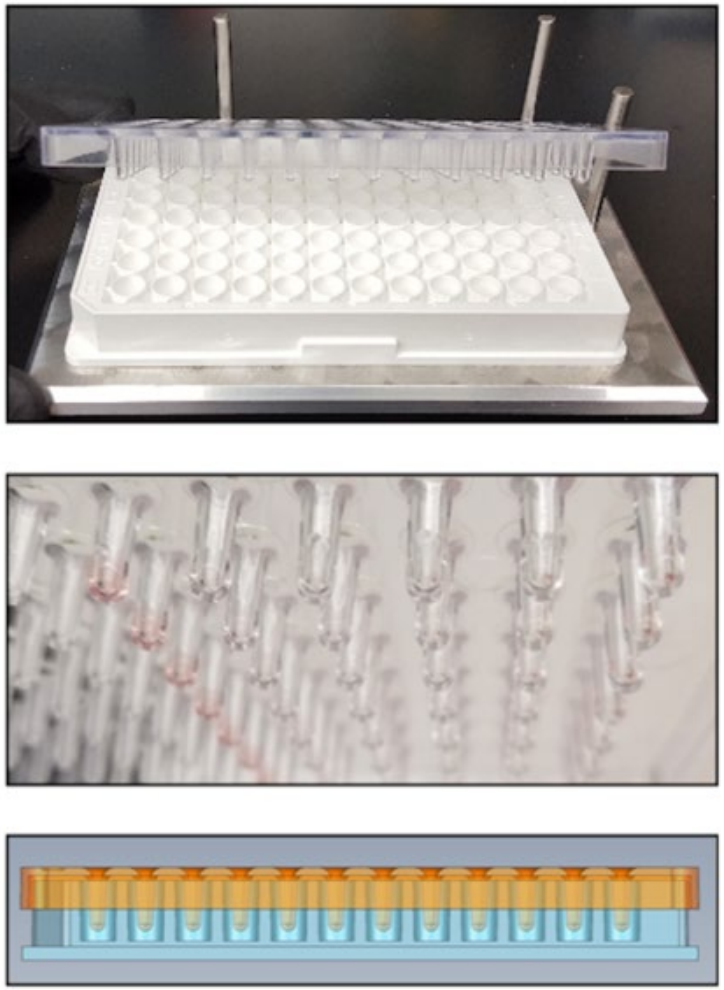


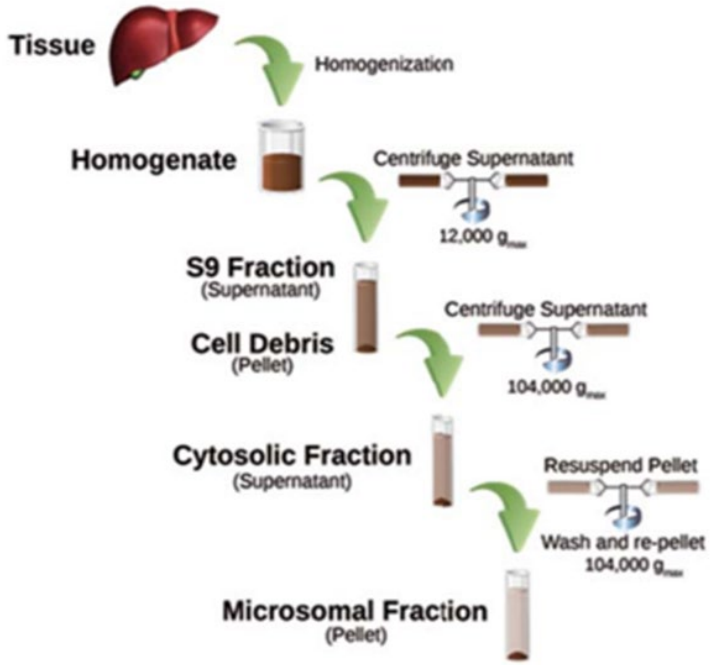




J Pharmacol Toxicol Methods. Jul-Aug 2018. DOI: 10.1016/j.vascn.2018.03.002

Extracellular Approach: Alginate Immobilization of Metabolic Enzymes (AIME)

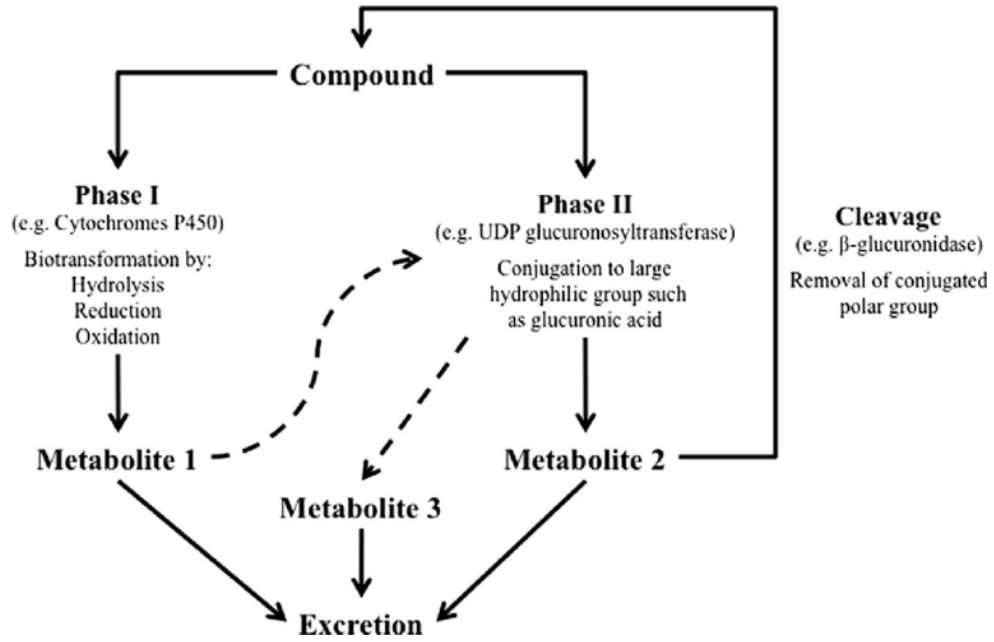


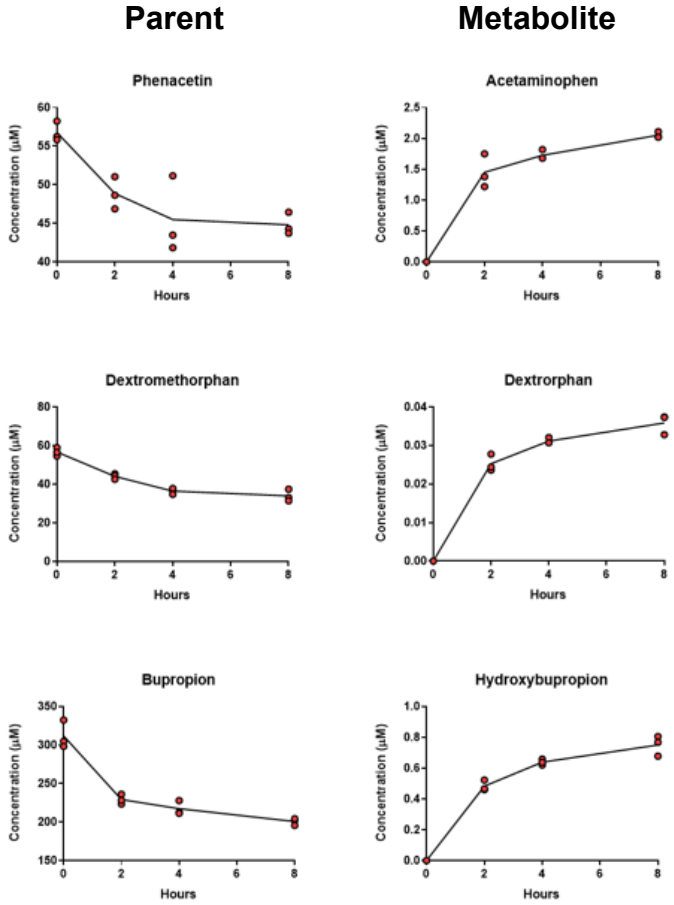


- **Liver Metabolism:** Phenobarbital/β-naphthoflavone-induced male Sprague Dawley rat hepatic S9.
- **Alginate Hydrogel:** Widely used in a variety of pharmaceutical and biomedical applications due to high biocompatibility, low toxicity, and mild gelation by divalent cations.
- **AIME:** The Alginate Immobilization of Metabolic Enzymes (AIME) platform consists of custom 96-well microplate lids containing solid supports attached to encapsulated hepatic S9-alginate microspheres.

Shimadzu Journal, 3(3), 64-68

Validation of Cytochrome P450 Metabolism





Key Points

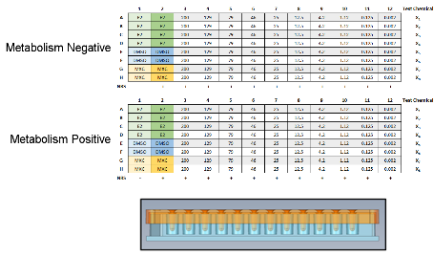
- AIME method optimized for Phase I metabolism.
- Metabolic activity validated across a diverse profile of CYPs with reference chemicals.

Substrate	Human	Rat
Phenacetin	CYP1A2	1A1, **1A2**
Bupropion	CYP2B6	**2B1**, 2B2, 2B3
Diclofenac	CYP2C9	**2C6**, 2C7, 2C11, 2C12, 2C13, 2C22, 2C23
Dextromethorphan	CYP2D6	2D1, **2D2**, 2D3, 2D4, 2D5, 2D18
Chlorzoxazone	CYP2E1	**2E1**

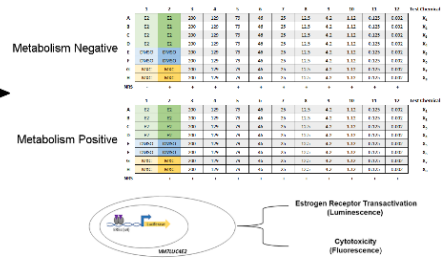
DOI:10.1007/s00338-014-1136-3

Retrofitting Metabolism to an Estrogen Receptor Transactivation Assay

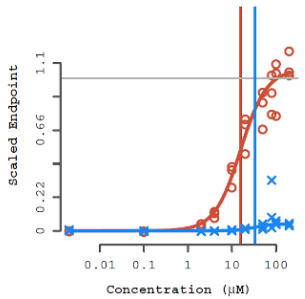
AIME Metabolism Assay



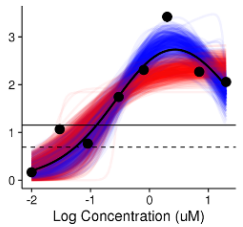
Estrogen Receptor Transactivation Assay



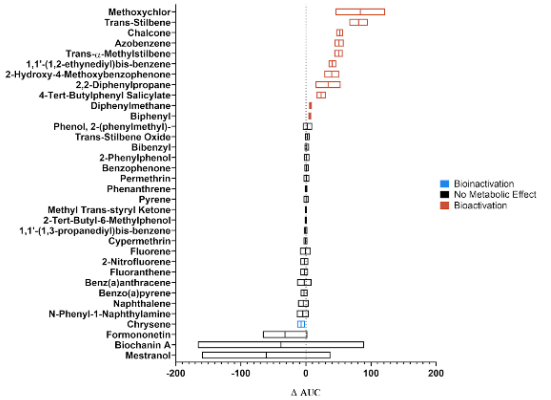
ToxCast Pipeline



Toxboot Uncertainty Quantification



Rank Metabolism-dependent Bioactivity

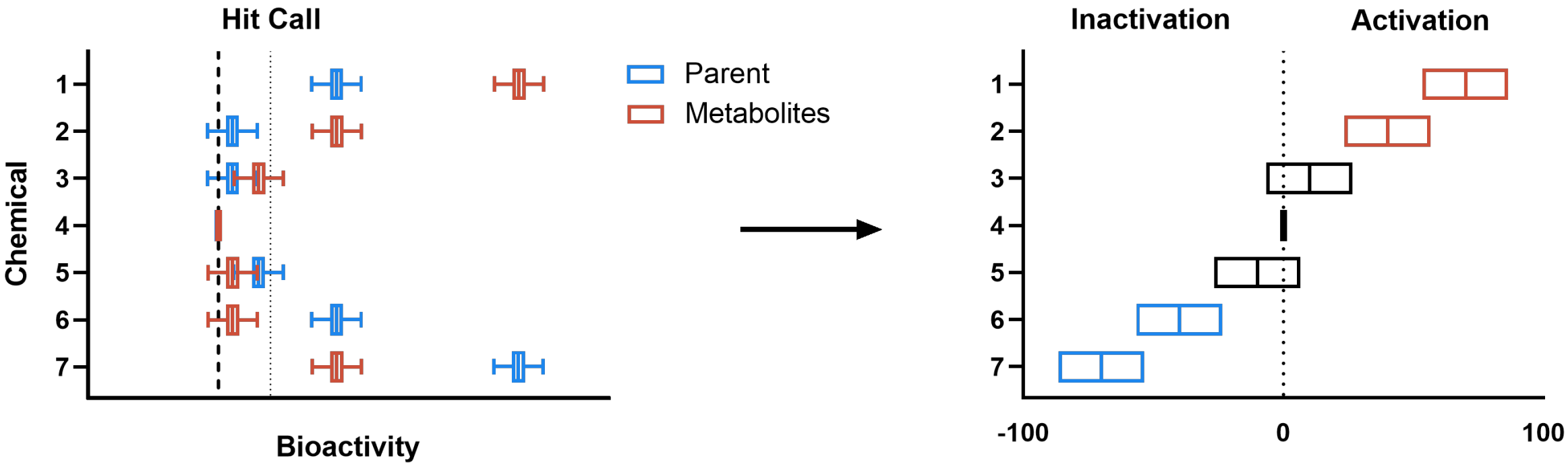


Study Highlights

- 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.

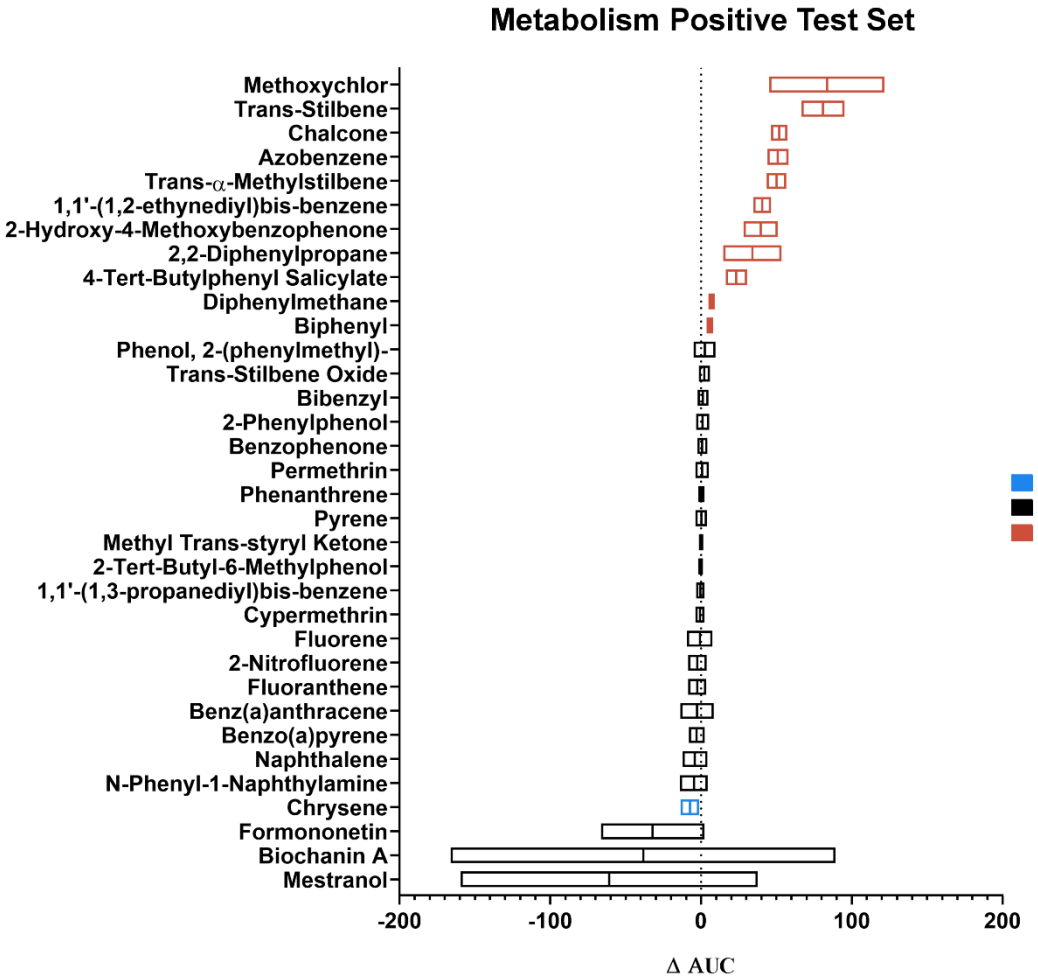
Toxicol Sci, Sep 2020, DOI:10.1093/toxsci/kfaa147

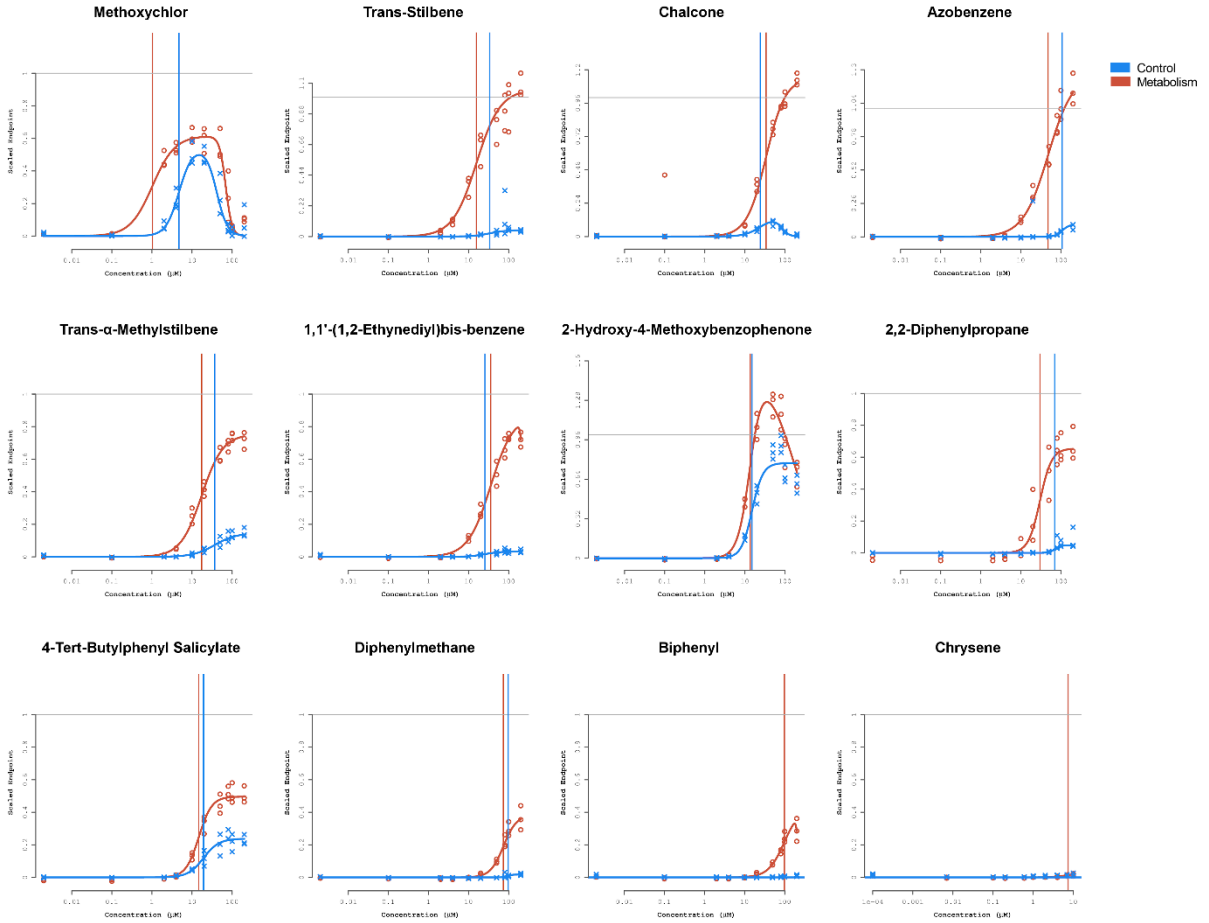
Toxboot Uncertainty Quantification: Statistical Analysis for Metabolism-dependent Effects



- A focus on false-positive and false-negative target assay effects alone omits a lot of important biology.
- Metabolism-dependent effects prioritized on a continuous scale to discriminate from target assay-dependent bioactivity thresholds.

AIME-coupled ERTA Metabolism Positive Test Set Screening

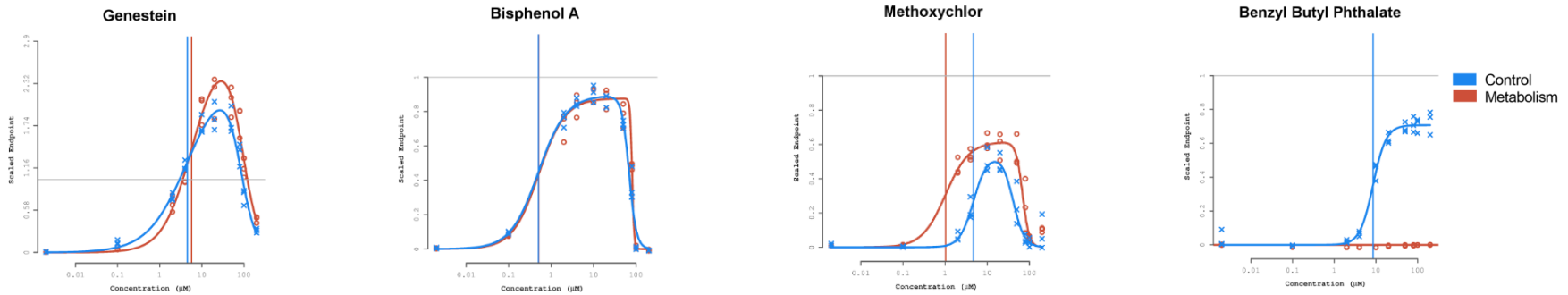




- 29/34 (85%) of parent chemicals from the positive test set were active in the absence of metabolism according to TCPL hit calls.
- 11/34 (32%) of chemicals exhibit significant metabolism-dependent bioactivation.

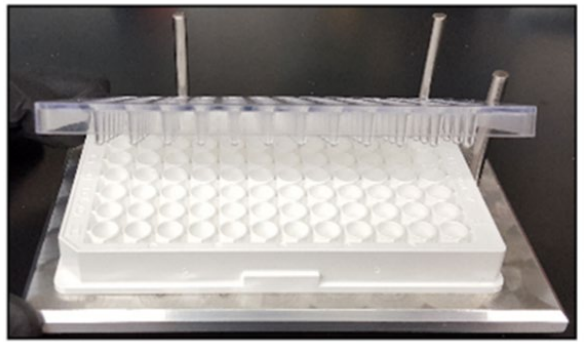
AIME - VM7Luc ERTA Assay: Relevance to the ToxCast ER Model and Uterotrophic Bioassay Data

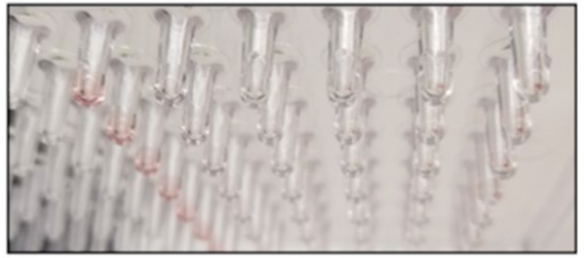
			ToxCast ER Model[a]	Uterotrophic Studies[b]			AIME - VM7Luc ERTA[c]						Concordance with In Vivo[d]		
CASRN	Chemical Name	Classification	AUC_Agonist	GL_Neg	GL_Pos	GL_WoE	Hitc_Met_Neg	Hitc_Met_Pos	$\Delta Hitc_{ER}$	ΔAUC	ΔAUC CI	Met_Effect	Met_Neg	Met_Pos	ΔMet
446-72-0	Genistein	Reference_Agonist	0.54	0	8	POS	1	1	0	27.96	[-1.37, 57.29]	NEG	1	1	0
80-05-7	Bisphenol A	Reference_Agonist	0.45	4	10	POS	1	1	0	1.57	[-46.01, 49.15]	NEG	1	1	0
72-43-5	Methoxychlor	Metabolism_Positive	0.25	1	3	POS	1	1	0	83.56	[45.44, 121.67]	POS	1	1	0
85-68-7	Benzyl butyl phthalate	Metabolism_Negative	0.18	1	0	NEG	1	0	-1	-73.48	[-78.91, -68.05]	POS	0	1	1

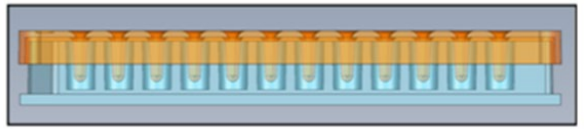


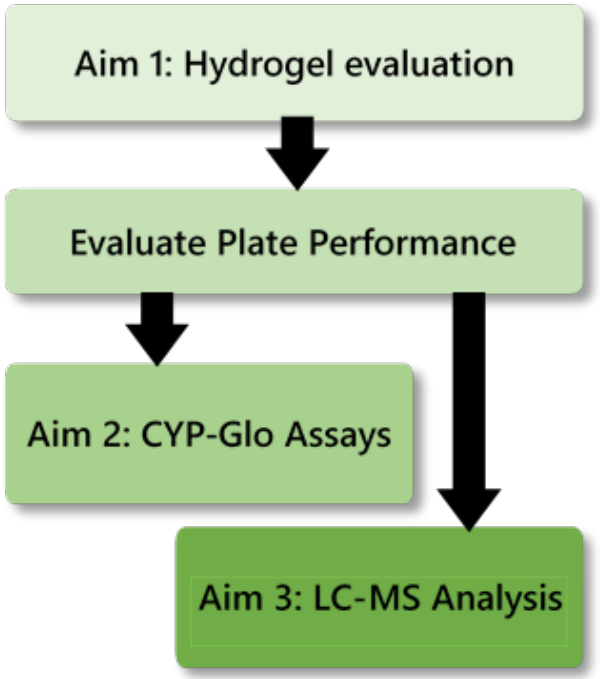
- Chemicals screened in the AIME-VM7Luc ERTA assay compared to ToxCast ER Model scores and Guideline-like Uterotrophic Studies (GL-UT) database.
- Comparison reveals cases of improved *in vitro* concordance with *in vivo* data.

Development of a Bioprinting Approach to Adapt the AIME Method for High-throughput Screening Applications



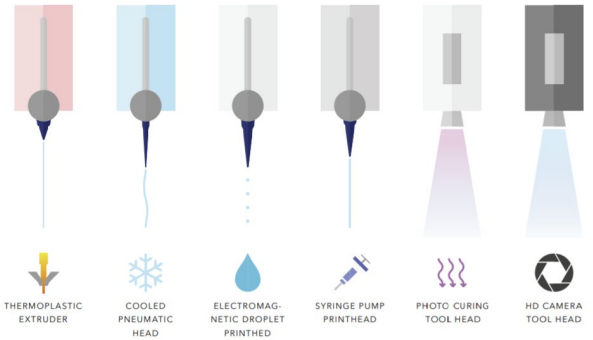






Goal: Adapt AIME method to an automated approach using bioprinting.

Approach: Evaluate various S9/hydrogel combinations, phase I and II optimization, and cross-linking approaches to increase workflow efficiency for metabolism screening.



References

- EPA New Approach Methods Workplan - (https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf)
- CSS Strategic Research Action Plan - (https://www.epa.gov/sites/production/files/2020-12/documents/css_fy19-22_strap-final_2020.pdf)
- NRC (2007). "Toxicity Testing in the 21st Century: A Vision and a Strategy." National Academies.– (http://nap.edu/11970. DOI 10.17226/11970)
- OECD (2014), Detailed Review Paper on the State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors, OECD Series on Testing and Assessment, No. 178, OECD Publishing, Paris, https://doi.org/10.1787/9789264221352-en.
- EPA Transform Tox Testing Challenge - (https://www.challenge.gov/assets/document-library/Transform-Tox-Testing-Challenge-Stage-2-Update1.pdf)
- Thomas RS, Bahadori T, Buckley TJ, Cowden J, Deisenroth C, Dionisio KL, Frithsen JB, Grulke CM, Gwinn MR, Harrill JA, Higuchi M, Houck KA, Hughes MF, Hunter ES, Isaacs KK, Judson RS, Knudsen TB, Lambert JC, Linnenbrink M, Martin TM, Newton SR, Padilla S, Patlewicz G, Paul-Friedman K, Phillips KA, Richard AM, Sams R, Shafer TJ, Setzer RW, Shah I, Simmons JE, Simmons SO, Singh A, Sobus JR, Strynar M, Swank A, Tornero-Valez R, Ulrich EM, Villeneuve DL, Wambaugh JF, Wetmore BA, Williams AJ. 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. PMID: 30835285; PMCID: PMC6542711.
- DeGroot DE, Swank A, Thomas RS, Strynar M, Lee MY, Carmichael PL, Simmons SO. mRNA transfection retrofits cell-based assays with xenobiotic metabolism. J Pharmacol Toxicol Methods. 2018 Jul-Aug;92:77-94. doi: 10.1016/j.vascn.2018.03.002. Epub 2018 Mar 16. PMID: 29555536.
- Deisenroth C, DeGroot DE, Zurlinden T, Eicher A, McCord J, Lee MY, Carmichael P, Thomas RS. The Alginate Immobilization of Metabolic Enzymes Platform Retrofits an Estrogen Receptor Transactivation Assay With Metabolic Competence. Toxicol Sci. 2020 Dec 1;178(2):281-301. doi: 10.1093/toxsci/kfaa147. PMID: 32991717.