Integration of Metabolic Competence with High-throughput Screening Assays

Chad Deisenroth, Ph.D.
Center for Computational Toxicology and Exposure
deisenroth.chad@epa.gov

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Disclaimer: The views expressed are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

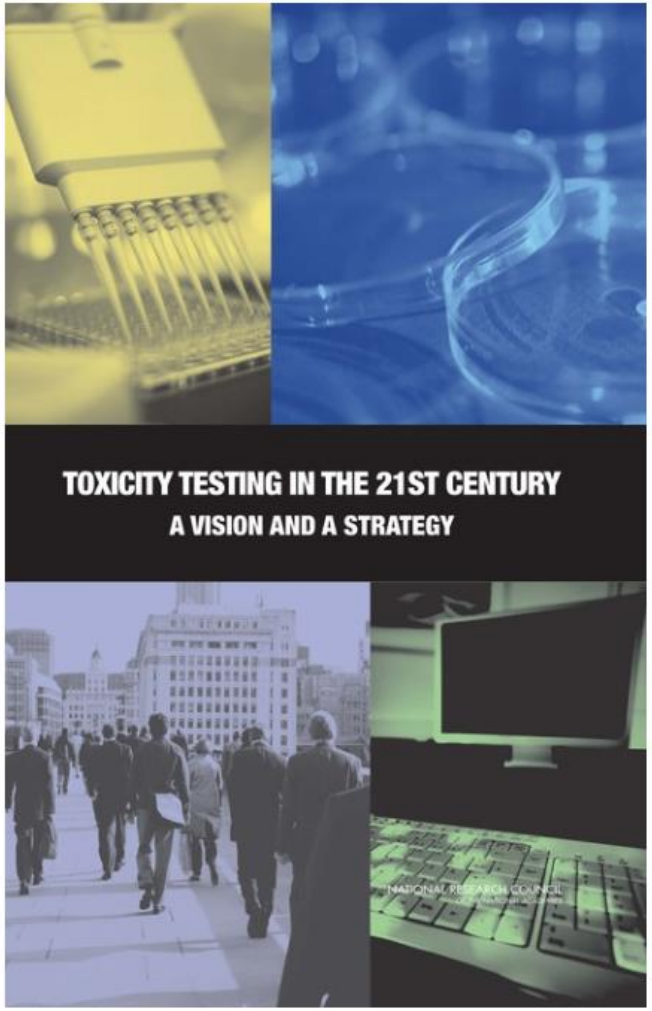
Chemical Safety Regulation – The Frank R. Lautenberg Chemical Safety for the 21st Century Act (2016)

- Amendment to the Toxic Substances Control Act (TSCA) of 1976, the nation's primary chemical management law.
- Mandatory requirement for the EPA to evaluate existing chemicals, establish risk-based safety standards, increase public transparency for chemical information, provide consistent source of funding for EPA to carry out responsibilities in the law.
- Section 4(h) in the new TSCA legislation requires –
  - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - Alternative approaches need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models.

U.S. EPA – Research Triangle Park, NC

https://www.congress.gov/114/plaws/publ182/PLAW-114publ182.pdf

Toxicity Testing in the 21st Century

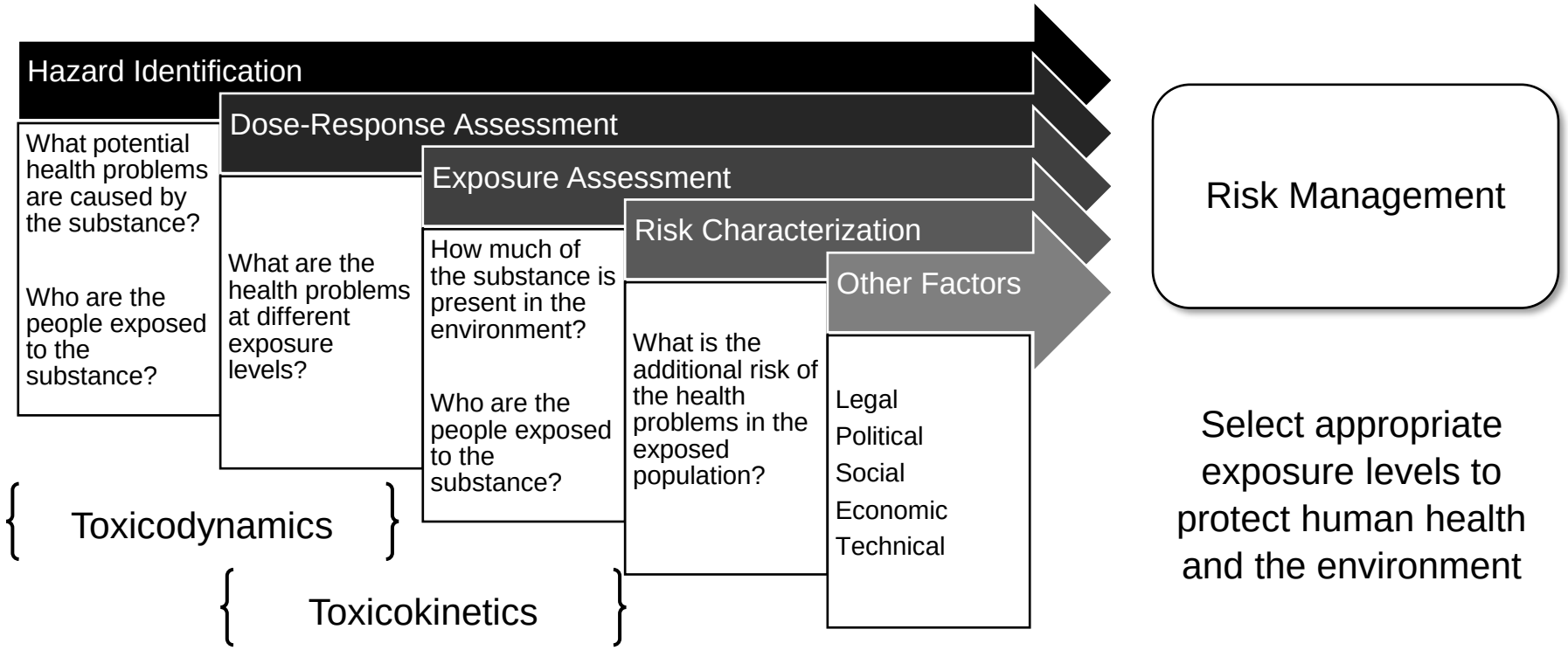


National Research Council released a report in 2007 calling for a genuine commitment to the reduction, refinement, and replacement of animal testing.

- Increase data relevance with human cell-based testing
- Speed up the data gathering process by transitioning away from animal models to high-throughput screening
- Focus on mechanisms of toxicity by integrating molecular biology with toxicology
- Incorporate elements of population susceptibility and exposure into every risk assessment
- Suggestions grounded in the political and economic landscape of global chemical testing

doi: 10.17226/11970

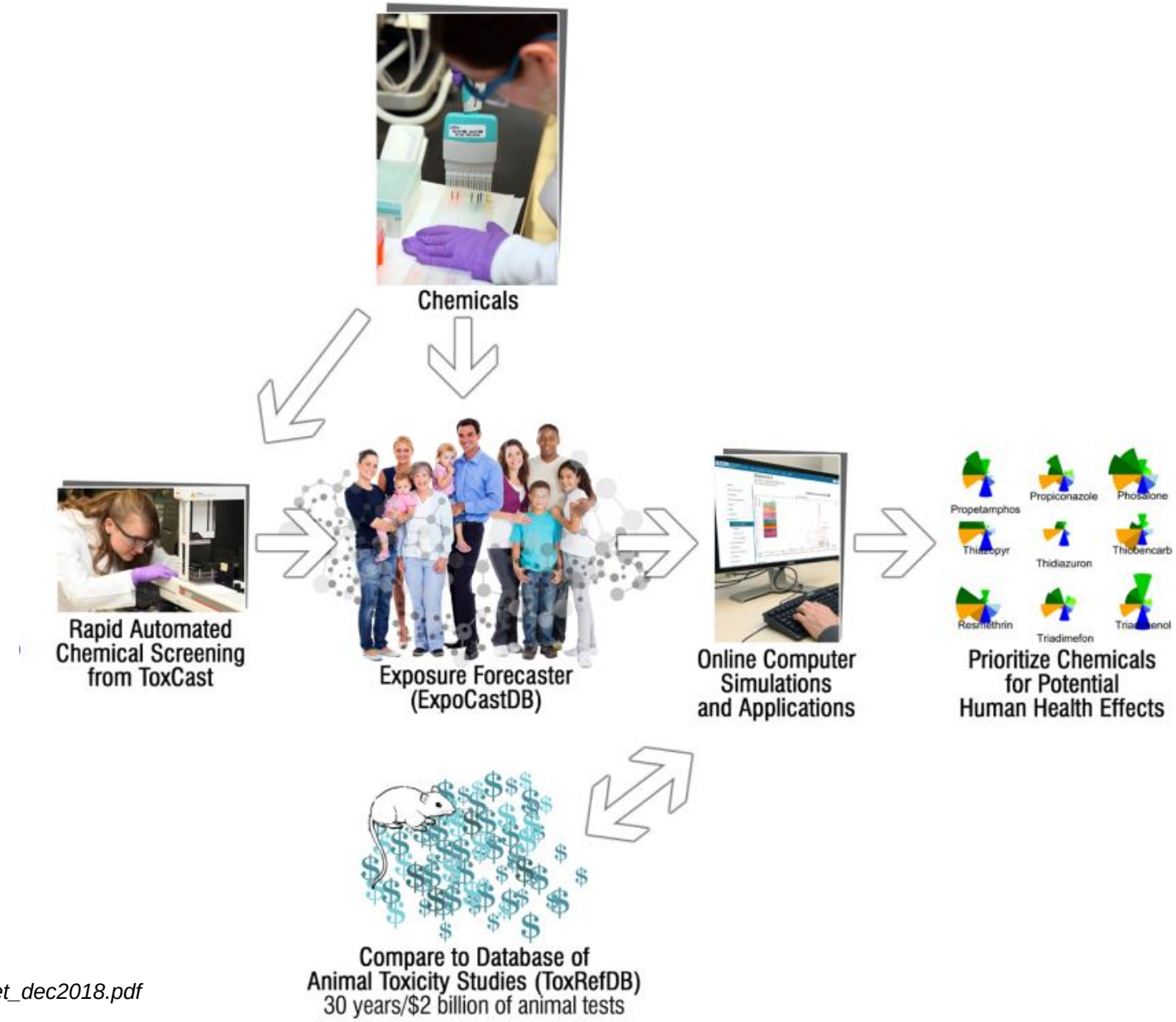
Chemical Risk Assessment and Management



- Toxicokinetics (TK): the movement and fate of a substance after it enters the body. Links substance exposure to internal tissue concentrations.
- Toxicodynamics (TD): interaction of a substance with a biological system. Links substance concentration to a biological response.
- Quantitative risk assessment evaluates hazard (TD), dose-response (TK/TD), and exposure (TK).

EPA Toxicity Forecaster (ToxCast™) Program

- Computational Toxicology (CompTox) research integrates advances in biology, biotechnology, chemistry, and computer science to identify potential chemical toxicants.
- Chemical dose and response can be extrapolated to a human exposure.
- The combined information helps prioritize chemicals based on potential human health risks.
- Using CompTox, thousands of chemicals can be quickly evaluated *in vitro* for potential risk.



https://www.epa.gov/sites/default/files/2019-01/documents/toxcast_factsheet_dec2018.pdf

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 capacity to address tissue- and organ-level effects.
- Minimal capability for addressing xenobiotic metabolism in *in vitro* test systems.

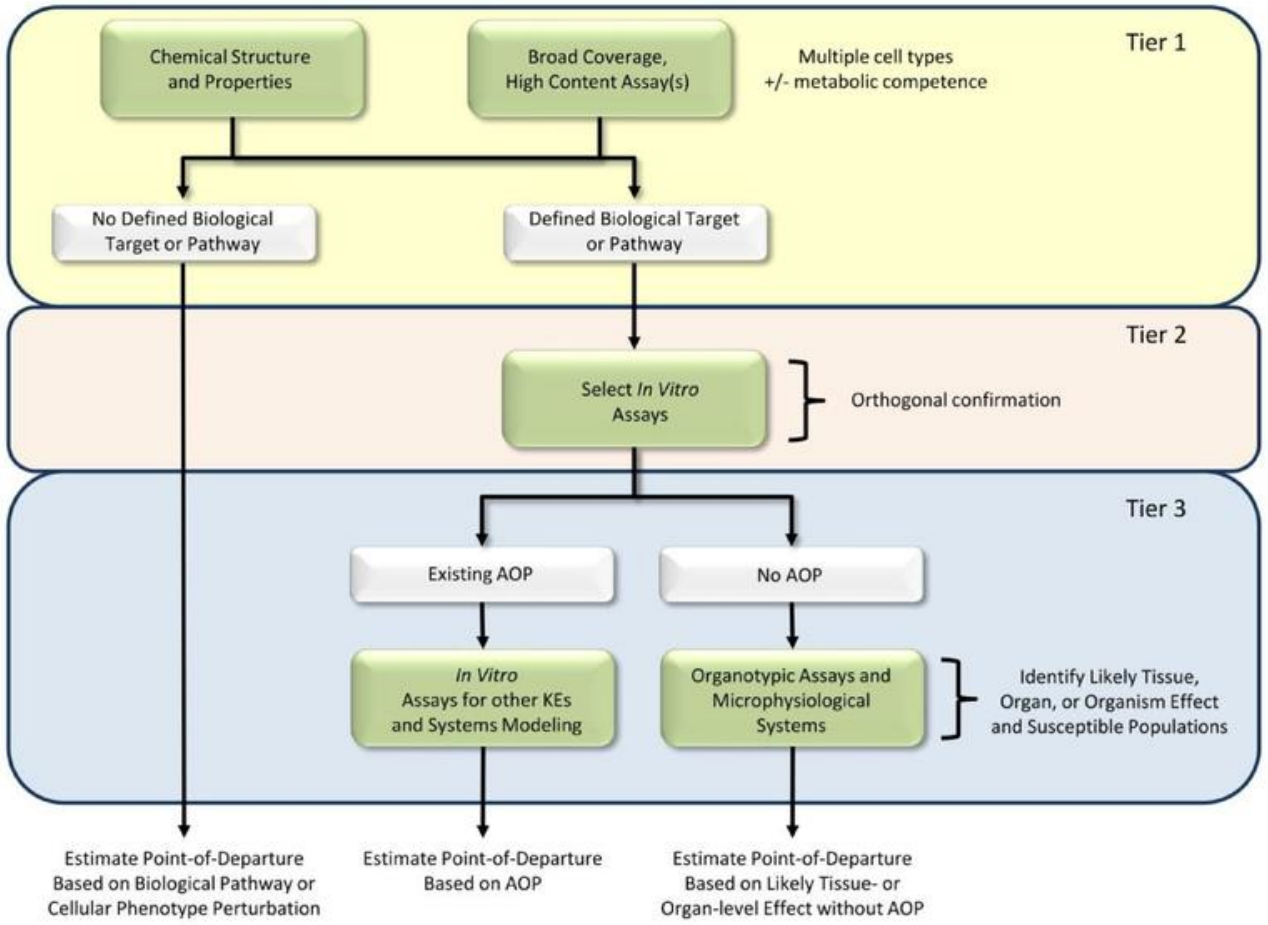
Possible metabolism solutions

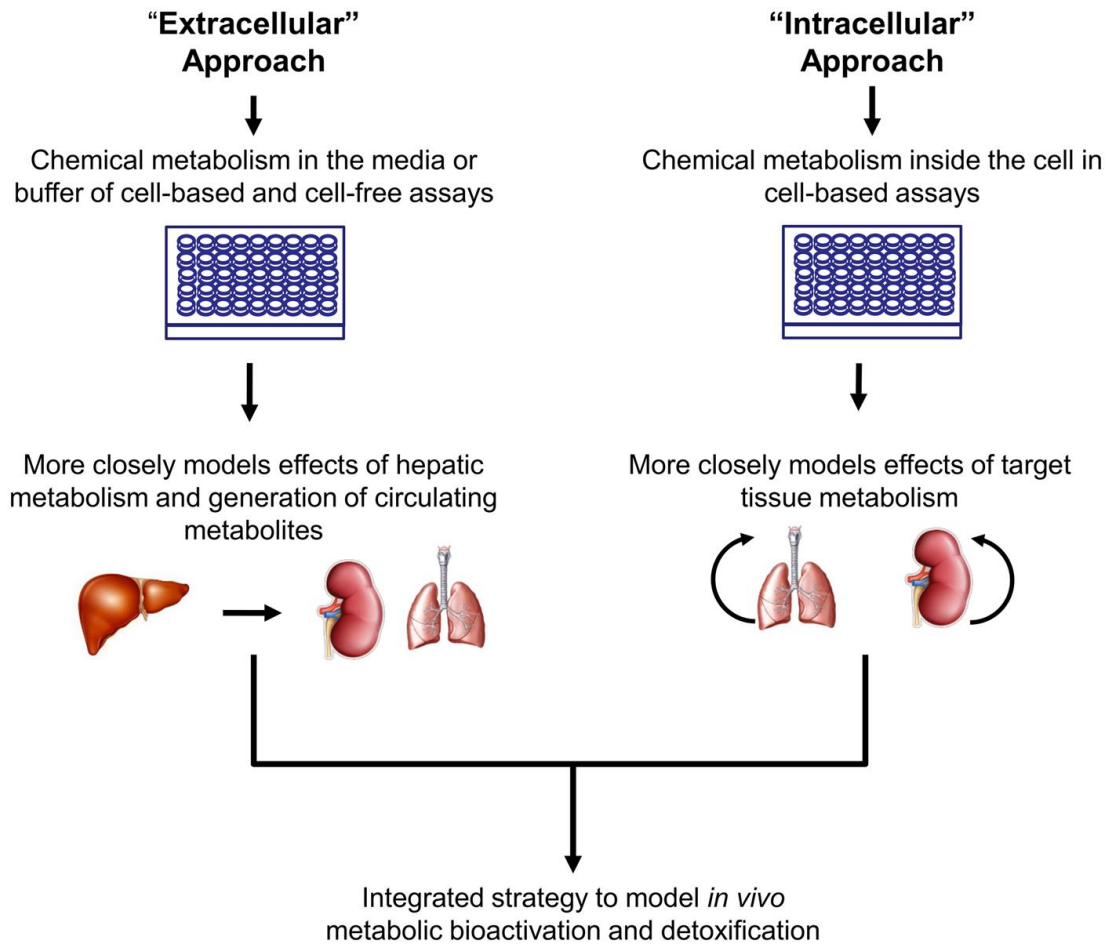
- Screen using computational predictions where possible.
- Retrofit *in vitro* assays with metabolic competence to identify metabolism-dependent effects and mechanisms of toxicity.

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

Hazard Identification Screening Paradigm





doi: 10.1093/toxsci/kfz058

Liver Metabolism

- Hepatic metabolism is the biotransformation of an endogenous or exogenous molecule by one or more enzymes to metabolites that are more hydrophilic and easily eliminated from the body.

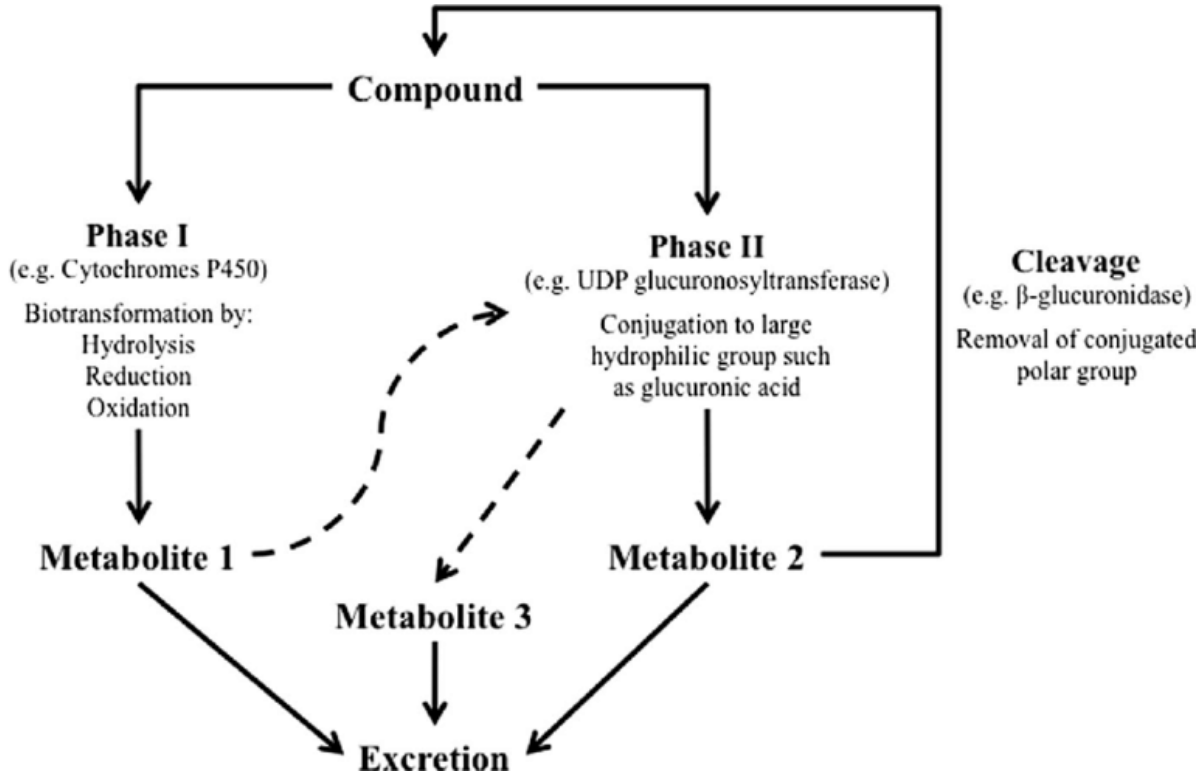
- Xenobiotic metabolism can produce metabolites that are more (bioactivation) or less (bioinactivated/detoxification) active than the parent molecule.

Phase 1 Enzymes – Introduce a reactive group into the molecule via oxidation, reduction, or hydrolysis.

- Cytochrome P450s
- Serine Hydrolases

Phase II Enzymes – Transfer of polar groups (e.g. glucuronide, glutathione, sulfate) onto the products of Phase I metabolism.

- UDP-glucuronosyltransferases
- Glutathione S-transferases
- Sulfotransferases
- N-Acetyltransferases



doi:10.1007/s00338-014-1136-3

Liver Metabolism: *In vitro* Tools

Many options.

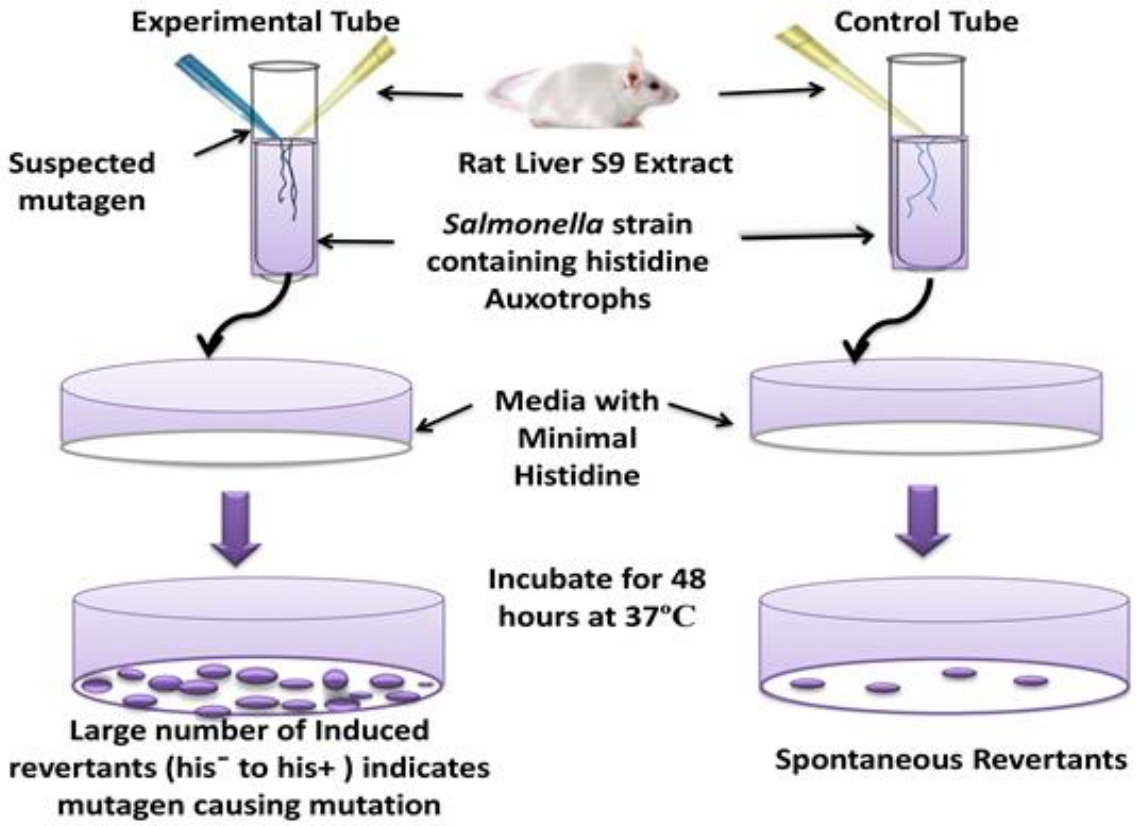
- Why is liver metabolism relevant to the scientific problem?
- How well does the tool recapitulate liver metabolism?
- What is the intended application?
- Who will use the data?

	Advantages	Limitations
Subcellular fractions (Asha and Vidyavathi, 2010)	Drug enzyme activities preserved Production of metabolites for structural analysis	Only suitable for short-term studies No cytosolic phase II enzyme reactions
Genetically engineered cells (Gomez-Lechon et al., 2008; Prakash et al., 2008)	One or more human enzymes expressed Available mainly for CYPs	Use for specific purposes only No physiological levels of enzymes
Hepatoma cell lines **(*e.g.* HepG2, HepaRG)** (Gerets et al., 2012; Sirenko et al., 2016)	High proliferation activity and good availability Stable metabolic performance Well characterized and abundant data available	Decreased drug enzyme activities Genotype instability
Stem cell-derived hepatocyte-like cells (HLCs) (Hay et al., 2008; Takayama et al., 2014; Gao and Liu, 2017)	Good availability Analysis of genetic polymorphisms Drug testing with patient-specific cell lines for personalized medicine Characterization of differentiation/maturation processes for potential *in vitro* or clinical use Establishment of disease models	Costly differentiation protocols Incomplete hepatic differentiation Lack of standardized methods for cell differentiation and characterization
Primary hepatocytes (Tostoes et al., 2011; Zeilinger et al., 2011, 2016; Bell et al., 2016; Pinheiro et al., 2017)	Obtained from whole livers or wedge biopsies Functions close to those of *in vivo* hepatocytes Suitable for interspecies and pharmacogenomic studies Induction/inhibition of drug metabolizing enzymes Representative of different lobular subpopulations Cryopreservation	Viability of 2-4 days in 2D cultures No bile canaliculus present Low human tissue availability Difficult recovery of cells and maintenance of function upon cryopreservation
Co-cultures of hepatocytes and NPCs (Proctor et al., 2017; Hafiz et al., 2020; Nudischer et al., 2020)	Improves cells functionality Allows to study specific hepatic injury mechanisms involving different cell types Closer to *in vivo* microenvironment	Higher complexity Lack of standardized methods for cell culture limiting inter-laboratory comparisons

Physiological relevance

doi: 10.3389/fcell.2021.626805

Classic Application of *In vitro* Liver Metabolism in Toxicity Testing - Evaluating Mutagenic Potential with the Ames Test

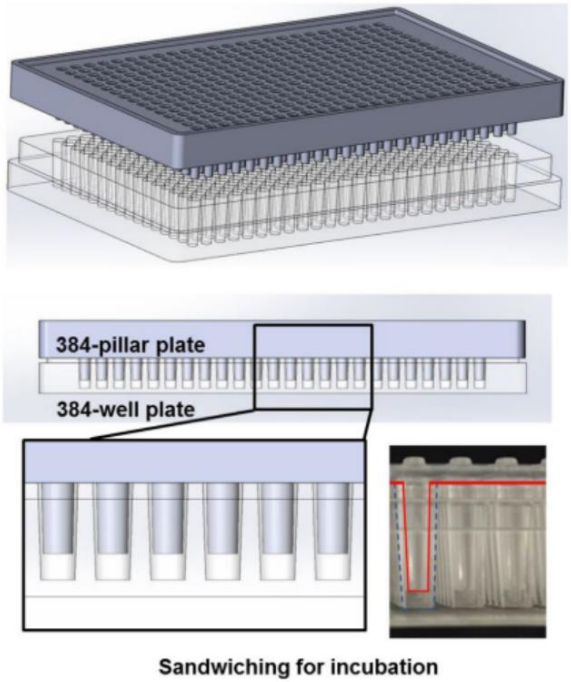


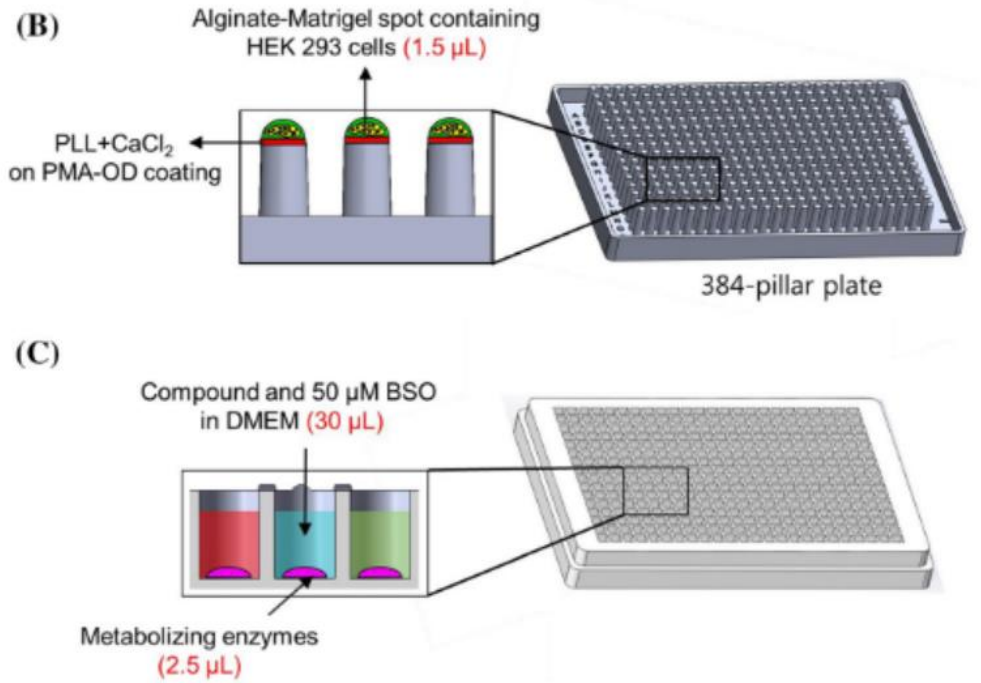
- The Ames test (or Bacterial Reverse Mutation test) is a biological assay to evaluate the mutagenic potential of chemical compounds.
- Subcellular liver fractions are commonly used to evaluate the function of parent compounds and metabolites.

doi: 10.21769/BioProtoc.2763

High-throughput metabolism-induced toxicity assays demonstrated on a 384-pillar plate

Kyeong-Nam Yu[1] · Soo-Yeon Kang[1] · Stephen Hong[1] · Moo-Yeal Lee[1]





- Human embryonic kidney (HEK) 293 cells in a mixture of alginate and Matrigel were printed on 384-pillar plates for membrane integrity and viability assays.
- Recombinant human Cytochrome P450s and UGTs were independently evaluated for Phase I and II hepatic metabolism-dependent effects.
- Metabolism augmented toxicity for select reference chemicals and revealed mechanisms of biotransformation.

(2018) doi: 10.1007/s00204-018-2249-1

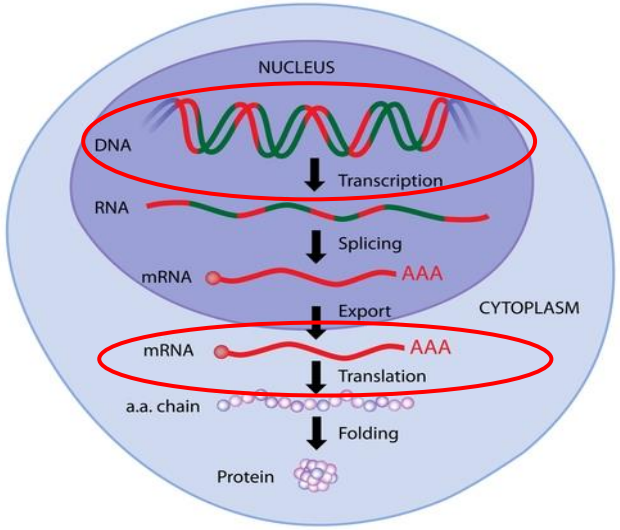
Journal of Pharmacological and Toxicological Methods
Volume 92, July–August 2018, Pages 77-94

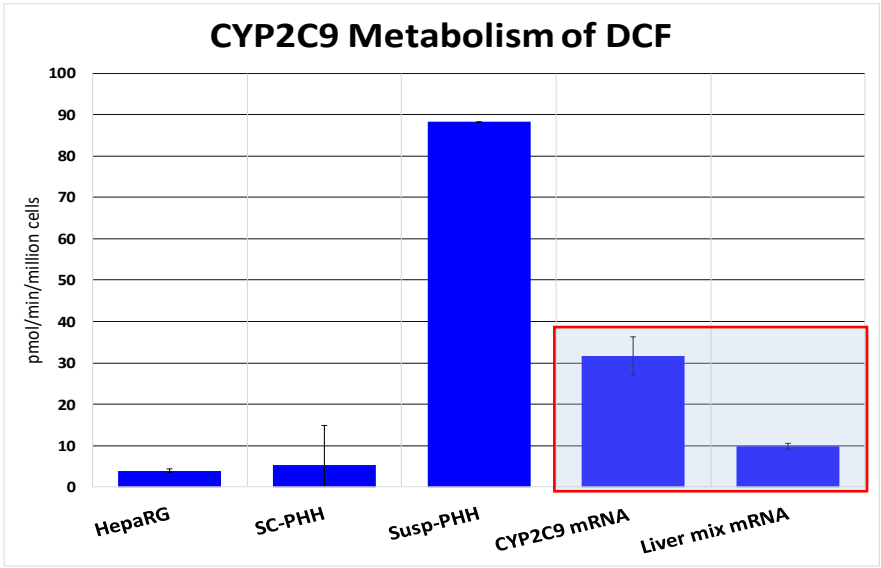
Research article

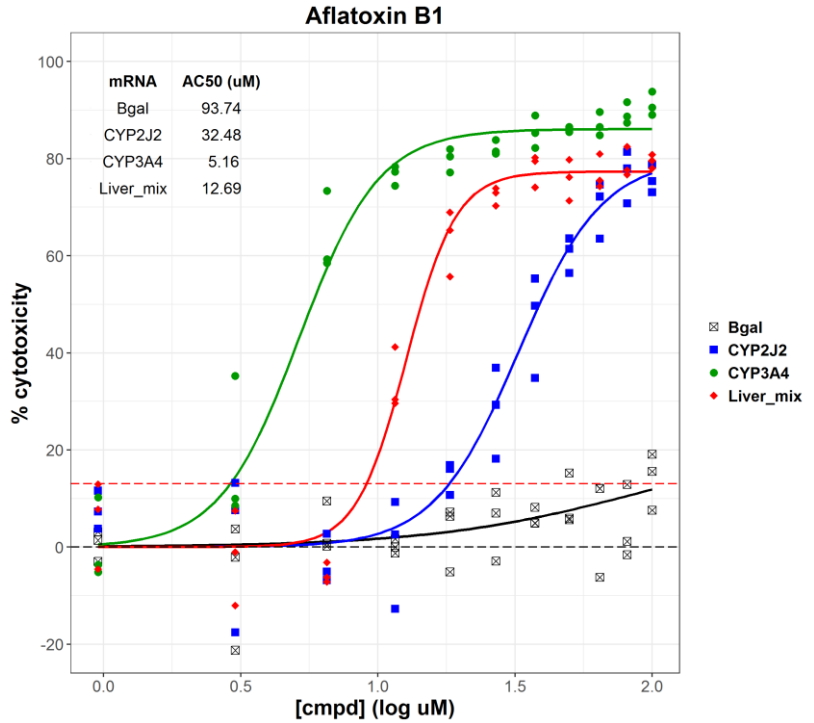
mRNA transfection retrofits cell-based assays with xenobiotic metabolism

Danica E. DeGroot [a], Adam Swank [b], Russell S. Thomas [a], Mark Strynar [c], Mi-Young Lee [d], Paul L. Carmichael [d], Steven O. Simmons [a]

- 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.
- User-defined composition and ratios of multiple input mRNAs.





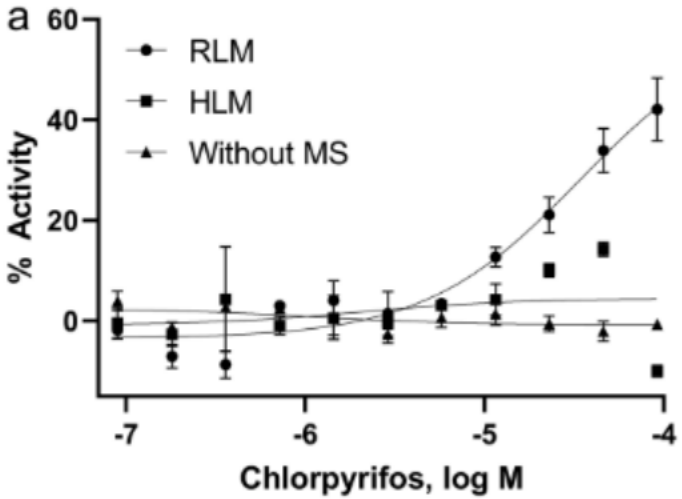


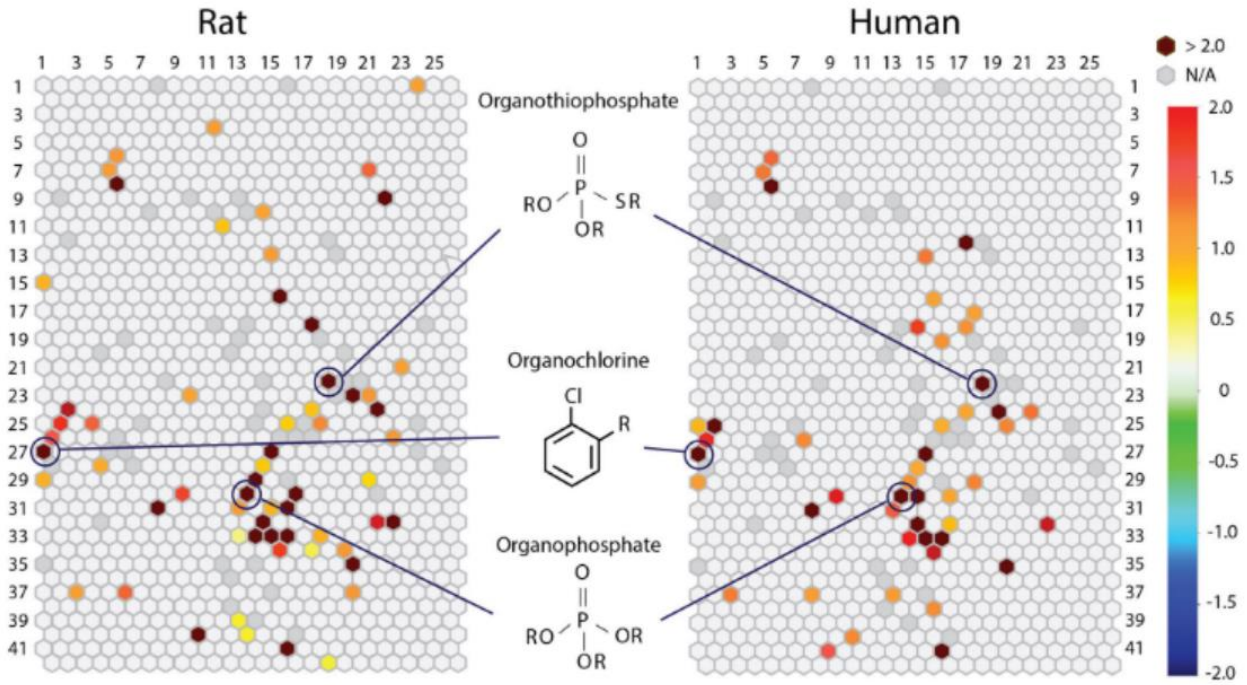
(2018) doi: 10.1016/j.vascn.2018.03.002

Toxicokinetics and Metabolism | Open Access | Published: 18 April 2022

Identification of environmental chemicals that activate p53 signaling after in vitro metabolic activation

Masato Ooka, Jinghua Zhao, Pranav Shah, Jameson Travers, Carleen Klumpp-Thomas, Xin Xu, Ruili Huang, Stephen Ferguson, Kristine L. Witt, Stephanie L. Smith-Roe, Anton Simeonov & Menghang Xia

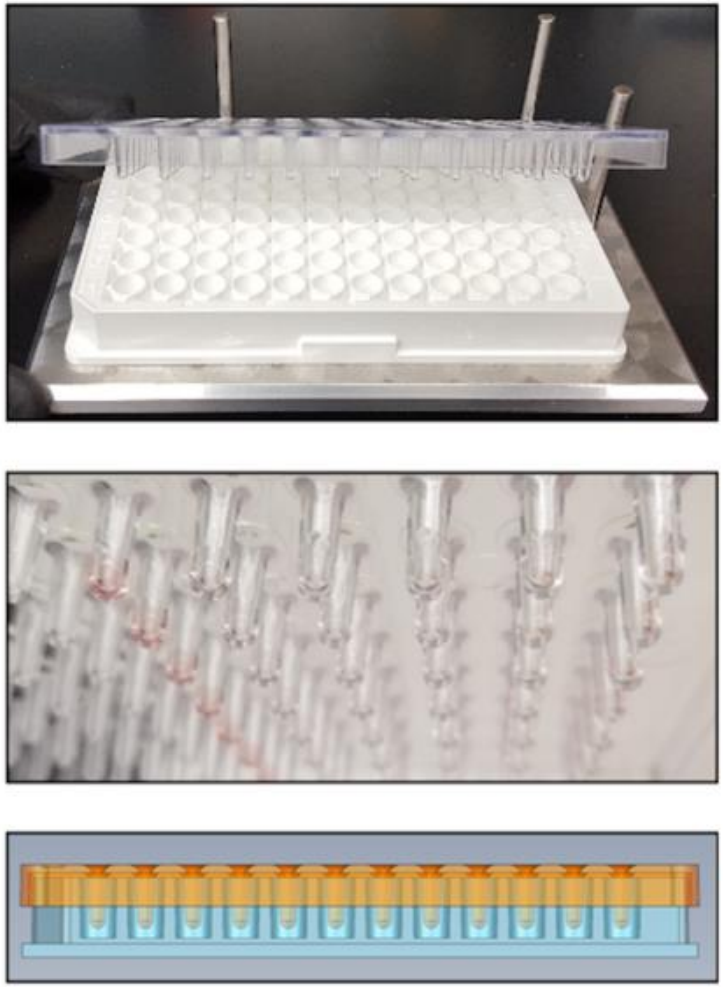


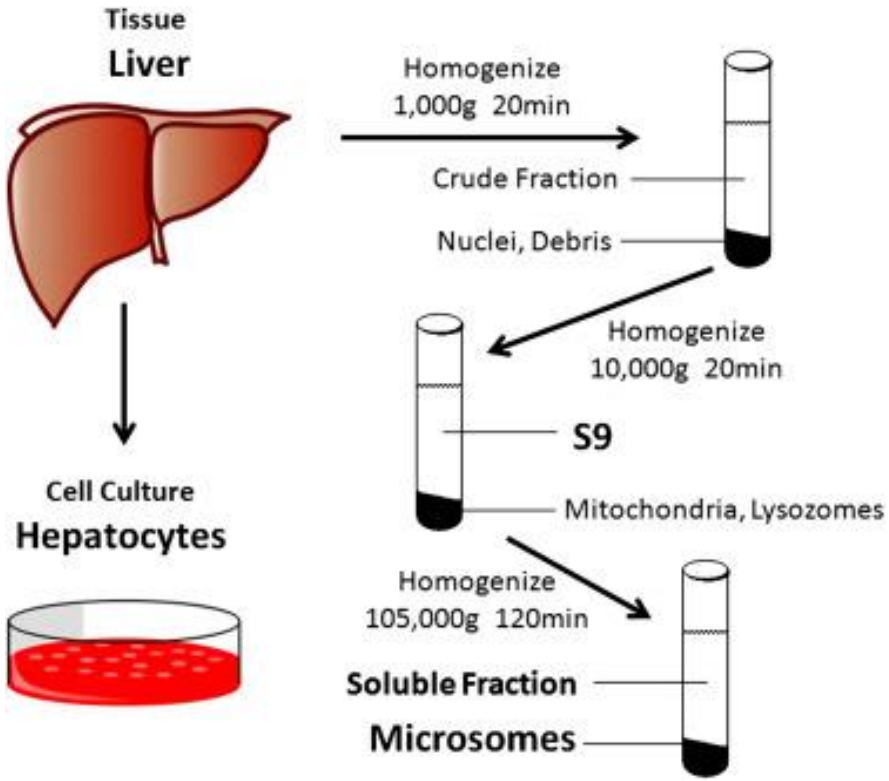


- Tox21 10K chemical library screened for metabolism-dependent effects in a p53 DNA damage assay for genotoxicity.
- Human (HLM) and Rat (RLM) liver microsomes added Phase I hepatic metabolism.
- *In silico* structural similarity analysis identified classes of chemicals that were bioactivated in rat and humans.

(2022) doi: 10.1007/s00204-022-03291-5

The Alginate Immobilization of Metabolic Enzymes (AIME) Method





- **AIME Method:** The AIME platform consists of custom 96- or 384-well microplate lids containing solid supports attached to encapsulated hepatic S9-alginate microspheres.
- **Application:** Compatible with many biochemical and cell-based assays.

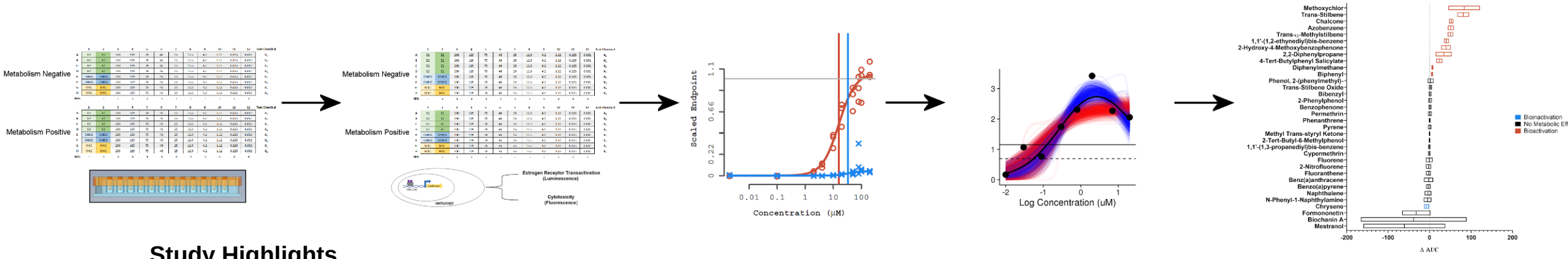
doi: 10.1016/B978-0-12-803620-4.00003-7.

TOXICOLOGICAL SCIENCES, 178(2), 2020, 281–301
doi: 10.1093/toxsci/kfaa147
Advance Access Publication Date: 29 September 2020
Research Article

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

Chad Deisenroth,*,1 Danica E. DeGroot,*,2 Todd Zurlinden,* Andrew Eicher,* James McCord,* Mi-Young Lee,†3 Paul Carmichael,† and Russell S. Thomas*

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



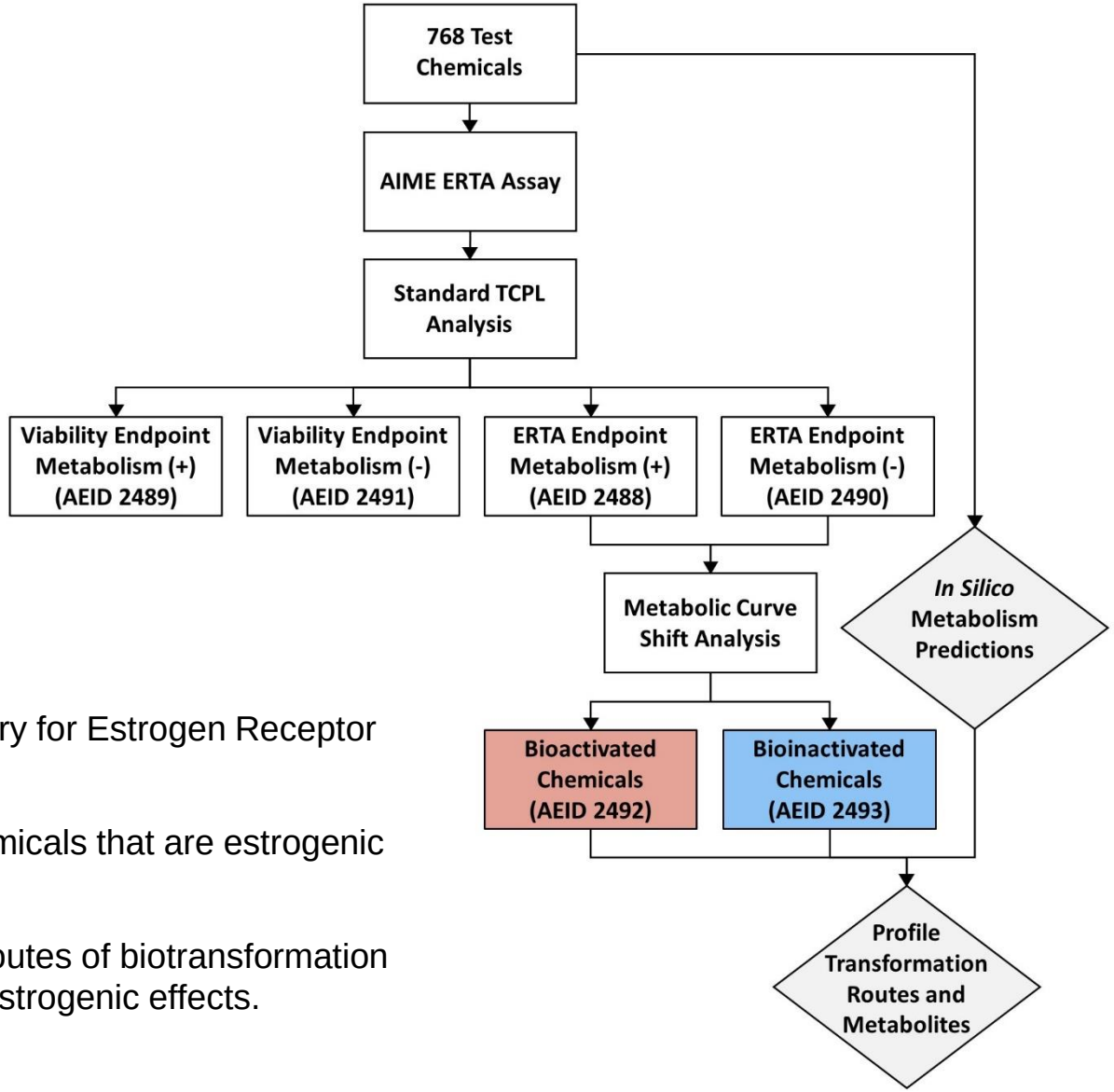
Study Highlights

- 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.

TOXICOLOGICAL SCIENCES, 187(1), 202
https://doi.org/10.1093/toxsci/kfac019
Advance Access Publication Date: 16 February
Research Article

Chemical Screening in an Estrogen Receptor Transactivation Assay With Metabolic Competence

Kristen Hopperstad, Danica E. DeGroot,[2] Todd Zurlinden, Cassandra Brinkman, Russell S. Thomas, and Chad Deisenroth[1]



Study Highlights

- **Chemical Screening**: Screened 768 chemicals from the ToxCast library for Estrogen Receptor 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.

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

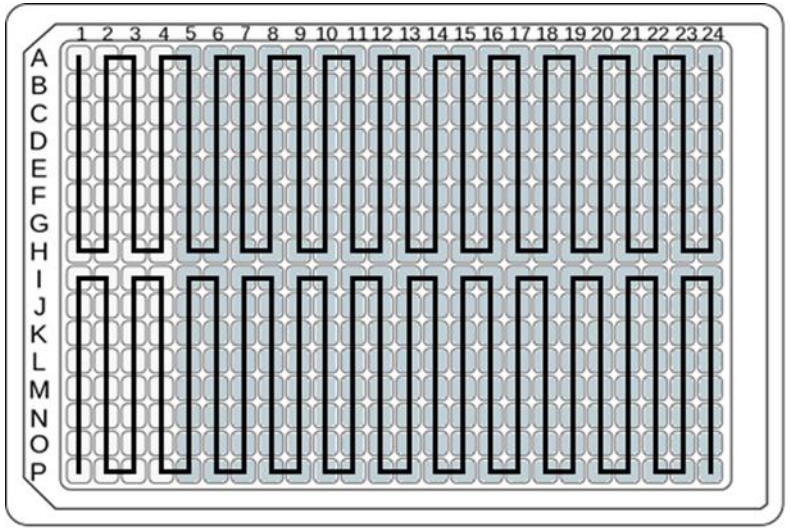
Parent and Metabolite Identifiers

Route of Transformation

Accumulation and Probability Classification

In Vitro Metabolism Data

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



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Ready for assay

- Goal: Adapt AIME method to an automated approach using bioprinting for routine application to high-throughput screening.
- The bioprinter method expands the functional capacity for hepatic phase I (CYPs) and phase II (UGTs, SULTs, GSTs) metabolic enzymes.

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Steve Simmons
Rusty Thomas

Paul Carmichael
Mi-Young Lee

Contact

deisenroth.chad@epa.gov