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Perspectives on the Use of High Throughput Profiling Assays in Next Generation Risk Assessment

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Disclaimer

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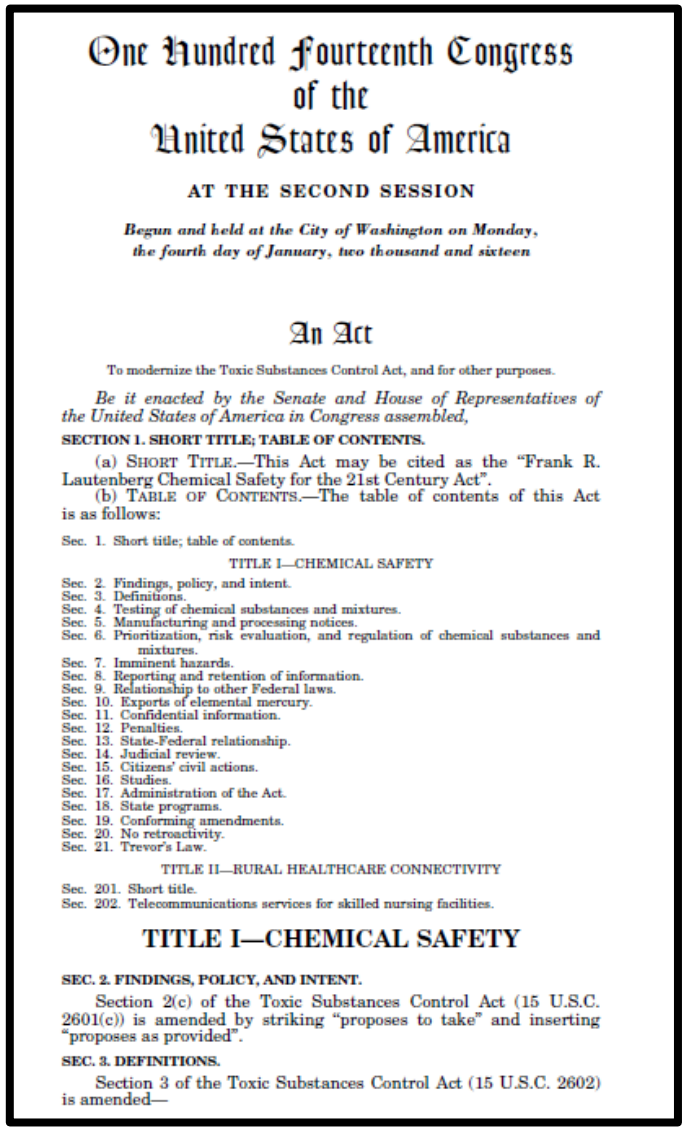
Outline

- **Background**
  - Emphasis on NAMs at US EPA
  - US EPA Blueprint for Computational Toxicology
- **High Throughput Transcriptomics (HTTr)**
- **High Throughput Phenotypic Profiling (HTPP)**
- **Potential Applications for HTTr- and HTPP-derived Molecular PODs**

Regulatory Driver for Development & Use of NAMs by US EPA



One Hundred Fourteenth Congress
of the
United States of America

AT THE SECOND SESSION

Begun and held at the City of Washington on Monday, the fourth day of January, two thousand and sixteen

An Act

To modernize the Toxic Substances Control Act, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE; TABLE OF CONTENTS.

(a) SHORT TITLE.—This Act may be cited as the "Frank R. Lautenberg Chemical Safety for the 21st Century Act".

(b) TABLE OF CONTENTS.—The table of contents of this Act is as follows:

Sec. 1. Short title; table of contents.

TITLE I—CHEMICAL SAFETY

Sec. 2. Findings, policy, and intent.
Sec. 3. Definitions.
Sec. 4. Testing of chemical substances and mixtures.
Sec. 5. Manufacturing and processing notices.
Sec. 6. Prioritization, risk evaluation, and regulation of chemical substances and mixtures.
Sec. 7. Imminent hazards.
Sec. 8. Reporting and retention of information.
Sec. 9. Relationship to other Federal laws.
Sec. 10. Exports of elemental mercury.
Sec. 11. Confidential information.
Sec. 12. Penalties.
Sec. 13. State-Federal relationship.
Sec. 14. Judicial review.
Sec. 15. Citizens' civil actions.
Sec. 16. Studies.
Sec. 17. Administration of the Act.
Sec. 18. State programs.
Sec. 19. Conforming amendments.
Sec. 20. No retroactivity.
Sec. 21. Trevor's Law.

TITLE II—RURAL HEALTHCARE CONNECTIVITY

Sec. 201. Short title.
Sec. 202. Telecommunications services for skilled nursing facilities.

TITLE I—CHEMICAL SAFETY

SEC. 2. FINDINGS, POLICY, AND INTENT.

Section 2(c) of the Toxic Substances Control Act (15 U.S.C. 2601(c)) is amended by striking "proposes to take" and inserting "proposes as provided".

SEC. 3. DEFINITIONS.

Section 3 of the Toxic Substances Control Act (15 U.S.C. 2602) is amended—

The Toxic Substances Control Act (TSCA), as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, directs EPA to:

1. **Reduce and replace**, to the extent practicable and scientifically justified, the use of vertebrate animals in the testing of chemical substances or mixtures;
2. Promote the development and timely incorporation of **alternative test methods or strategies** that do not require new vertebrate animal testing

"Alternative test methods" – Tools of the Trade

1. ***Computational toxicology and bioinformatics.***
2. ***High-throughput screening methods.***
3. Testing of categories of chemical substances.
4. ***Tiered testing methods.***
5. ***In vitro studies.***
6. Systems Biology.
7. ICCVAM or OECD validated assays.
8. Industry consortia that develop information submitted under this title.

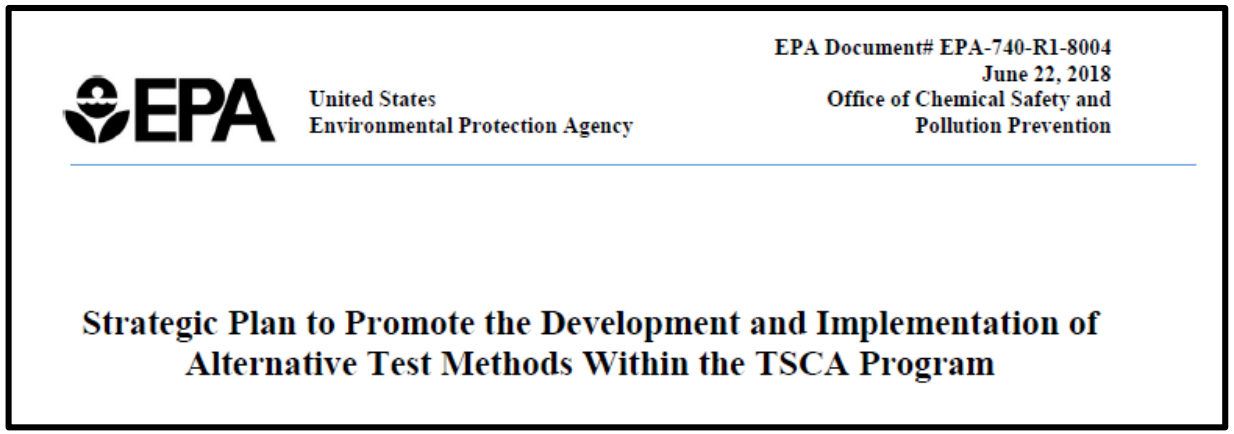
"Alternative test methods" → "New Approach Methods (NAMs)"

Any technology, methodology, approach or combination thereof that can be used to provide information on chemical hazard and risk that avoids the use of intact animals.

2016

https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

Emphasis on NAMs at US EPA

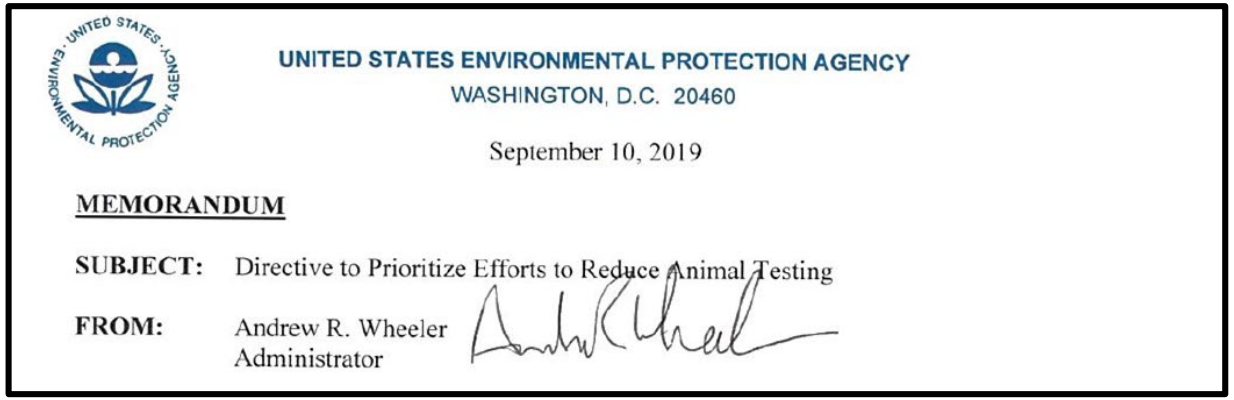


EPA Document# EPA-740-R1-8004
June 22, 2018
Office of Chemical Safety and Pollution Prevention

United States Environmental Protection Agency

Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program

Outlines strategic plan for the reduction of testing in vertebrates for chemicals regulated under TSCA.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

Directs leadership at US EPA [OSCPP and ORD] to prioritize efforts that will demonstrate measurable reduction of animal testing while ensuring protection of human health and environment.

Describes US EPA's roadmap and tangible steps to pursuing and achieving animal use reduction goals while ensuring that the Agency's regulatory, compliance and enforcement activities remain fully protective of human health and the environment.

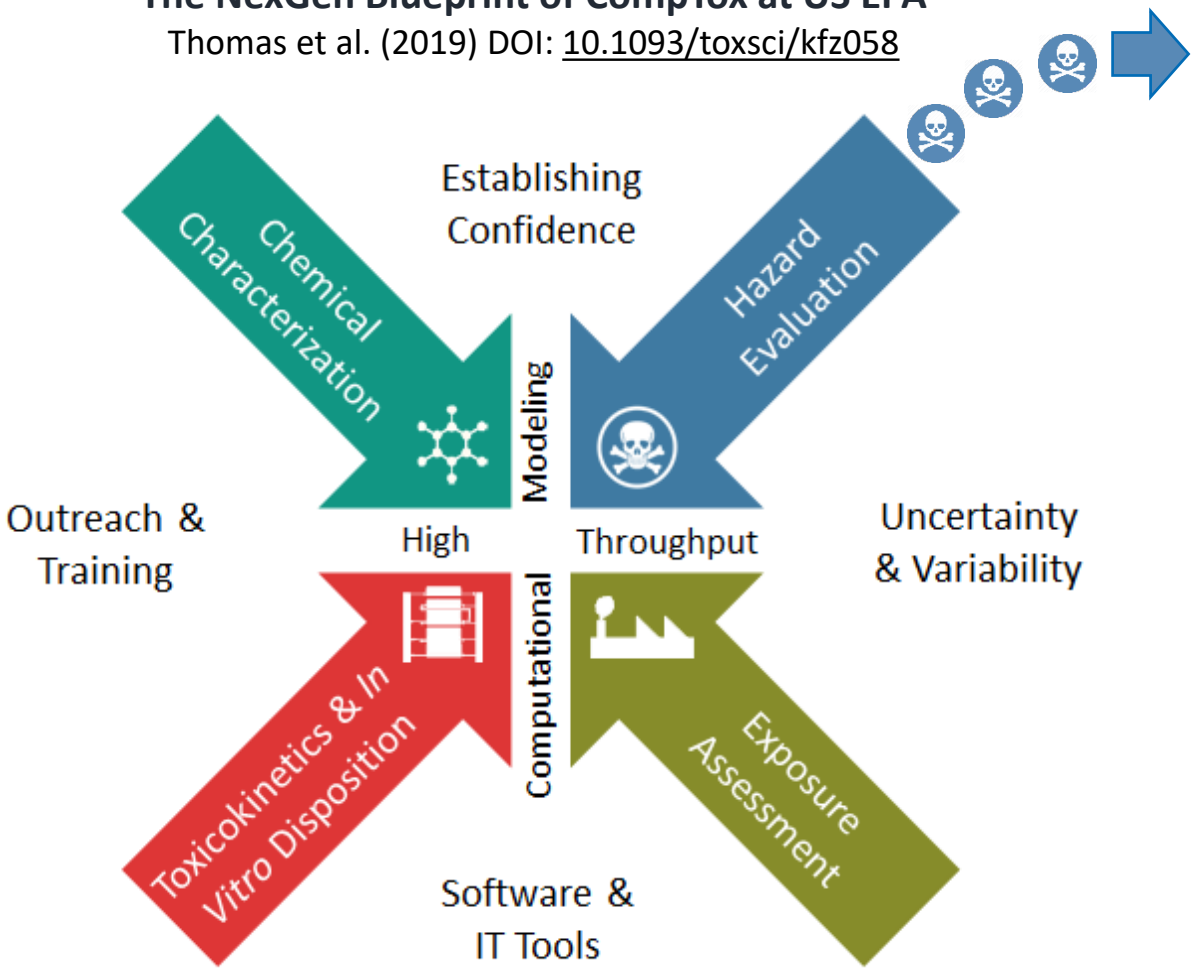
2016 — 2018 — 2019 — 2020

https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing

Computational Toxicology Research Areas

The NexGen Blueprint of CompTox at US EPA

Thomas et al. (2019) DOI: 10.1093/toxsci/kfz058



- **ToxCast:** Uses targeted high-throughput screening (HTS) assays to expose living cells or isolated proteins to chemicals and assess bioactivity and potential toxic effects.

	# of assays	# of chemicals	Types of chemicals
Phase 1 *(2007 – 2009)*	500	300	Mostly pesticides
Phase 2 *(2009 – 2013)*	700	2,000	Industrial, consumer product, food use, "green"

- Mostly targeted assays (*chemical X → target Y*).
- Incomplete coverage of human biological space.
- **New Strategy for Hazard Evaluation:** Improve efficiency and increase biological coverage by using broad-based (i.e. non-targeted) assays that cast the broadest net possible for capturing the potential molecular and phenotypic responses of human cells in response to chemical exposures.

NAMs-Based Tiered Hazard Evaluation Approach (1)

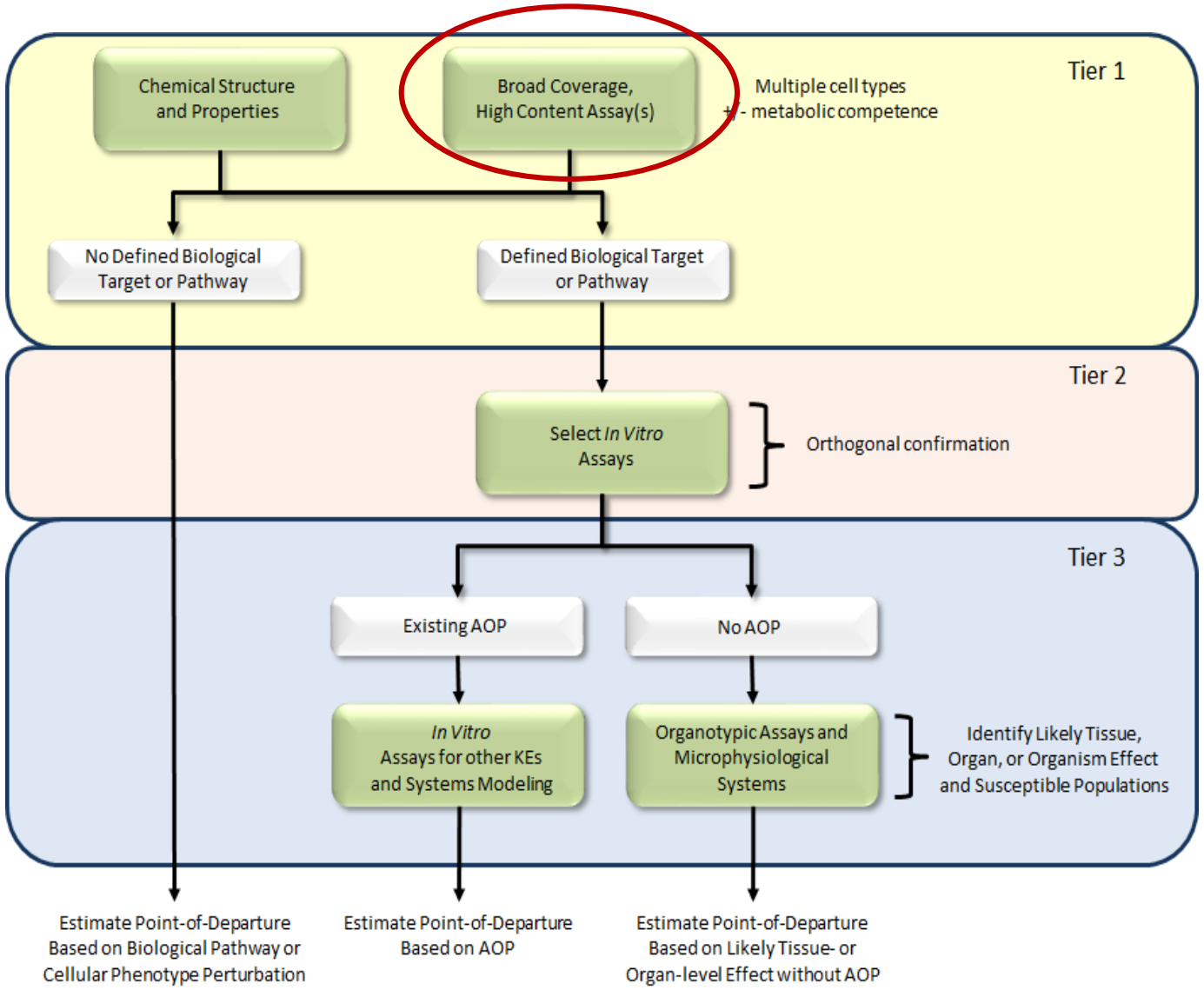
High throughput profiling (HTP) assays are proposed as the first tier in a NAMs-based hazard evaluation approach.

HTP Assay Criteria:

1. Yield bioactivity profiles that can be used for **potency estimation, mechanistic prediction** and evaluation of **chemical similarity**.
2. Compatible with multiple human-derived culture models.
3. Concentration-response screening mode.
4. Cost-effective.

To date, EPA has identified and implemented two HTP assays that meet this criteria.

- **High-Throughput Transcriptomics [HTTr]**
- **High-Throughput Phenotypic Profiling [HTPP]**



The NexGen Blueprint of CompTox at US EPA
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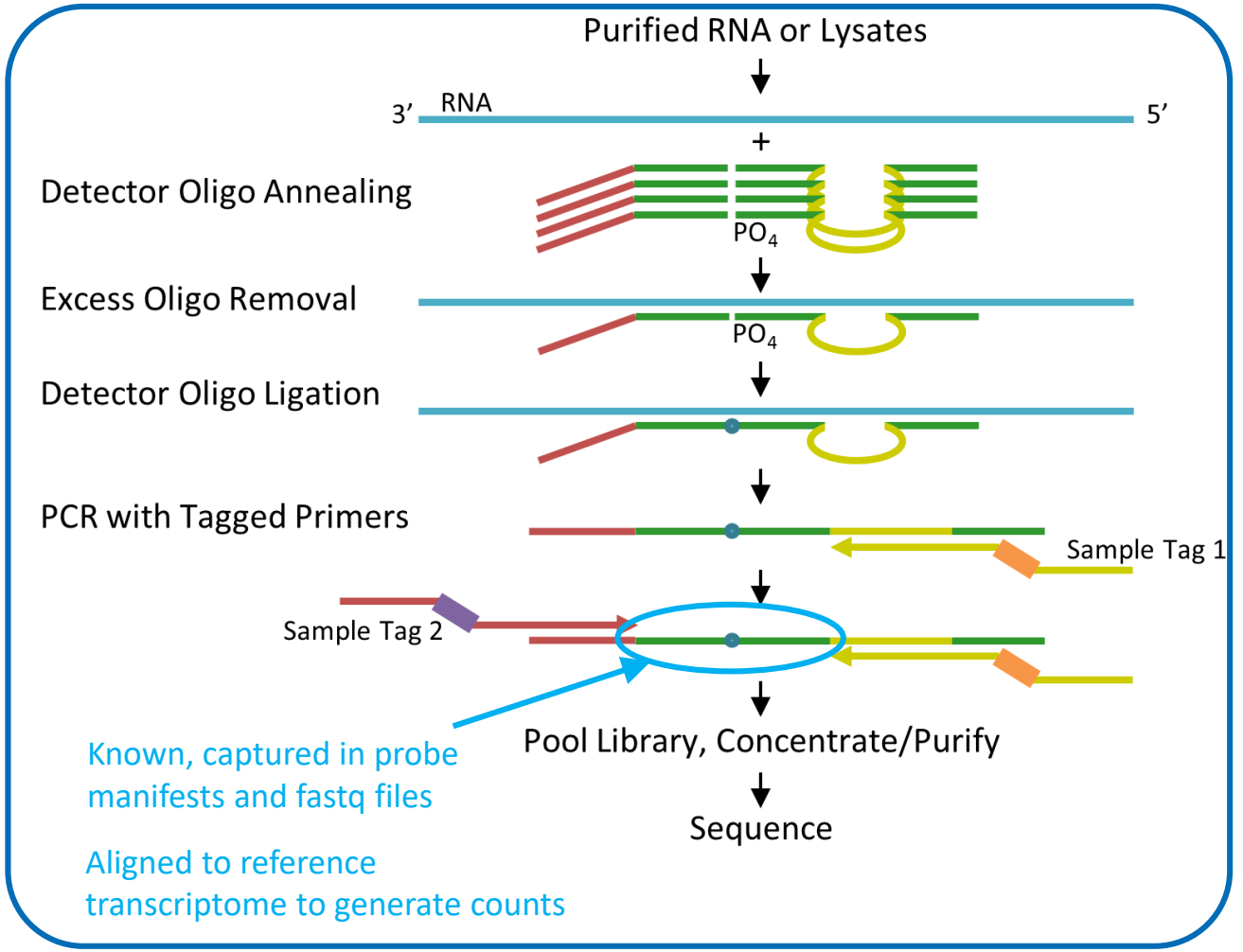
High-Throughput Transcriptomics (HTTr)

Templated Oligo with Sequencing Readout (TempO-Seq)

- The **TempO-Seq** human whole transcriptome assay measures the expression of greater than 20,000 transcripts.
- Requires only picogram amounts of total RNA per sample.
- Compatible with purified RNA samples or **cell lysates**.
- Lysates are barcoded according to sample identity and combined in a single library for sequencing using industry standard instruments.
- Scalable, targeted assay:
  - 1) specifically measures transcripts of interest
  - 2) ~50-bp reads for all targeted genes
  - 3) requires less flow cell capacity than RNA-Seq

TempO-Seq Assay Illustration



Yeakley et al. (2017) DOI: 10.1371/journal.pone.0178302

MCF7 Pilot Experimental Design

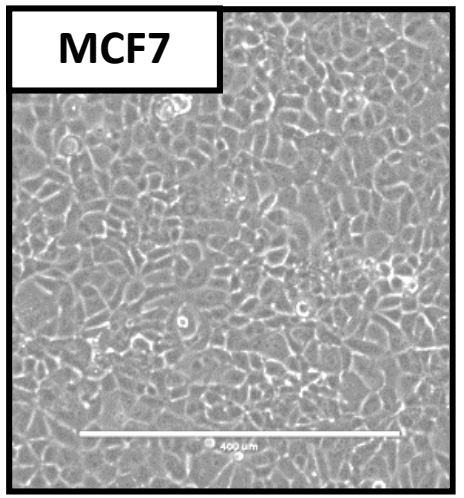
High-Throughput Transcriptomics Platform for Screening Environmental Chemicals

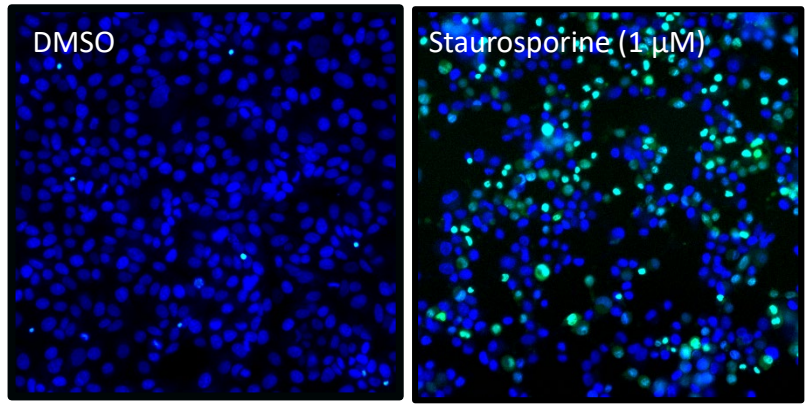
TOXICOLOGICAL SCIENCES, 2021, 1–22

doi: 10.1093/toxsci/kfab009
Advance Access Publication Date: 4 February 2021
Research Article

Joshua A. Harrill,*,1 Logan J. Everett,* Derik E. Haggard,*,† Thomas Sheffield,*,† Joseph L. Bundy,* Clinton M. Willis,*,‡ Russell S. Thomas,* Imran Shah,* and Richard S. Judson*

Parameter	Multiplier	Notes
Cell Type(s)	1	MCF7
Assay Formats:	2	High-Throughput Transcriptomics Cell Viability
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	44	ToxCast chemicals
Time Points:	1	6 hours
Concentrations:	8	3.5 $\log_{10}$ units; semi $\log_{10}$ spacing
Biological Replicates:	3	Independent cultures





CellEvent Caspase 3/7

Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

MCF7 Pilot Chemical List

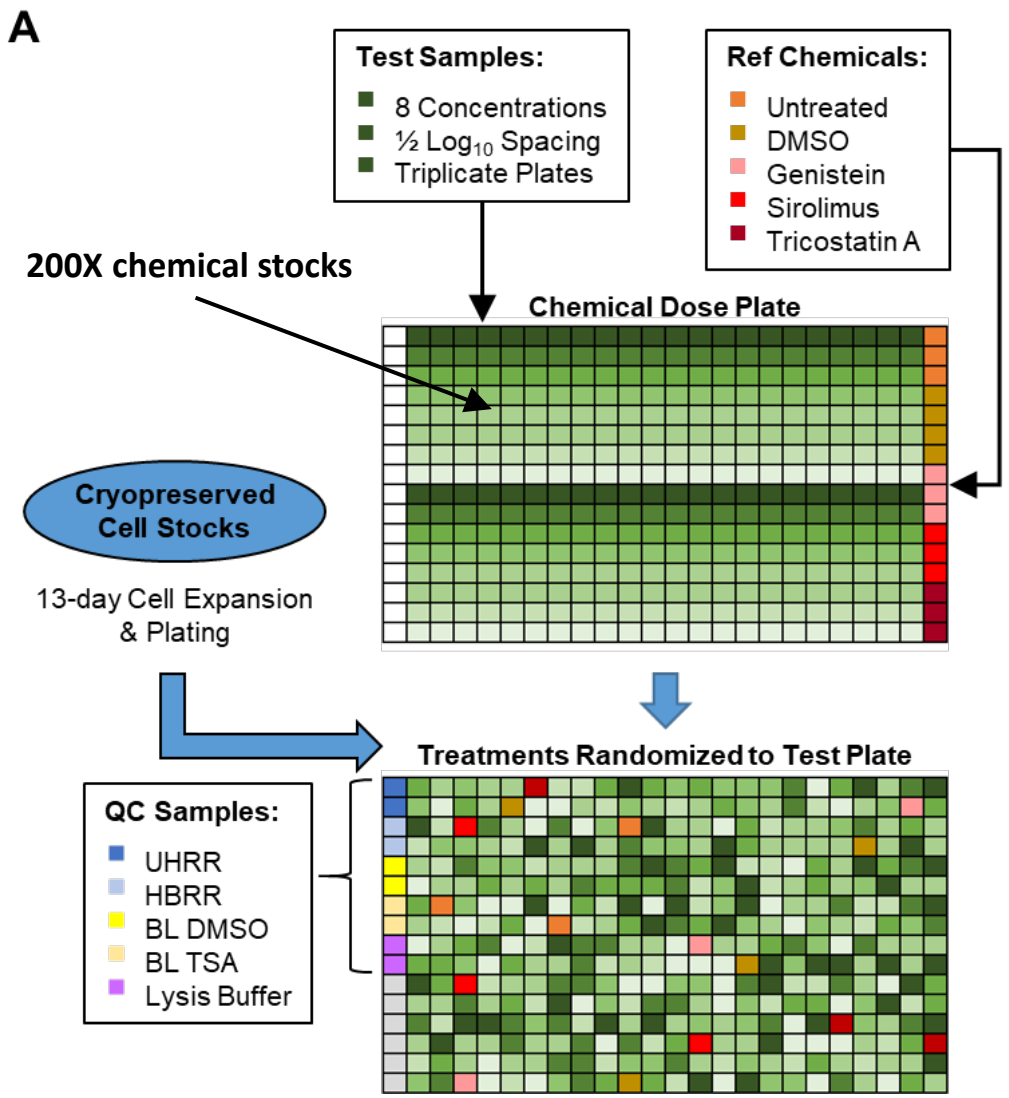
Table 1. Chemicals Used in the Study

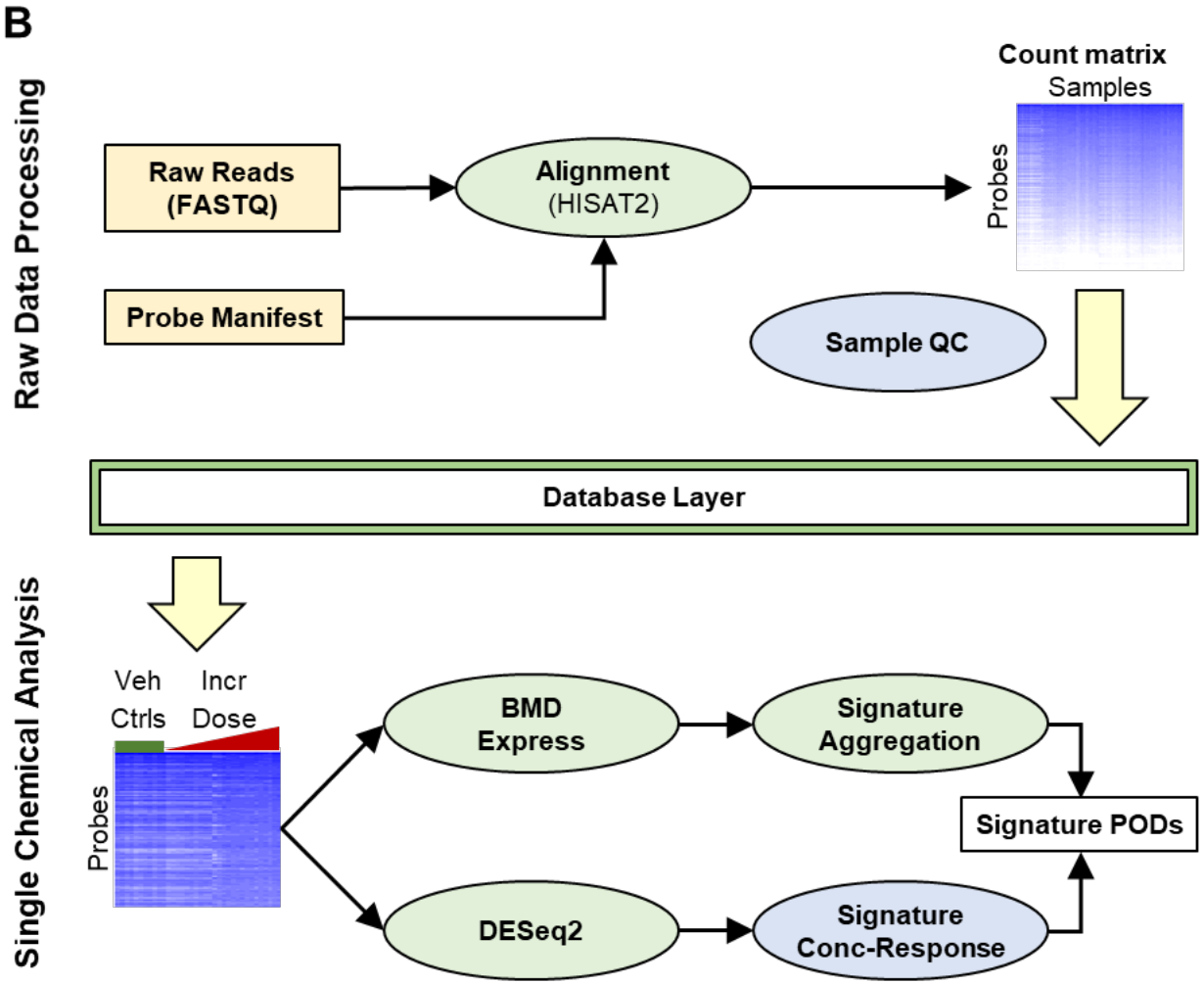
Name	Target Annotation
Cyproterone acetate	AR antagonist
Flutamide	AR antagonist
Nilutamide	AR antagonist
Vinclozolin	AR antagonist
Amiodarone hydrochlorid	Blocks myocardial calcium, potassium and sodium channels
Cladribine	DNA synthesis inhibitor
4-Cumylphenol	ER agonist
4-Nonylphenol, branched	ER agonist
Bisphenol A	ER agonist
Bisphenol B	ER agonist
4-Hydroxytamoxifen	ER antagonist
Clomiphene citrate (1:1)	ER antagonist
Fulvestrant	ER antagonist
Cyproconazole	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor
Imazalil	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor
Prochloraz	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor
Propiconazole	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor
Atrazine	Herbicide, photosystem II inhibitor
Cyanazine	Herbicide, photosystem II inhibitor
Simazine	Herbicide, photosystem II inhibitor
Butafenacil	Herbicide, PPO inhibition
Fomesafen	Herbicide, PPO inhibition
Lactofen	Herbicide, PPO inhibition
Lovastatin	HMGCR inhibitor
Simvastatin	HMGCR inhibitor
Maneb	Inhibition of metal-dependent and sulfhydryl enzyme systems
Thiram	Inhibition of metal-dependent and sulfhydryl enzyme systems
Ziram	Inhibition of metal-dependent and sulfhydryl enzyme systems
Reserpine	Inhibition of the ATP/Mg2+ pump
Rotenone	Mitochondria (complex I inhibitor)
Pyraclostrobin	Mitochondria (complex III inhibitor)
Trifloxystrobin	Mitochondria (complex III inhibitor)
Fenpyroximate (Z, E)	Mitochondrial electron transport inhibitor
Clofibrate	PPARα agonist, upregulates extrahepatic lipoprotein lipase
Fenofibrate	PPARα agonist, upregulates extrahepatic lipoprotein lipase
Farglitazar	PPARγ agonist
Perfluorooctanoic acid (PFOA)	PPARγ, PPARα agonist
Perfluorooctanesulfonic acid (PFOS)	PPARγ, PPARα agonist
Troglitazone	PPARγ, PPARα agonist
Cycloheximide	Protein synthesis inhibitor
Bifenthrin	Sodium channel modulator
Cypermethrin	Sodium channel modulator
Tetrac	T4 synthesis inhibitor
3,5,3′-triiodothyronine	THR agonist

- Chemicals were selected that cover a broad range of molecular targets with some redundancy within target class.
- Intentionally selected some chemicals whose molecular targets are not expressed in MCF7 cells (or in mammalian tissues).

Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

HTTr Experimental Design and Bioinformatics Workflow





Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

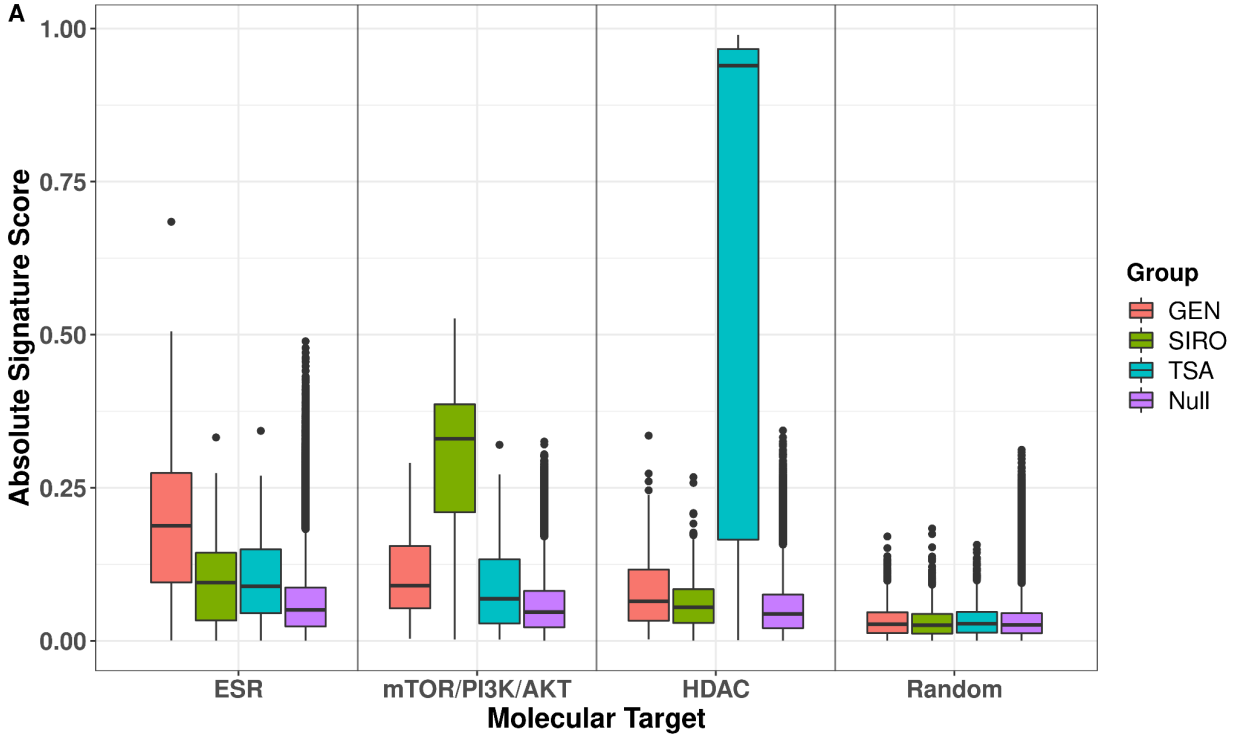
Concentration-Response Modeling of Gene Signatures

- Understanding the biological meaning of changes in gene expression for **10,000 – 20,000 genes** is difficult.
- Analyzing responses at the level of the gene signature aids in **data interpretation**.
- Takes into account coordinated changes in gene expression that may not be identified using gene level fitting approaches.
- **Examples of signature types**:
  - Genes that are perturbed in diseased tissue vs. healthy tissue.
  - Genes perturbed by gene knockdowns / knockouts.
  - Genes perturbed by drugs or other chemicals with known (or unknown) mechanisms.
- **Example use:**
  - If an unknown *chemical X* perturbs genes that are also perturbed by a well-characterized chemical with a specific mechanism of action, then one can infer the *chemical X* may affect the same molecular target(s).
- **CCTE signature collection:**
  - Compiled from many public sources (MSigDB[1], BioPlanet[2], DisGeNET[3], Connectivity Map[4]) → ~10,000 signatures
  - **For CMAP signatures:**
    - Identify the top 100 up- and down-regulated genes.
    - Score each "up" and "down" signature separately.
    - Combine into a single score ($Score_{UP} – Score_{Down} = Score_{Combined}$)

[1] *Liberzon et al., Bioinformatics. 2011 Jun 15;27(12):1739-40*
[2] *Huang et al., Front Pharmacol. 2019 Apr 26;10:445*
[3] *Pinero et al., Database (Oxford). 2015 Apr 15;2015:bav028*
[4] *Subramanian et al., Science. 2006 Sep 29;313(5795):1929-35.*

Signature Scoring for HTTr Assay Performance Assessment

A



B

	Signature	Absolute Score	Direction
Genistein	RYAN_ESTROGEN_RECEPTOR_ALPHA	0.6844617	+
	CMAP equilin 1.5e-05 100 5542 100	0.5054424	+
	CMAP fulvestrant 1e-08 100 1417 100	0.4857079	-
	CMAP fulvestrant 1e-06 100 6811 100	0.4519683	-
	BHAT_ESR1_TARGETS_NOT_VIA_AKT1	0.4270213	+
Sirolimus	CMAP sirolimus 1e-07 100 5437 100	0.5265263	+
	CMAP sirolimus 1e-07 100 6409 100	0.5166319	+
	CMAP sirolimus 1e-07 100 8359 100	0.5001286	+
	CMAP wortmannin 1e-06 100 577 100	0.4990501	+
	CMAP wortmannin 1e-08 100 1423 100	0.4971645	+
Trichostatin A	CMAP vorinostat 1e-05 100 817 100	0.9899307	+
	CMAP trichostatin A 1e-07 100 8791 100	0.9869679	+
	CMAP trichostatin A 1e-07 100 6547 100	0.9854099	+
	CMAP trichostatin A 1e-06 100 8056 100	0.9852016	+
	CMAP trichostatin A 1e-07 100 7972 100	0.9849593	+

- Signature scoring using the single sample Gene Set Enrichment Analysis (ssGSEA) approach (Barbie et al. 2009)
- The “correct” target classes were identified for reference chemical treatments.

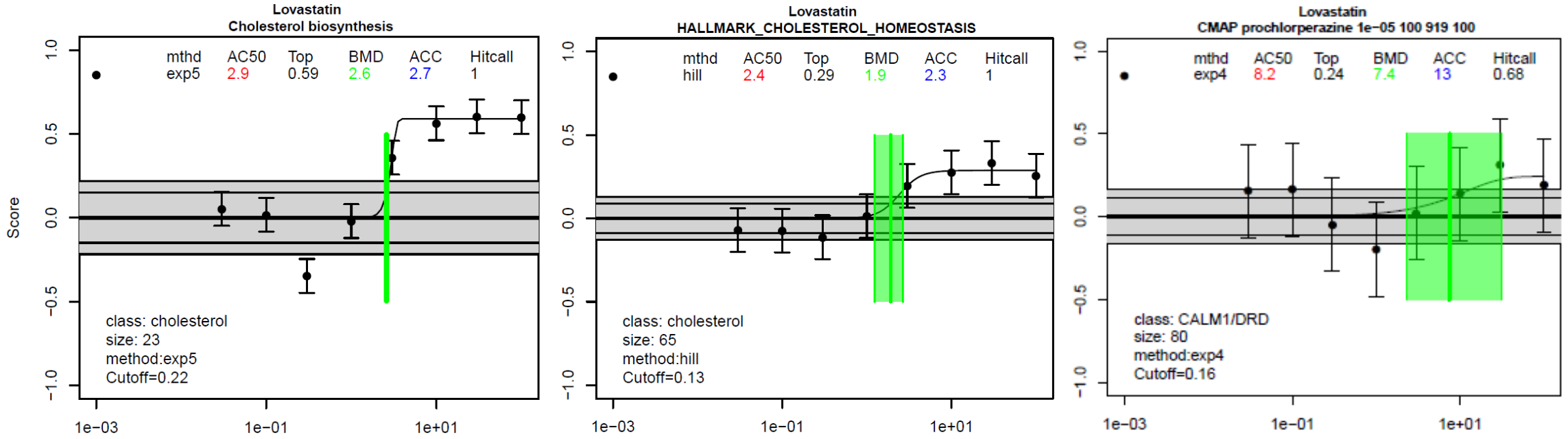
Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009
Barbie et al. (2009) DOI: 10.1038/nature08460

Concentration-Response Modeling of Signature Scores

Concentration response modeling of signature scores using ***tcplfit2*** (https://rdrr.io/github/USEPA/CompTox-ToxCast-tcplFit2/)

New and/or improved functionality of *tcplfit2* (versus *tcpl*):

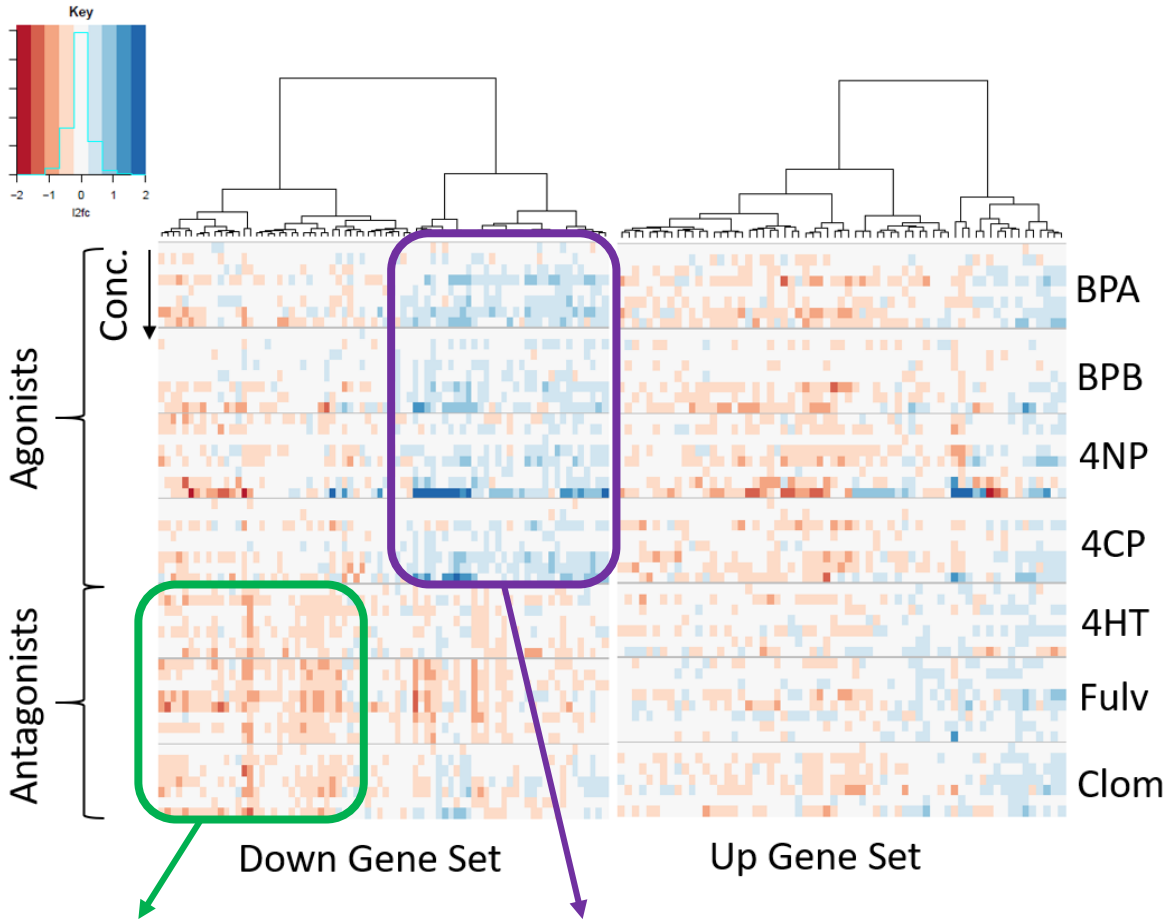
- All curve forms from *tcpl* and BMDExpress are included.
- Calculates benchmark concentrations (BMCs) in addition to AC50s.
- Models in the "up" and "down" direction.
- Provides continuous hit calls for identifying high confidence and low confidence hits.



Concentration-Response Modeling of Signature Scores (2)

A

Fulvestrant Signature
(Top 100 Up & Down Genes)



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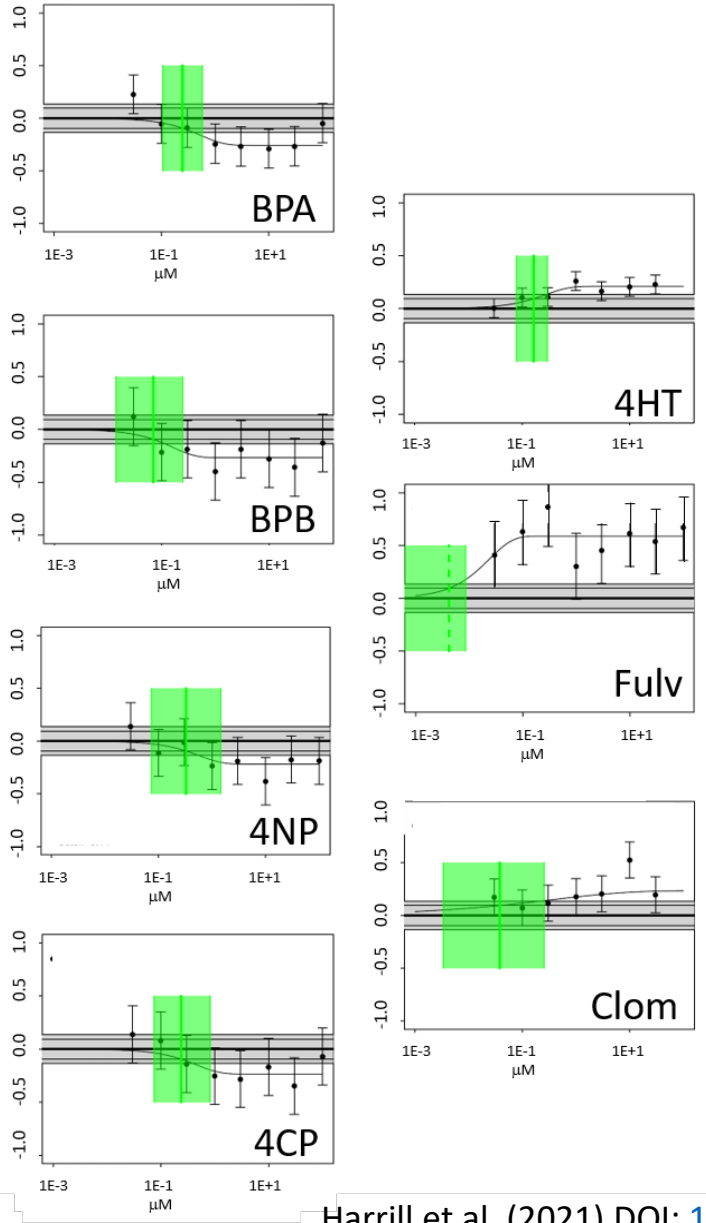


These gene level data are noisy!

Signature level results display correct directionality!

The expression of fulvestrant signature "down" genes goes down following ER antagonist treatment

The expression of fulvestrant signature "down" genes goes up following ER agnoist treatment

Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

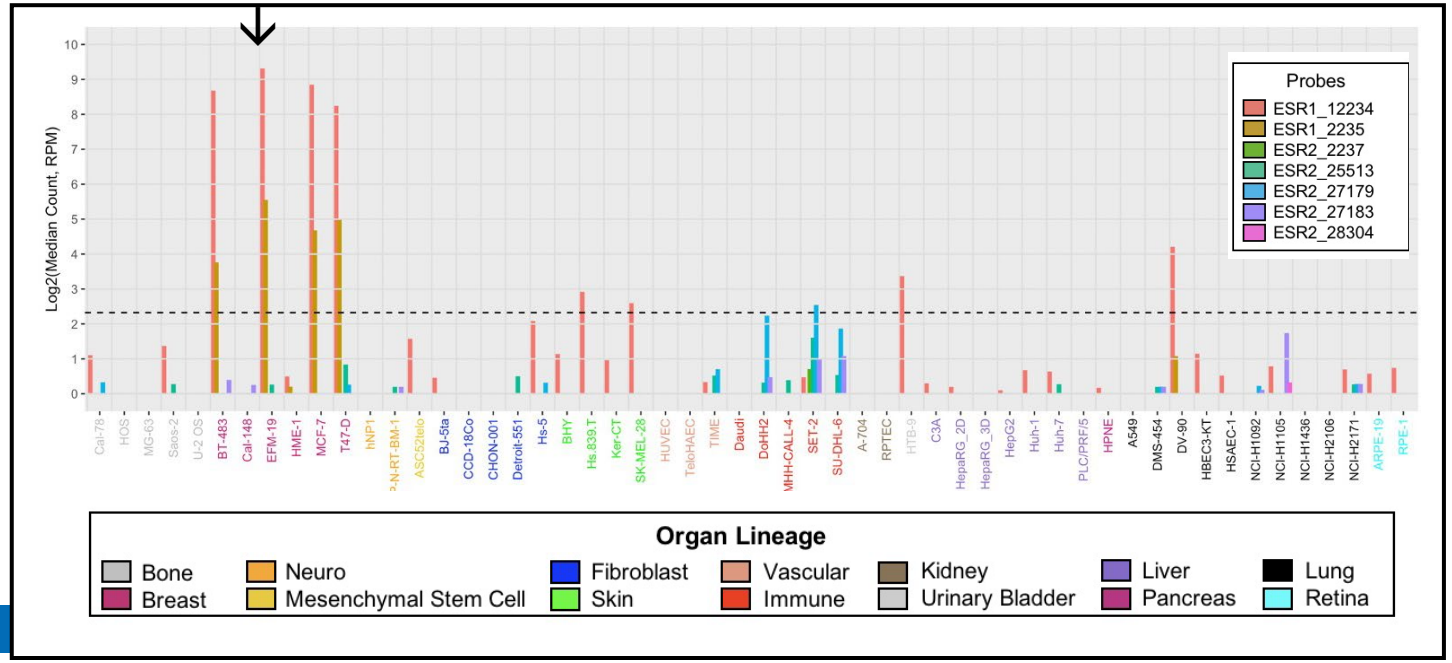
Comparison of Transcriptional BPACs to ER Model

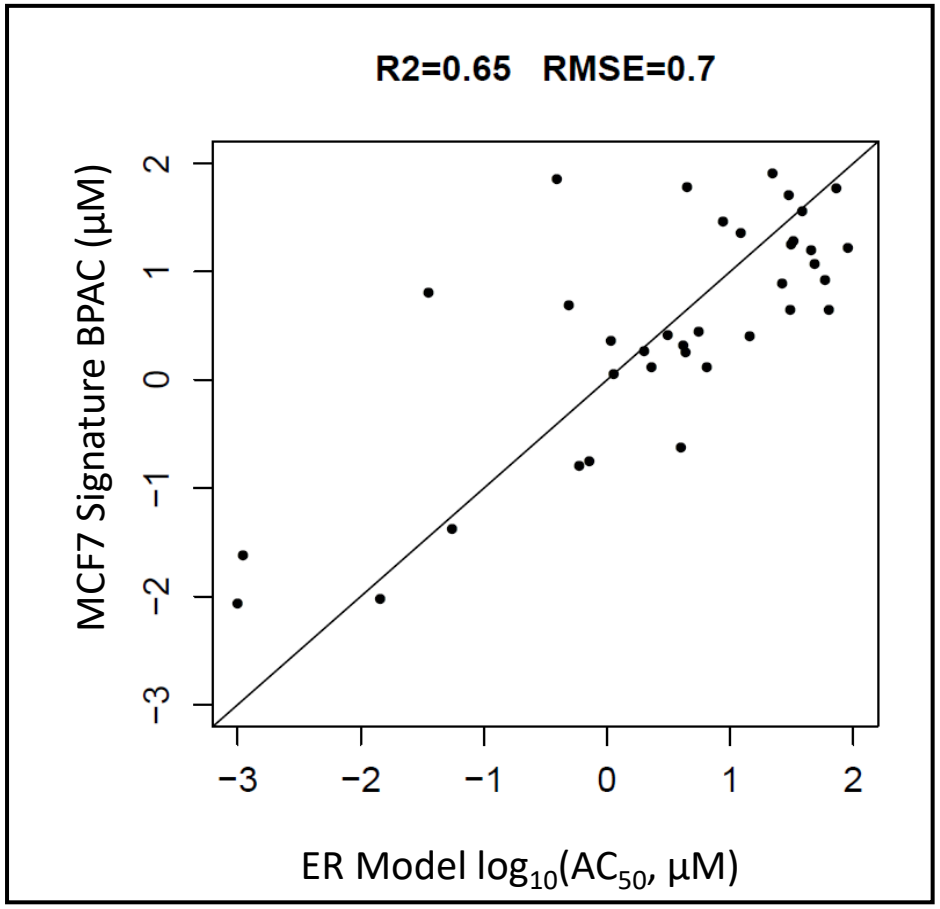
- US EPA has developed a battery of 18 ToxCast assays to predict activity at the estrogen receptor (Brown et al. (2015) DOI: 10.1021/acs.est.5b02641)

- Log_{10} AC_{50} values from the ToxCast ER model assays were compared to transcriptomic signature BPACs in MCF7 cells for a collection of 37 estrogenic chemicals.

- Signature-based BPACs are concordant with ER model predictions. →

- Estrogen receptor is also abundantly expressed in MCF7 cells (and other breast-derived cell lines).





Harrill et al., (unpublished). DO NOT CITE OR QUOTE

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High-Throughput Phenotypic Profiling (HTPP)

High Throughput Phenotypic Profiling (HTPP) with Cell Painting

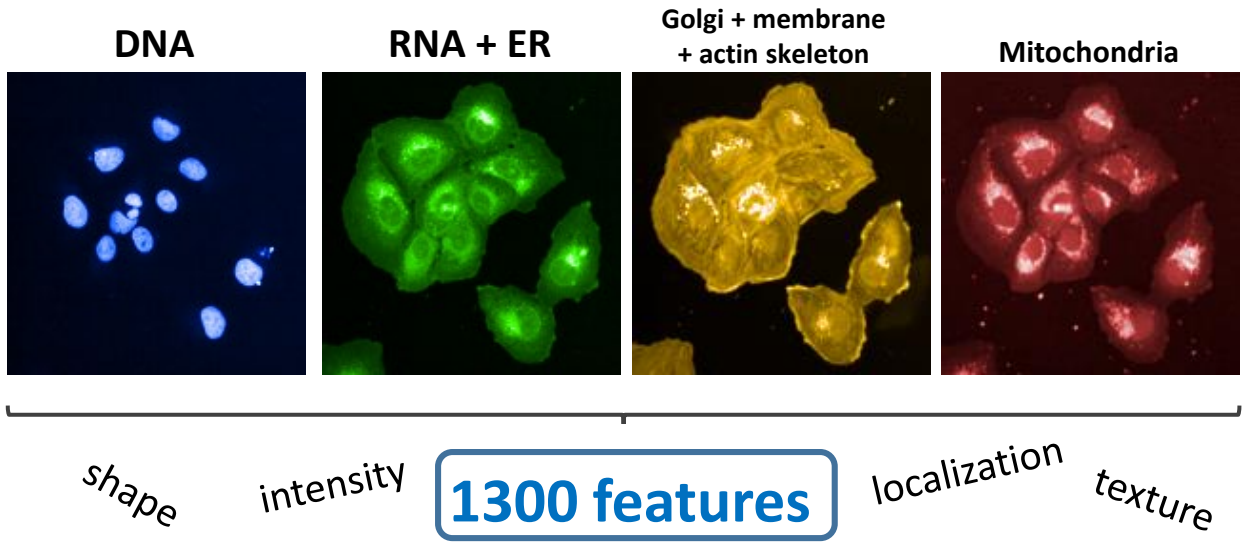
- **Cell Painting** is a profiling method that measures a large variety of phenotypic features in fluoroprobe labeled cells *in vitro*

  (Bray et al. (2016) DOI: 10.1038/nprot.2016.105)

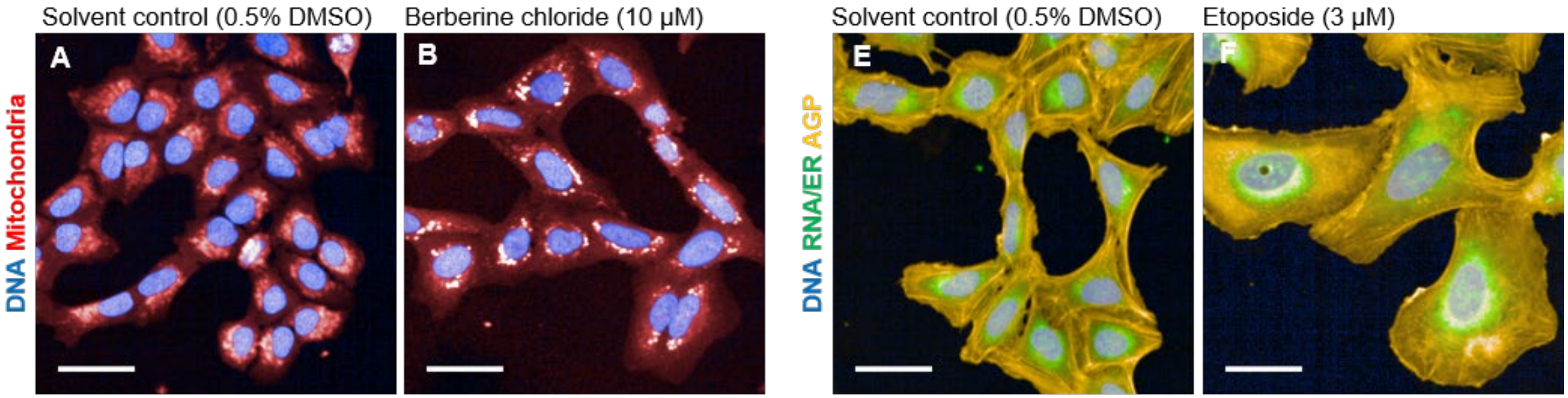
- Previous Uses:
  - Drug discovery
  - Compound efficacy and toxicity screening
  - Mechanism-of-action identification
  - Chemical grouping
  - Functional genomics

- Efficient and **cost-effective** method for evaluating the bioactivity of environmental chemicals.

Marker	Cellular Component	Labeling Chemistry	Labeling Phase	Opera Phenix	
				Ex.	Em.
Hoechst 33342	Nucleus	Bisbenzamide probe that binds to dsDNA	Fixed	405	480
Concanavalin A – AlexaFluor 488	Endoplasmic reticulum	Lectin that selectively binds to α-mannopyranosyl and α-glucopyranosyl residues enriched in rough endoplasmic reticulum		435	550
SYTO 14 nucleic acid stain	Nucleoli	Cyanine probe that binds to ssRNA		435	550
Wheat germ agglutinin (WGA) – AlexaFluor 555	Golgi Apparatus and Plasma Membrane	Lectin that selectively binds to sialic acid and N-acetylglucosaminyl residues enriched in the trans-Golgi network and plasma membrane		570	630
Phalloidin –AlexaFluor 568	F-actin (cytoskeleton)	Phallotoxin (bicyclic heptapeptide) that binds filamentous actin			
MitoTracker Deep Red	Mitochondria	Accumulates in active mitochondria	Live	650	760

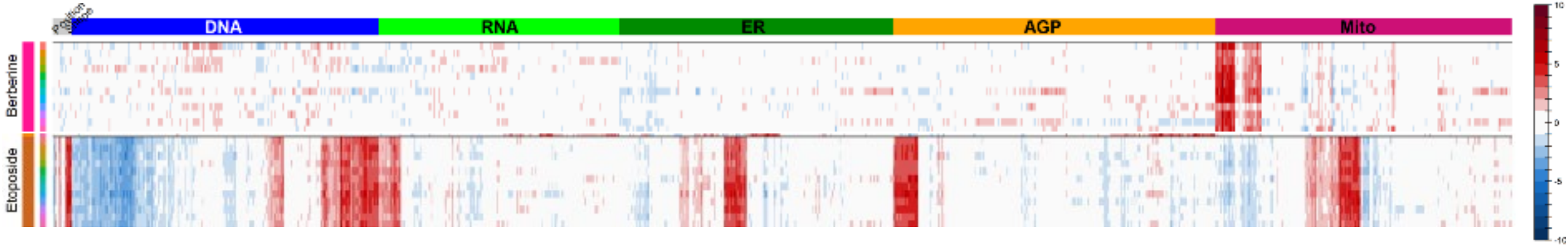


Chemicals Produce Distinct Quantifiable Phenotypes



→ Mitochondrial compactness/texture

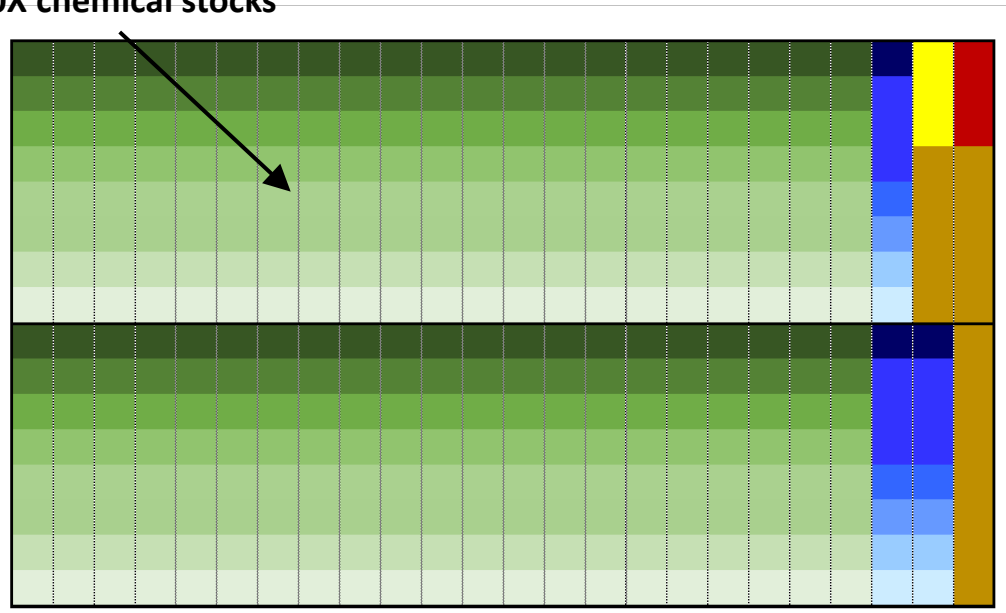
→ Cells are larger

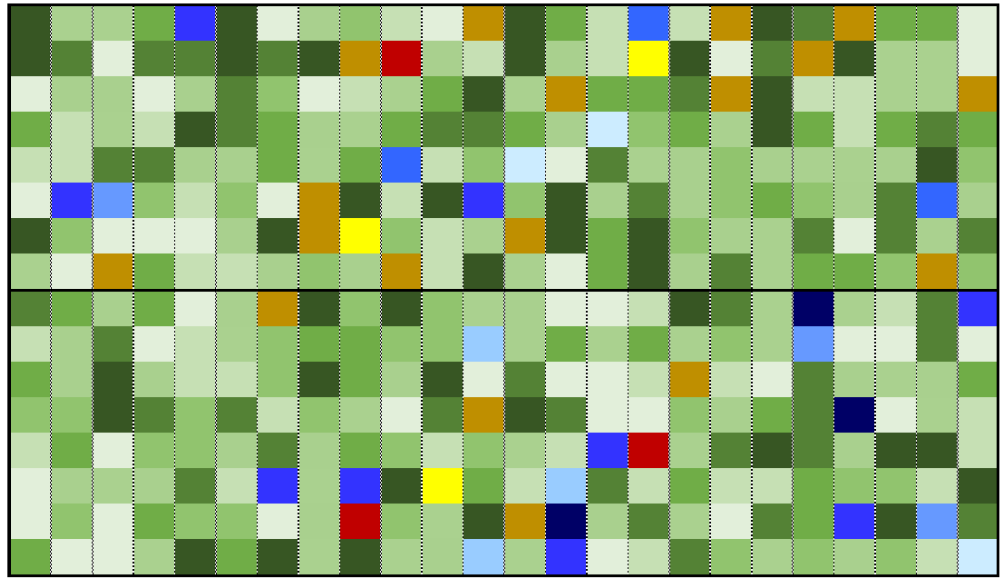


- Repeated testing of reference chemicals demonstrates reproducibility of Cell Painting phenotypes.

adapted from Nyffeler et al. (2020) DOI: 10.1016/j.taap.2019.114876

HTPP Screening Dose Plate Design (U-2 OS Cells)





Label	Reference Chemicals:	Molecular Mechanism-of-Action	Test Concentrations
A	Etoposide	DNA topoisomerase inhibitor	0.03 - 10 µM
B	all-trans-Retinoic Acid	Retinoic acid receptor agonist	0.0003 – 1 µM
C	Dexamethasone	Glucocorticoid receptor agonist	0.001 – 3 µM
D	Trichostatin A	Histone deacetylase inhibitor	1 µM
E	Staurosporine	Cytotoxicity control	1 µM
F	DMSO	Vehicle control	0.5 %

HTPP Data Analysis Pipeline

Data reduction

cell-level data

Normalization
MAD normalization

$$\frac{\text{cell value} - \text{median}_{DMSO}}{1.4826\ \text{MAD}_{DMSO}}$$

normalized cell-level data

Aggregation
median

well-level data

Standardization
Z transformation

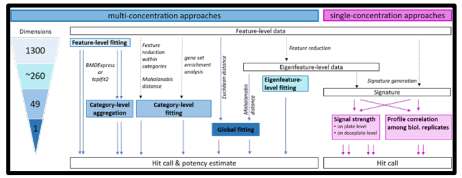
Cell Count Info
Conc. > 50% cell loss

scaled well-level data

clipped well-level data

-20 -15 -10 -5 0 5 10 15 20

Concentration Response Modeling



Feature z-scores
Latent variables

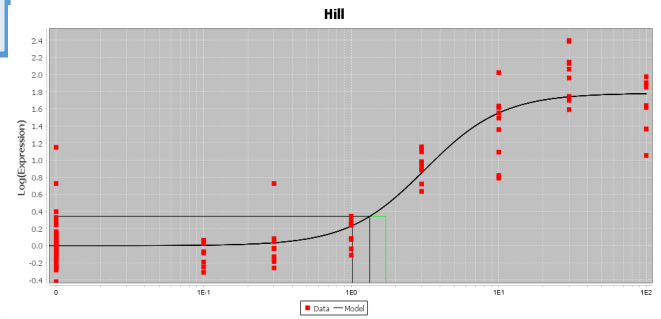
(See Next Slide)

Nyffeler et al. (2021). DOI: 10.1177/2472555220950245

Fit Multiple Curve Shapes

Best Model Selection

BMC

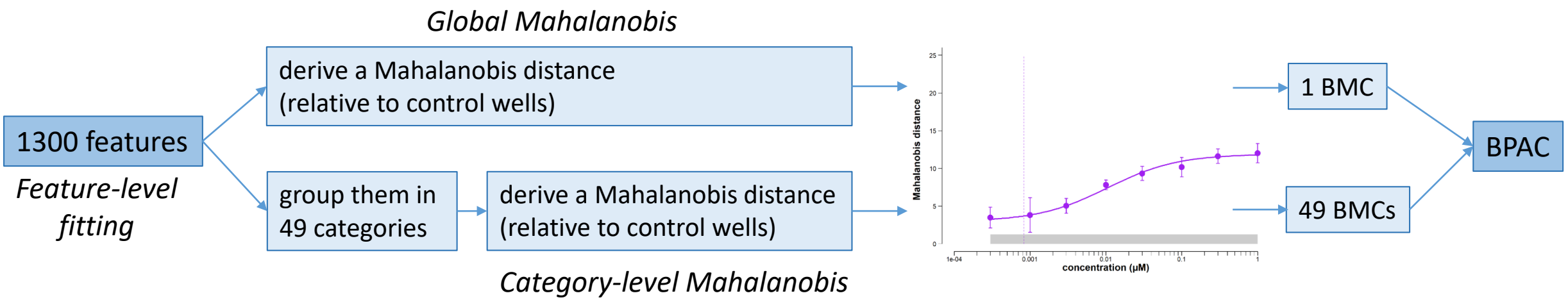


Berberine chloride
Mito_Cells_Morph_STAR

Mahalanobis Distance Modeling of HTPP Data

Mahalanobis Distance (D_M):

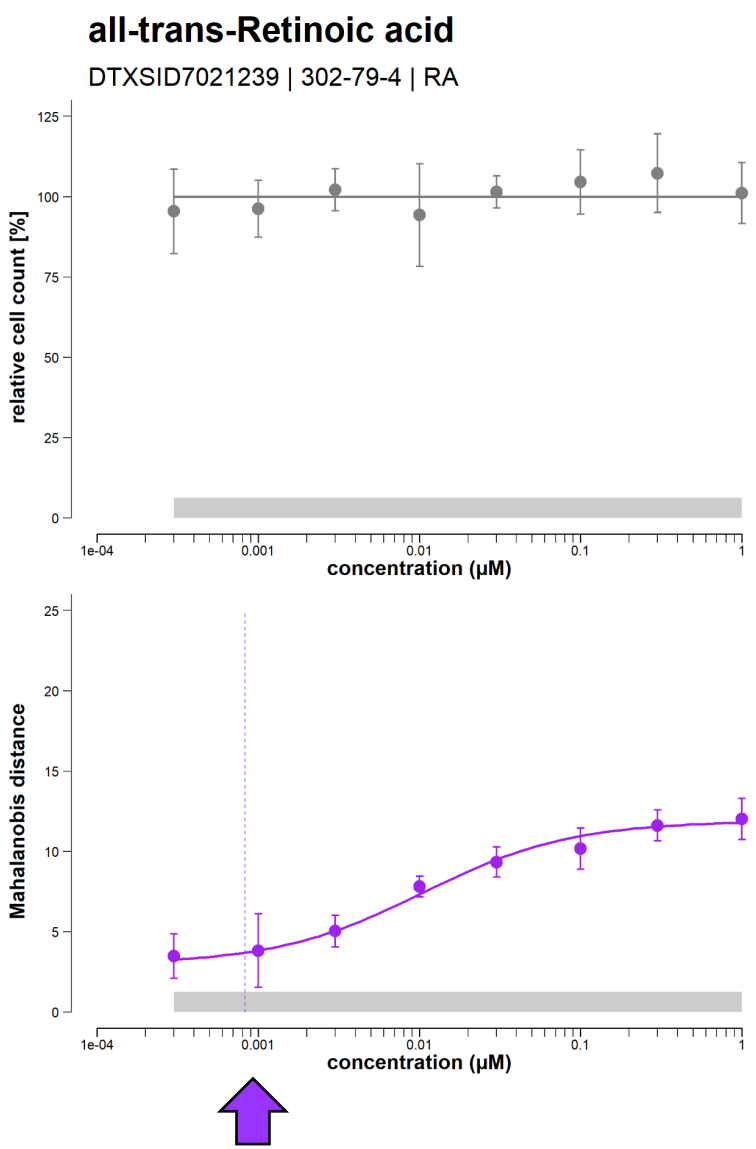
- A multivariate distance metric that measures the distance between a point (vector) and a distribution.
- Takes into account inherent correlations in phenotypic feature data



- Chemicals where a BMC can be determined using either the global or category D_M approach are considered active.
- The minimum of the global or most sensitive category BMC is the **Phenotype Altering Concentration (PAC).**
- Feature level results are used to compare bioactivity profiles across chemicals.

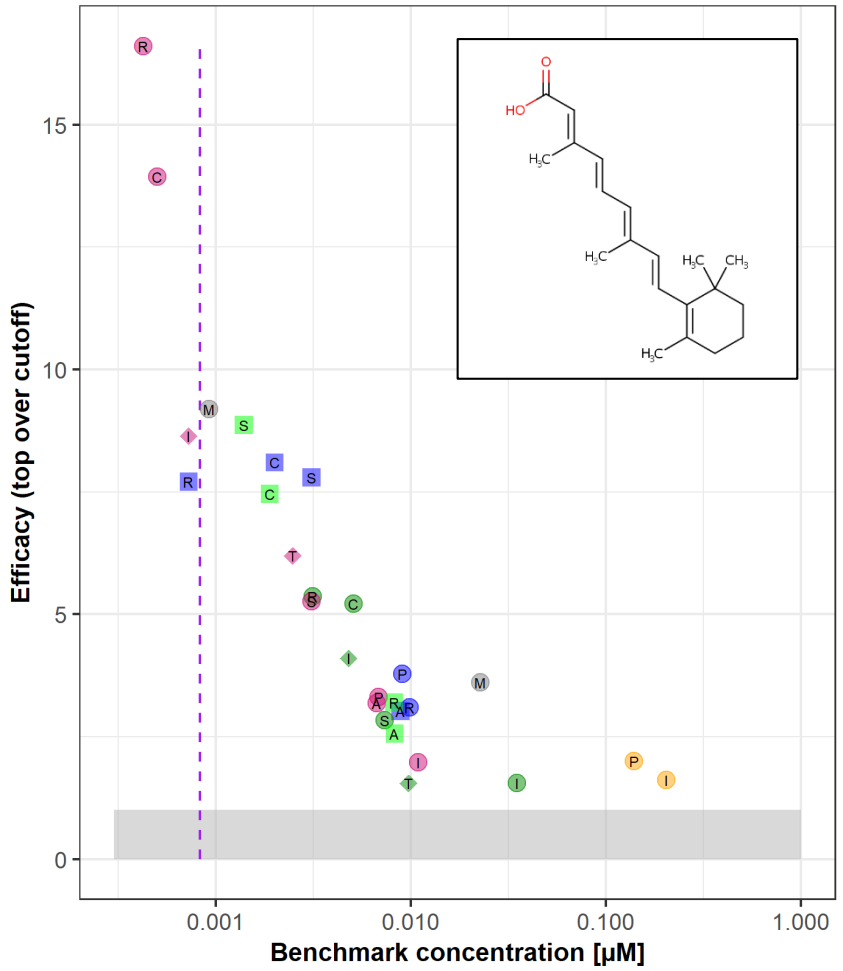
Adapted from Nyffeler et al. (2021). DOI: 10.1177/2472555220950245

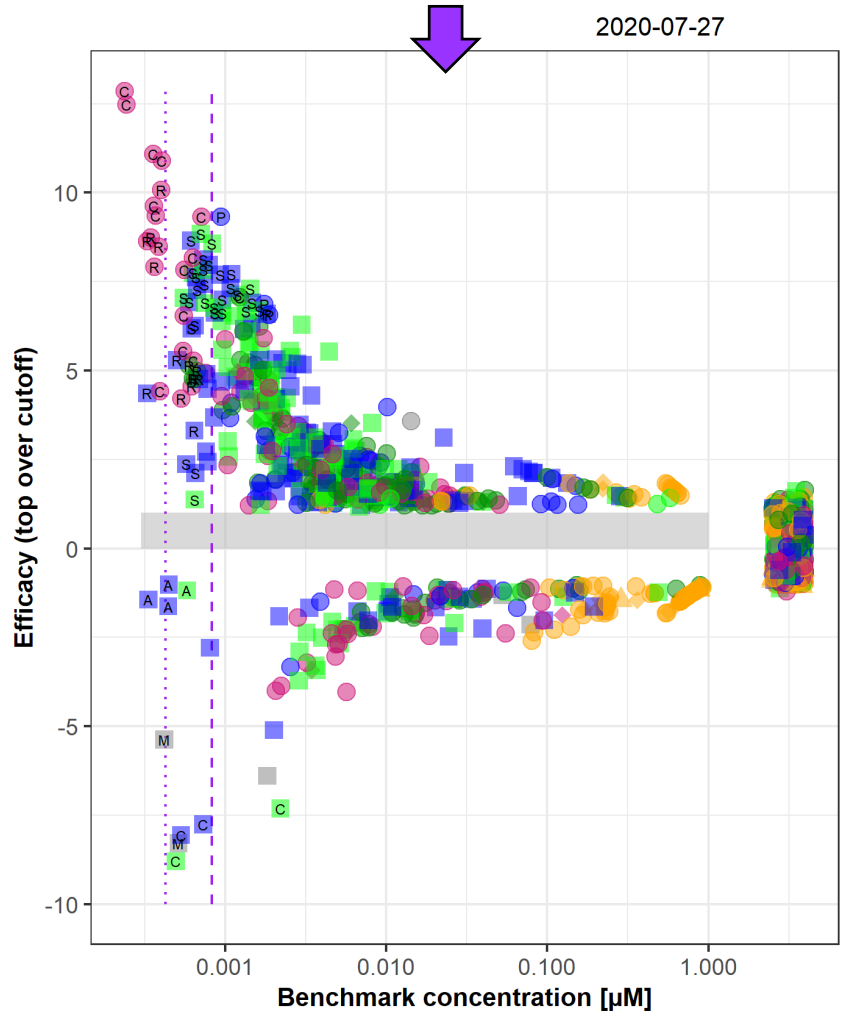
Summarization of Concentration-Response Modeling of HTPP Data



Benchmark Concentration

Profile of Phenotypic Effects



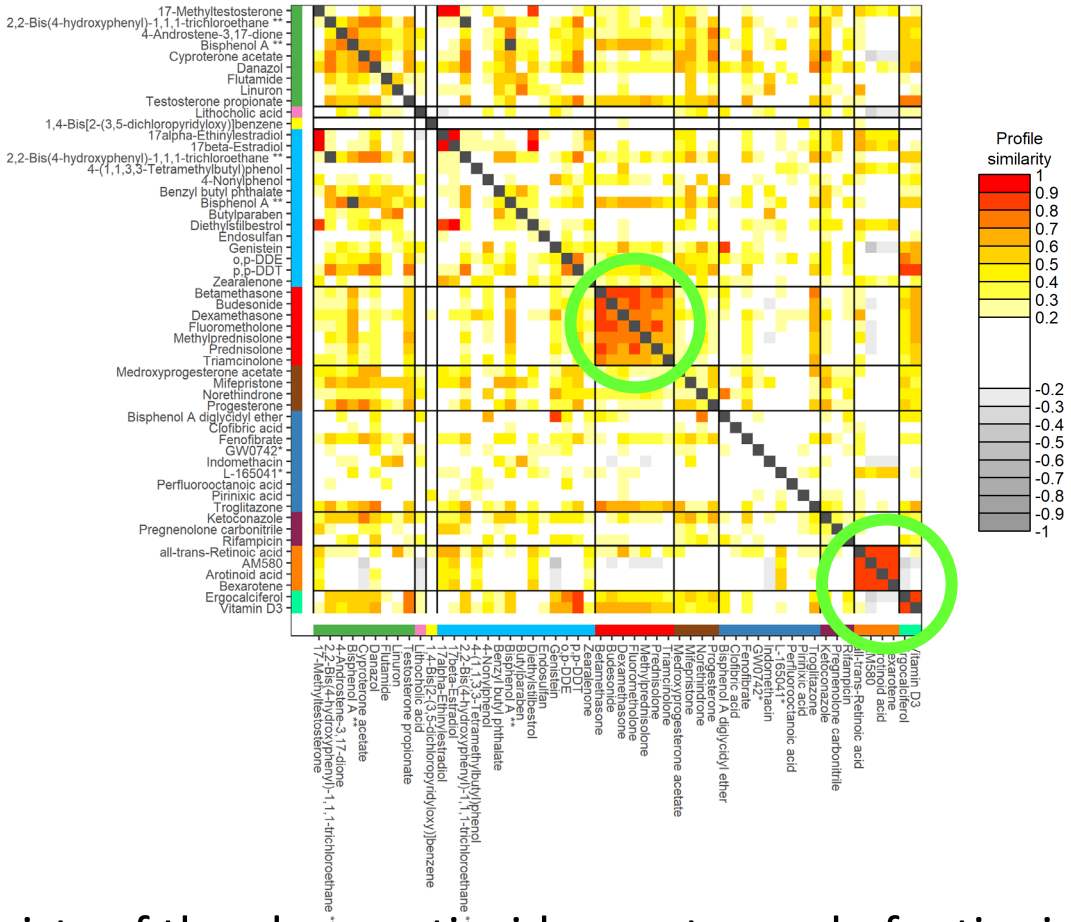


Phenotypic Profile Similarity with Nuclear Receptor Modulators

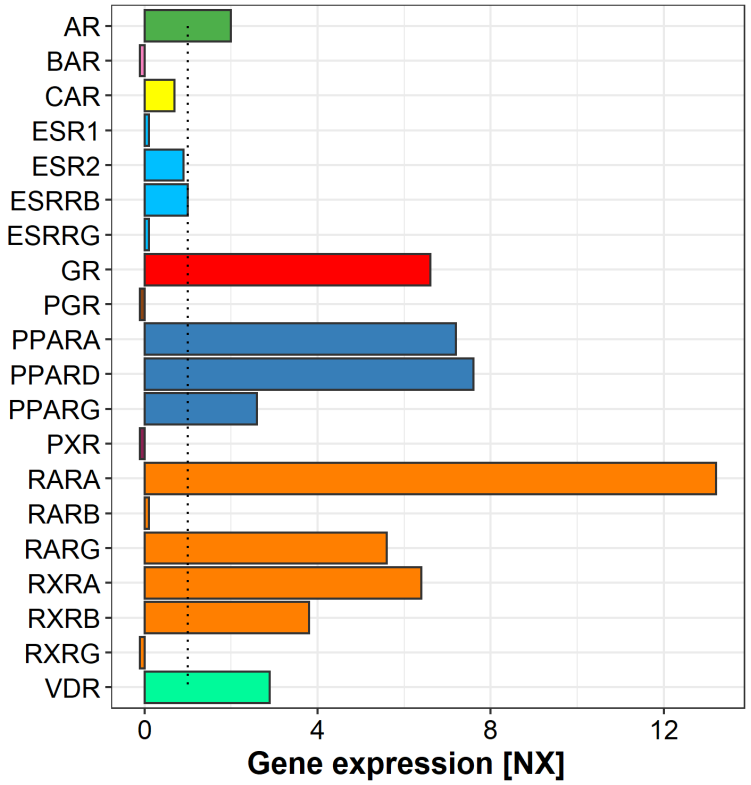
target

- AR
- BAR
- CAR
- ESR
- GR
- PGR
- PPAR
- PXR
- RAR
- VDR

Biological similarity in HTPP



Gene expression in U-2 OS



- Agonists of the glucocorticoid receptor and of retinoic acid receptors display characteristic profiles
- Expression of a target does not guarantee that characteristic profiles are observed (e.g. PPAR)

EPA United States Environmental Protection Agency

Potential Applications for HTTr- and HTPP-Derived Molecular PODs

HTP Screening Experimental Designs

Parameter	Multiplier	Notes			
Chemicals	462	APCRA case study chemicals			
Cell Types	4	U-2 OS		HepaRG-2D	MC-7
Assay Formats	2	HTPP	HTTr	HTTr	HTTr
Exposure Durations	Variable	24 HR	24 HR	24 HR	6 HR
Concentrations:	8	3.5 log_{10} units; ~half-log_{10} spacing			
Biological Replicates:	Variable	4	3	3	3

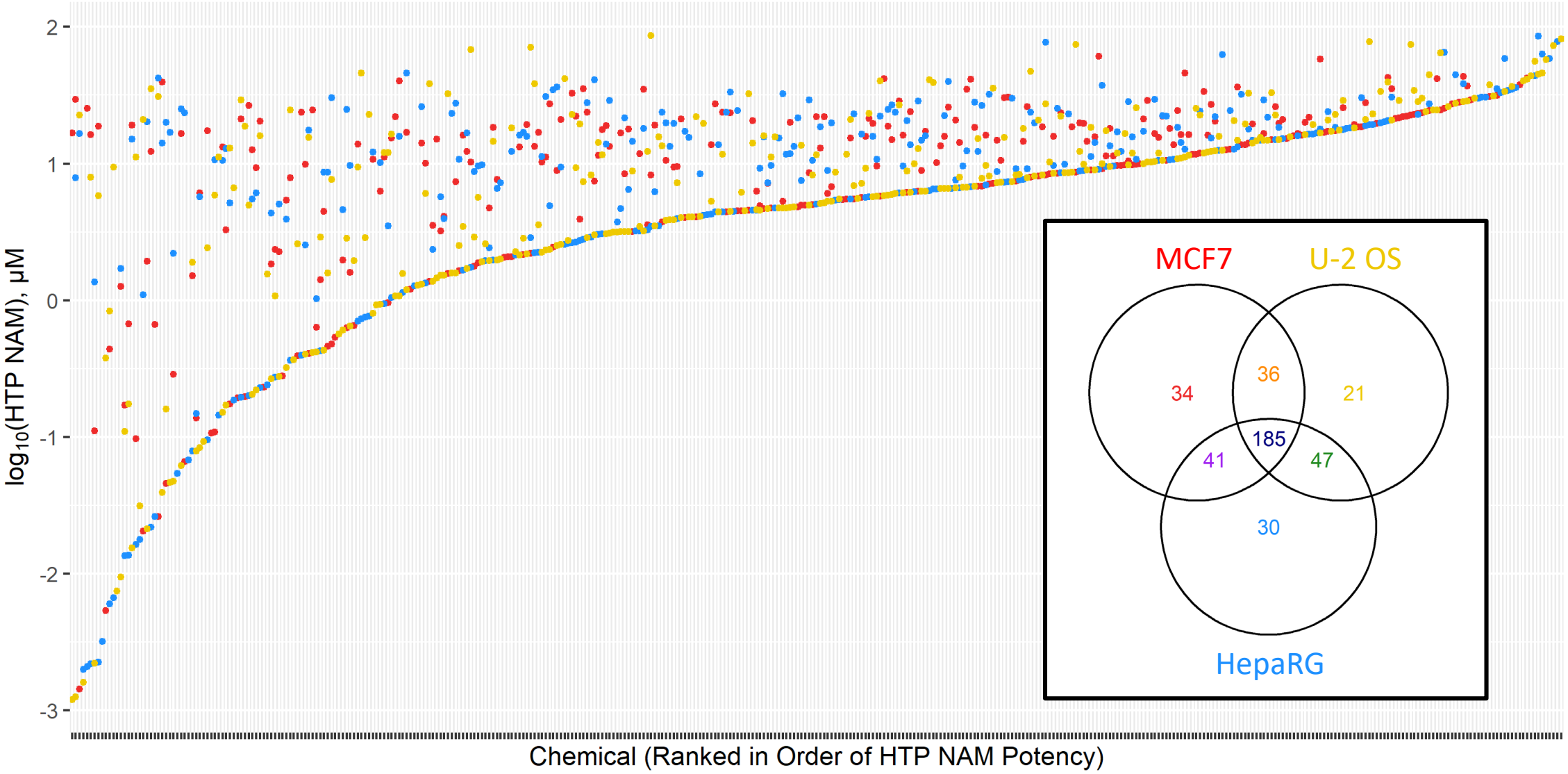
APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

International collaboration of regulatory scientists focused on next generation chemical risk assessment including **deriving quantitative estimates of risk based on NAM-derived potency information and computational exposure estimates.**

APCRA Chemicals → PK parameters necessary for *in vitro* to *in vivo* extrapolation (IVIVE)
in vivo toxicity data

Kavlock et al. (2018)
Chem. Res. Tox; 31(5): 287-290

Comparison of Screening Results Across Cell Lines



- Molecular POD defined as the minimum potency observed in HTP NAM assays across three cell types.

In Vitro to *In Vivo* Extrapolation (IVIVE) Using High-Throughput Toxicokinetic (httk) Modeling

Predicted exposure

Exposure predictions (EPA ExpoCast)

- Systematic Empirical Evaluation of Models (SEEM) version 3
- Inferred from human biomonitoring data, production volume and use categories (industrial / consumer use)

New approach methodologies (NAMs)

Toxicological threshold of concern (TTC)

HTP Potency Estimate (µM)

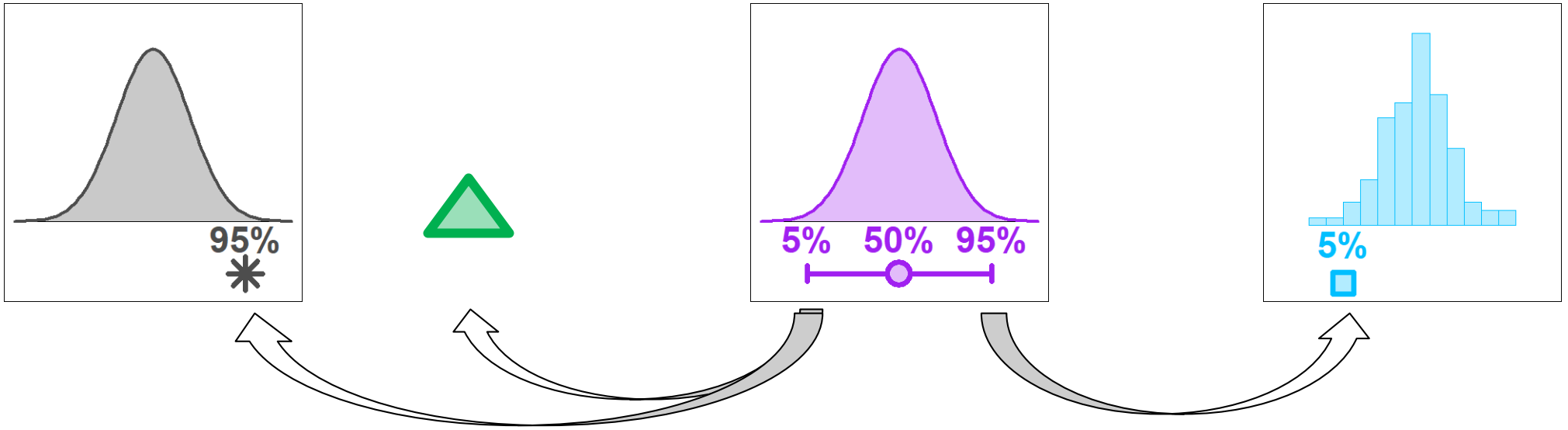
***In vitro*-to-*in vivo* extrapolation (IVIVE)**
high-throughput toxicokinetics (httk)

HTP AED (mg/kg bw/day)

***in vivo* point-of-departure**

Database of *in vivo* effect values (EPA – ToxValDB)

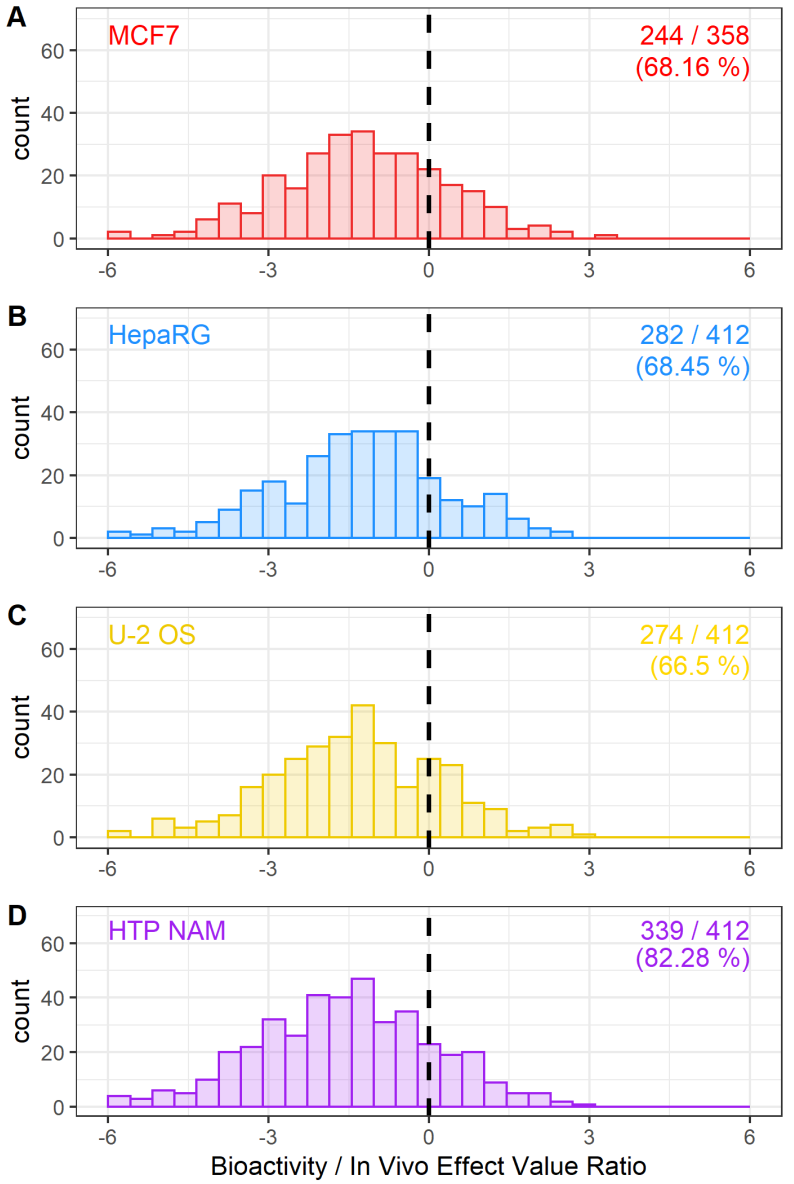
- Mammalian species
- oral exposures
- Various study types
- NOEL, LOEL, NOAEL, LOAEL
- mg/kg/day

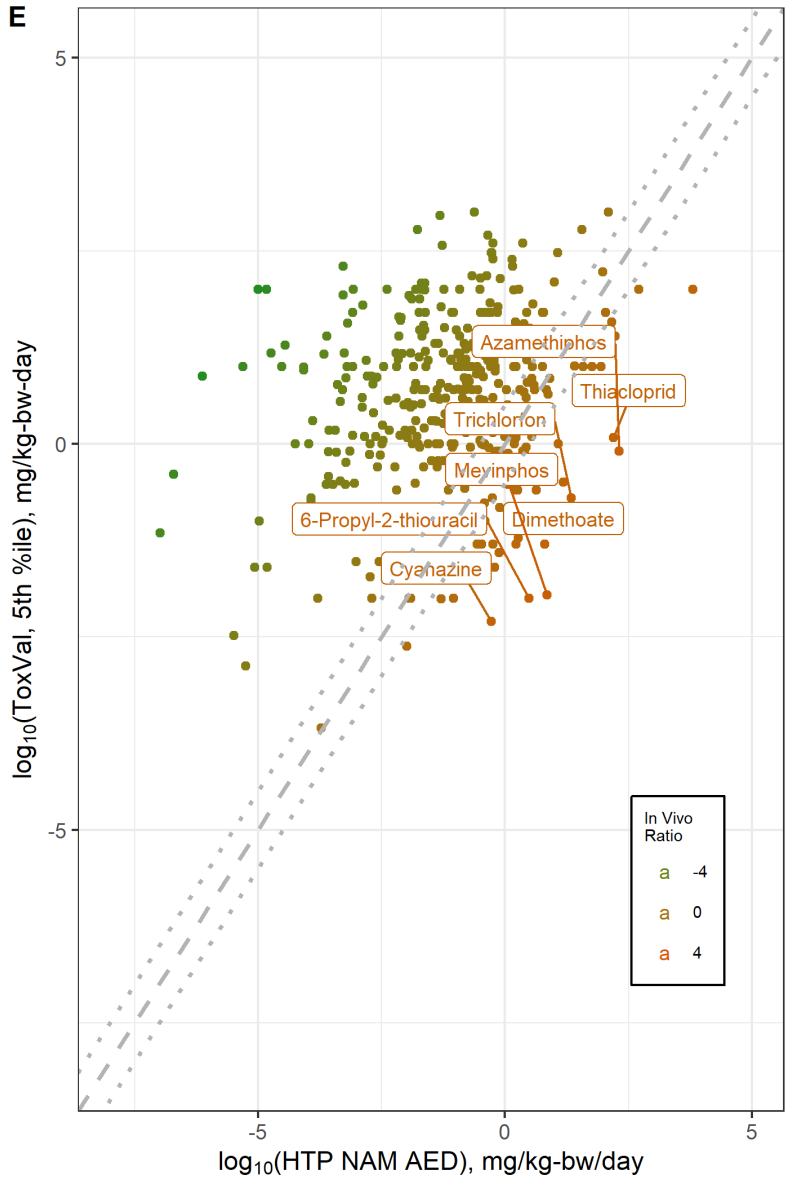


POD: point-of-departure
AED: administered equivalent dose

Bioactivity / *In Vivo* Effect Value Ratio Analysis

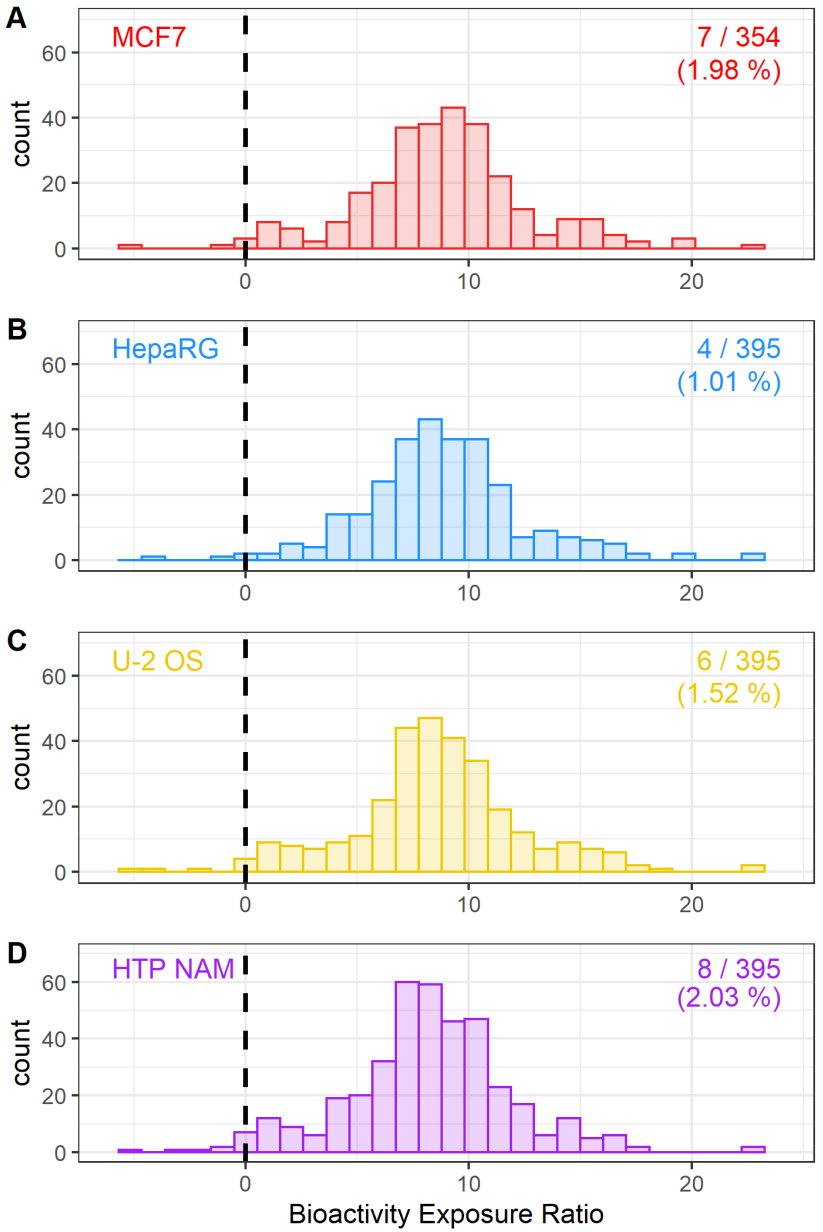
- **Negative ratios** indicate that AEDs derived from HTP NAMs molecular PODs are **conservative** surrogates for traditional *in vivo* PODs.
- When cell lines are considered individually, **~66-68%** of chemicals had negative ratios.
- When considered in combination, the number and percentage of chemicals with negative ratios **increased (82.3 %)**.
- Paul-Friedman et al. (2020)[a]:
  - Using ToxCast, **89 %** of APCRA chemicals had negative ratios.
- Positive ratios observed for several organophosphate and carbamate pesticides.

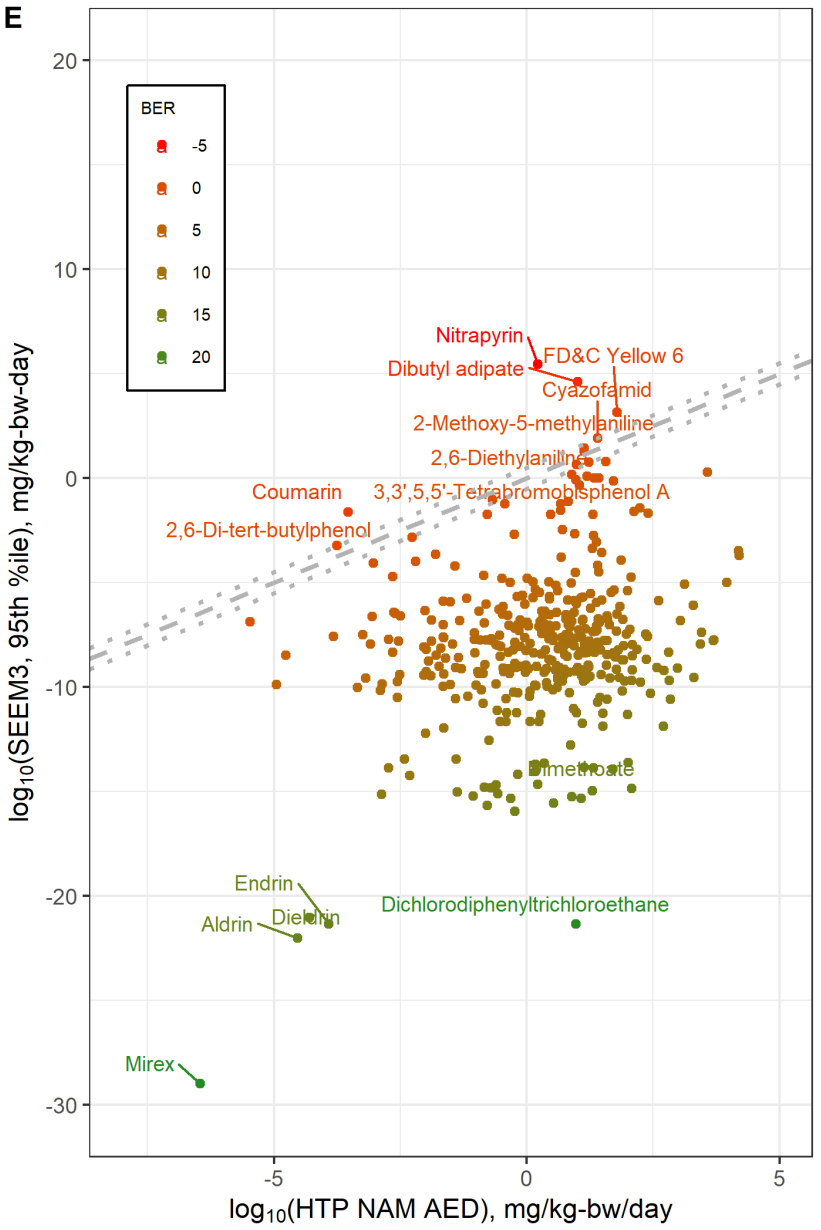




Bioactivity Exposure Ratio (BER) Analysis

- **Negative ratios** indicate a potential for human exposure to chemicals in a range that is bioactive in vitro.
- When cell lines are considered individually, **~1-2%** of chemicals had negative ratios.
- When considered in combination, the percentage of chemicals with negative ratios **did not appreciably change**.
- Positive ratios observed for several chemicals found in consumer products.
- Most extreme negative ratios associated with banned or limited use organochlorine pesticides.





Perspectives on the Use of HTP Assays at US EPA (1)

U.S. Environmental Protection Agency (EPA) Board of Scientific Counselors (BOSC)

Chemical Safety for Sustainability (CSS) and Human Health Risk Assessment (HHRA) Subcommittee

Meeting Summary

April 10–12, 2019

Charge Question 3 - HHRA has been collaborating with CSS on laying the foundation for future risk assessments. Please comment on the extent to which HHRA research is prepared to use novel data streams and tools, such as those from CSS, to advance the future of assessment science.

- For complex, comprehensive assessments, the HHRA program should base the design of such assessments on a systems biology model (or models), such as AOPs or modes of action (MOAs). The new data streams from the CSS program will largely provide biological activity profiling information, including quantitative predictions of bioactivity. Thus, information from such CSS program data streams (e.g., high throughput, high content, biological activity profiling transcriptomics, and high content phenotypic profiling) are anticipated to be most useful in understanding potential bioactivity associated with early or intermediate key events in such systems biology models.

Example of Deployment of HTP Assays at US EPA

Related Topics: Safer Chemicals Research

United States Environmental Protection Agency

PFAS Chemical Lists and Tiered Testing Methods Descriptions

"Panel of new approach methods to screen for potential liver, developmental neurotoxicity, developmental toxicity, immunotoxicity and mitochondrial toxicity as well as to better predict the disposition and excretion of PFAS from the body."

Tiered Testing Methods

Toxicological Response	Assay	Assay Endpoints	Purpose
Hepatotoxicity	3D HepaRG assay	Cell death and transcriptomics	Measure cell death and changes in important biological pathways
Developmental Toxicity	Zebrafish embryo assay	Lethality, hatching status and structural defects	Assess potential teratogenicity
Immunotoxicity	Bioseek Diversity Plus	Protein biomarkers across multiple primary cell types	Measure potential disease and immune responses
Mitochondrial Toxicity	Mitochondrial membrane potential and respiration (HepaRG)	Mitochondrial membrane potential and oxygen consumption	Measure mitochondrial health and function
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption	ACEA real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
General Toxicity	Attagene cis- and trans-Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in hepatotoxicity
	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
	High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology
Toxicokinetic Parameter	Assay	Assay Endpoints	Purpose
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to plasma protein	Measure amount of free chemical in the blood

"Results from the [NAMs] testing will be used to prioritize (tier) PFAS for risk assessment, provide support for gap-filling approaches such as chemical read-across and to inform further testing."

Assay	Assay Endpoints	Purpose
High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology

https://www.epa.gov/chemical-research/pfas-chemical-lists-and-tiered-testing-methods-descriptions#2

Summary and Conclusions

- **High-Throughput Profiling:** Developed experimental designs and scalable laboratory workflows for high-throughput transcriptomics and high-throughput phenotypic profiling of environmental chemicals that can be used in multiple human-derived cell types.
- **Potency Estimation:** Developed high-throughput concentration-response modeling workflows to identify thresholds for perturbation of gene expression (e.g. BPACs) and cell morphology (e.g. PACs).
- **IVIVE:** Potency estimates can be converted to administered equivalent doses (AEDs) using high-throughput toxicokinetic modeling.
- **Bioactivity to *In Vivo* Effect Value Ratio Analysis:** AEDs derived from HTP assays were conservative compared to traditional PODs a majority of the time. Performance improved to ~80% when results from multiple cell types were considered in combination.
- **Bioactivity to Exposure Ratio (BER) Analysis:** AEDs derived from HTP assays were compared to high-throughput exposure predictions. There were very few chemicals where AEDs were within the range of exposure predictions.
- **Comparison to ToxCast:** Applications using HTP NAMs potencies as input yielded comparable results compared to the use of ToxCast NAMs potencies.

Acknowledgements

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