Concentration-Response Analysis of Transcriptomics Data from *In Vitro* Screening Studies and Applications for Chemical Safety Assessments

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Overview

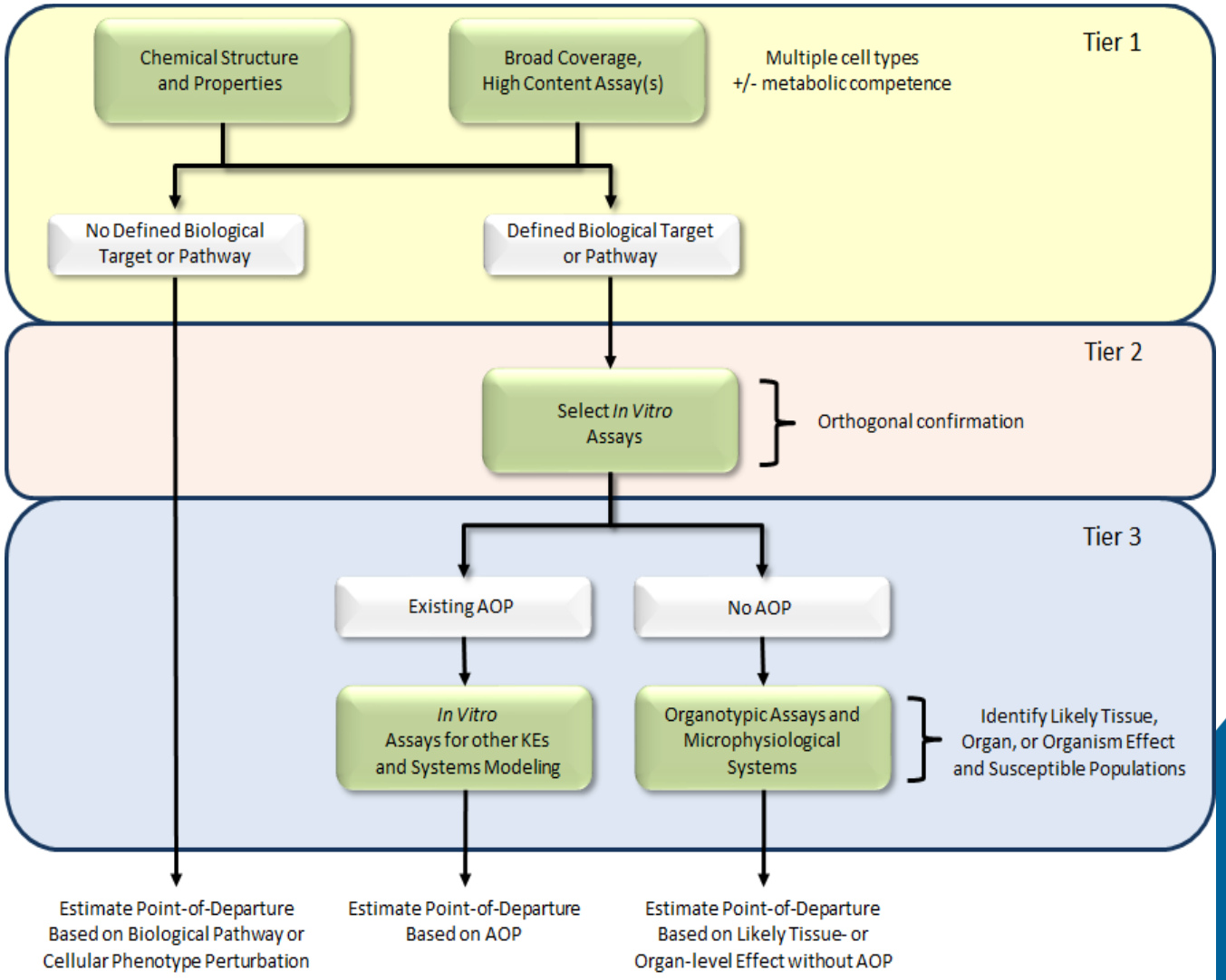
- **Background**
  - NAMs-based Tiered Toxicity Testing Strategy
  - TempO-Seq Assay for High-Throughput Transcriptomics (HTTr) Screening
  - CCTE HTTr Data Landscape
  - CCTE HTTr Bioinformatics Pipeline

- ***In Vitro* Screening Studies**
  - Volatiles Screening
  - HTTr Screening of APCRA Case Study Chemicals.

NAMs-based Tiered Toxicity Testing Strategy

- **New Approach Methodologies (NAMs)** are 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.
- Increasing efficiency and declining cost of generating whole transcriptome profiles has made **high-throughput transcriptomics (HTTr)** a practical NAM for *in vitro* chemical screening.
- The resulting data can potentially be used for **potency estimation**, **mechanistic prediction** and evaluation of **chemical similarity**.

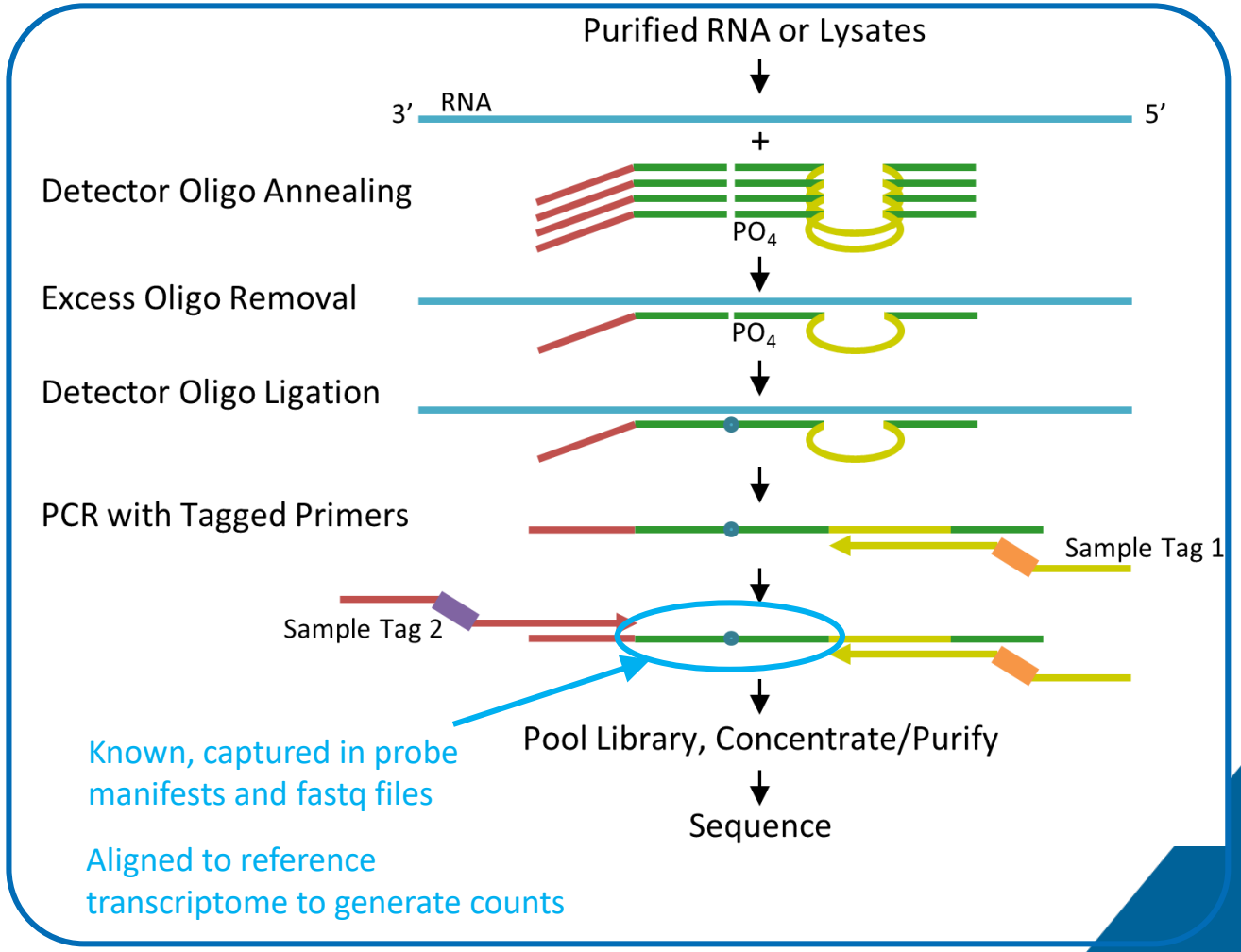
The NexGen Blueprint of CompTox as USEPA Tox. Sci. 2019; 169(2):317-322



Templated Oligo with Sequencing Readout (TempO-Seq)

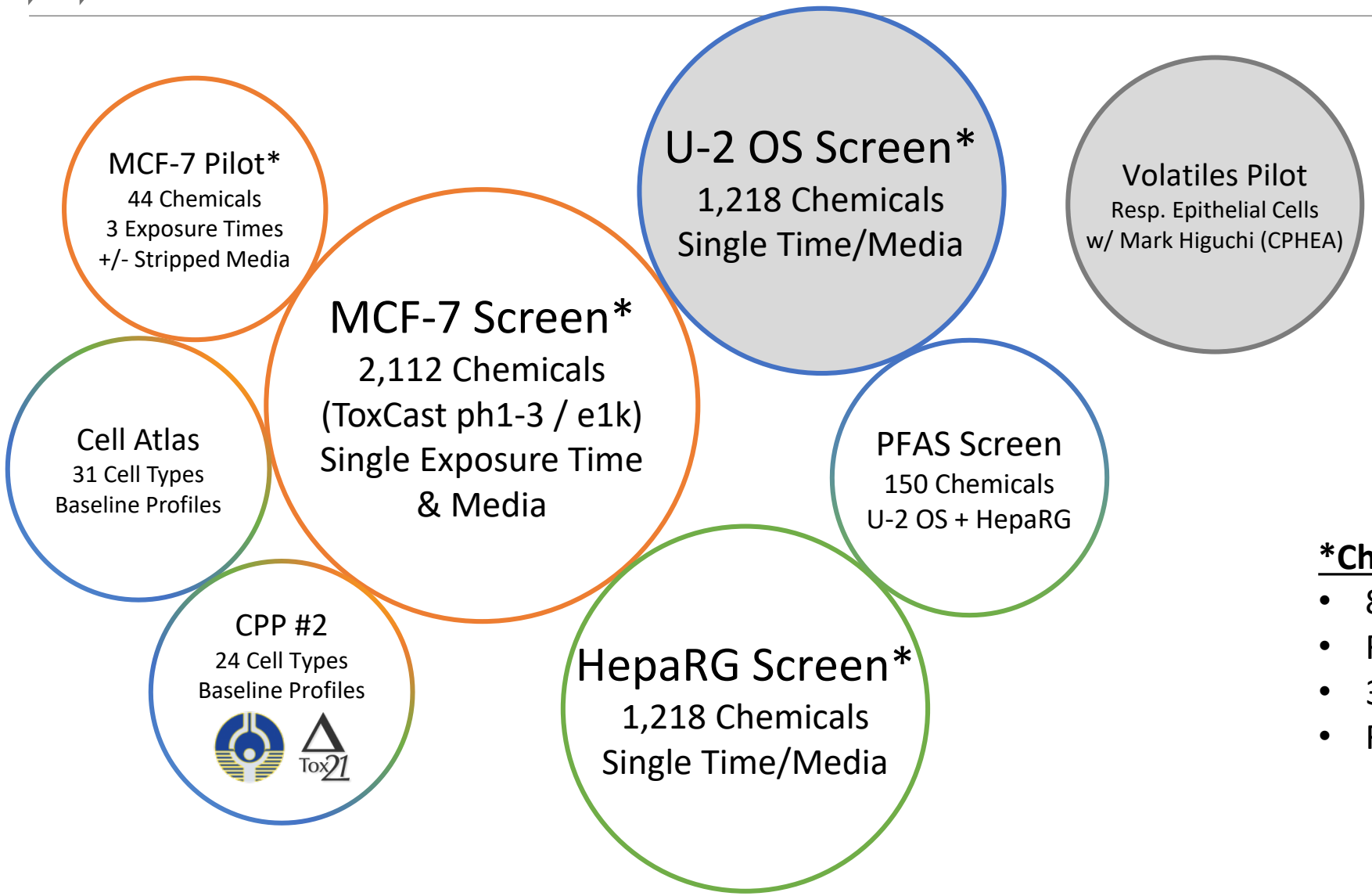
- The **TempO-Seq** human whole transcriptome assay measures the expression of greater than 20,000 transcripts.
- Requires only picogram amounts of total RNA per sample.
- Compatible with purified RNA samples or **cell lysates**.
- Lysates are barcoded according to sample identity and combined in a single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

TempO-Seq Assay Illustration



Yeakley, et al. PLoS ONE 2017

HTTr Data Landscape at US EPA

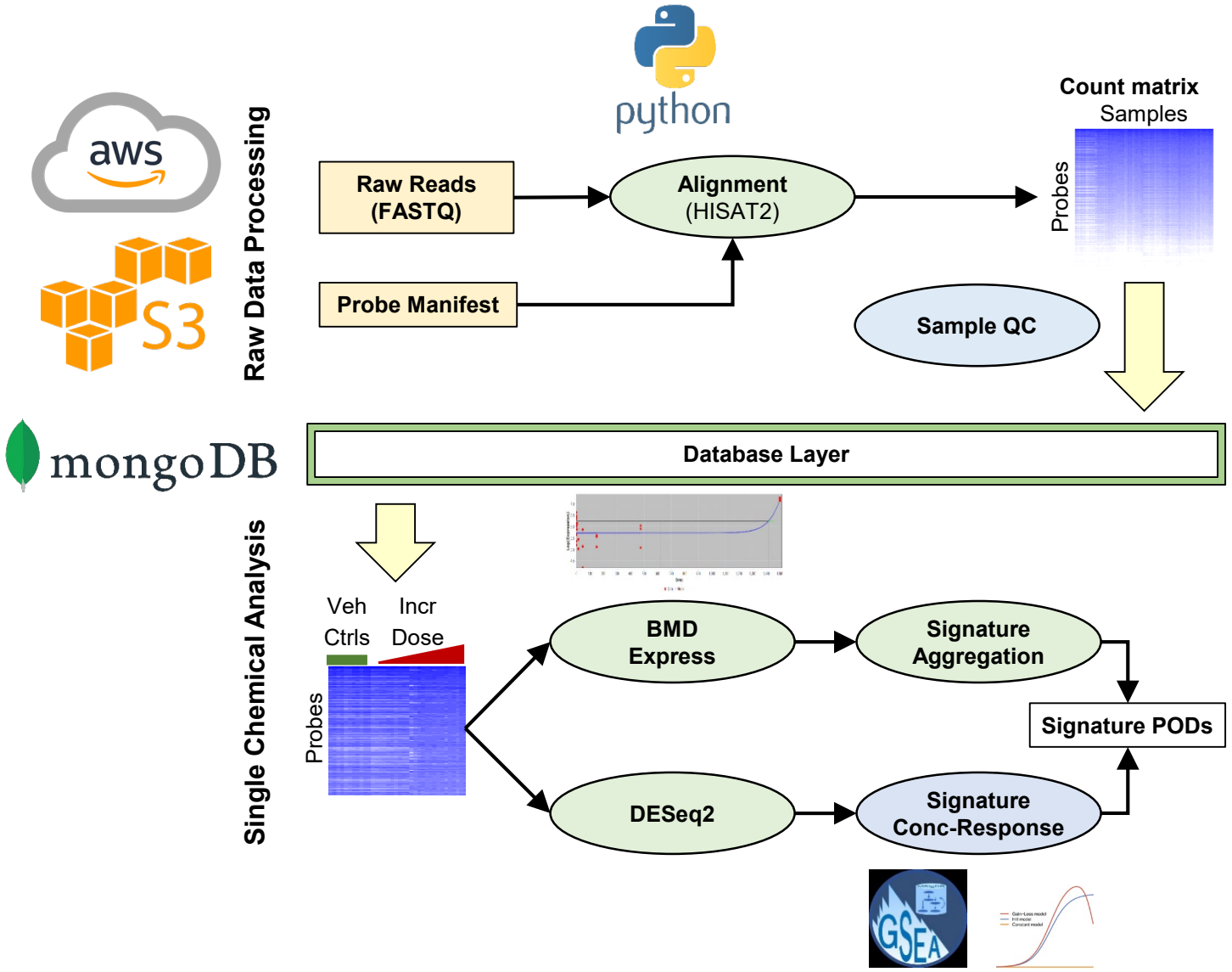


***Chemical Exposures:**

- 8 Concentration Series
- Regular Log10 Spacing
- 3 Replicates per Conc
- Randomized Plate Layout

Slide courtesy of Logan Everett

HTTr Bioinformatics Pipeline



- Code managed with git/BitBucket
- Database layer helps manage larger screens, protect/backup data
- Many data steps performed independently for each test chemical:
  - Removal of low signal probes
  - Normalization
  - DESeq2 analysis

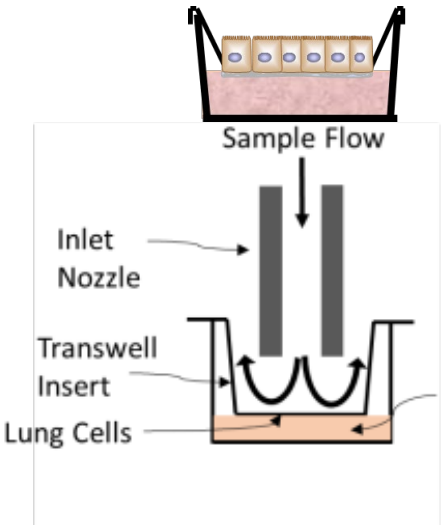
Slide courtesy of Logan Everett

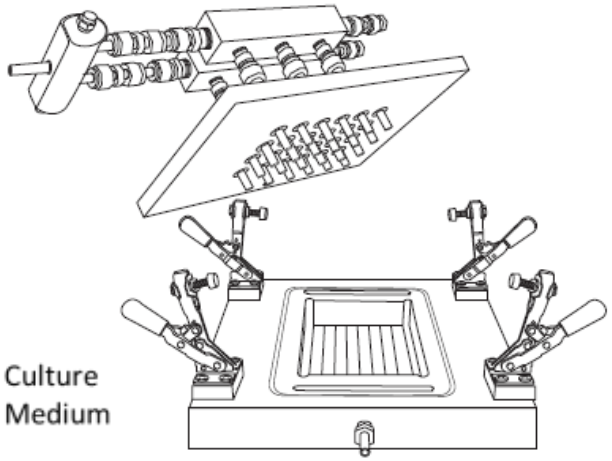
In Vitro HTTr Screening of Volatile Chemicals

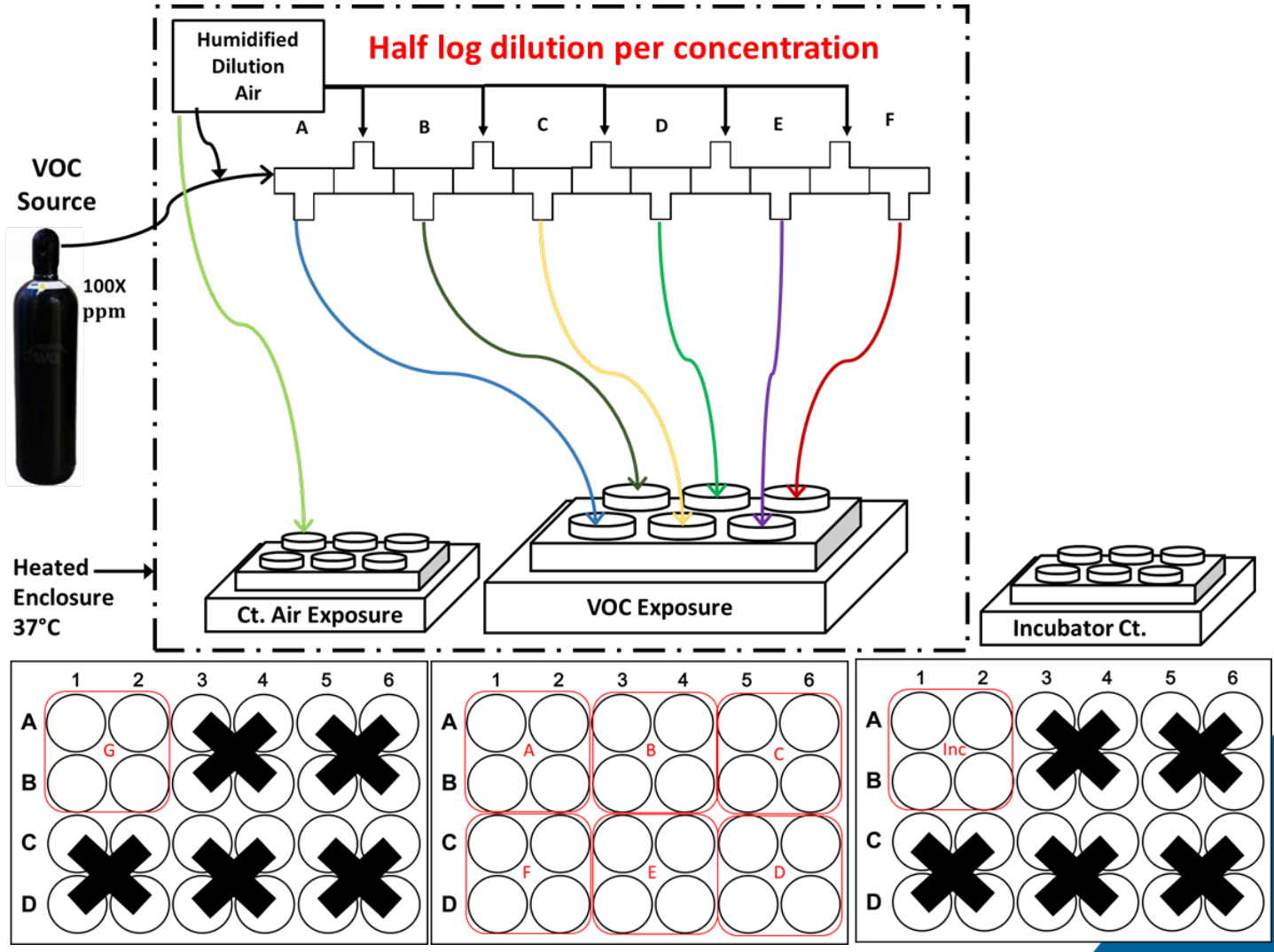
Direct exposure of human-derived cells cultured at air-liquid interface (ALI) to volatile chemicals to generate molecular point-of-departures (POD)

Experimental Design	Description
Cell Types	Primary Human Bronchial Epithelial Cells* BEAS-2B cells
Test Chemical	1,3-Butadiene, Acetaldehyde, Carbon Tetrachloride* Acrolein, Trichloroethylene*, Dichloromethane* Formaldehyde, 1-Bromopropane*
Exposure Regimen	• 6 concentrations, sham control, incubator control
Exposure Duration	• 2 hours, Assays conducted 4h post exposure
Technical Replicates	• TempO-Seq, n=2; Viability, n=2; Cytotoxicity, n=4
Biological Replicates	• Exposures per cell type conducted over three days, n=3
Assay Formats	• TempO-Seq • Cytotoxicity [LDH Release, Cell Titer Glo]

Cell Culture Exposure System (CCES)







Pre-exposure

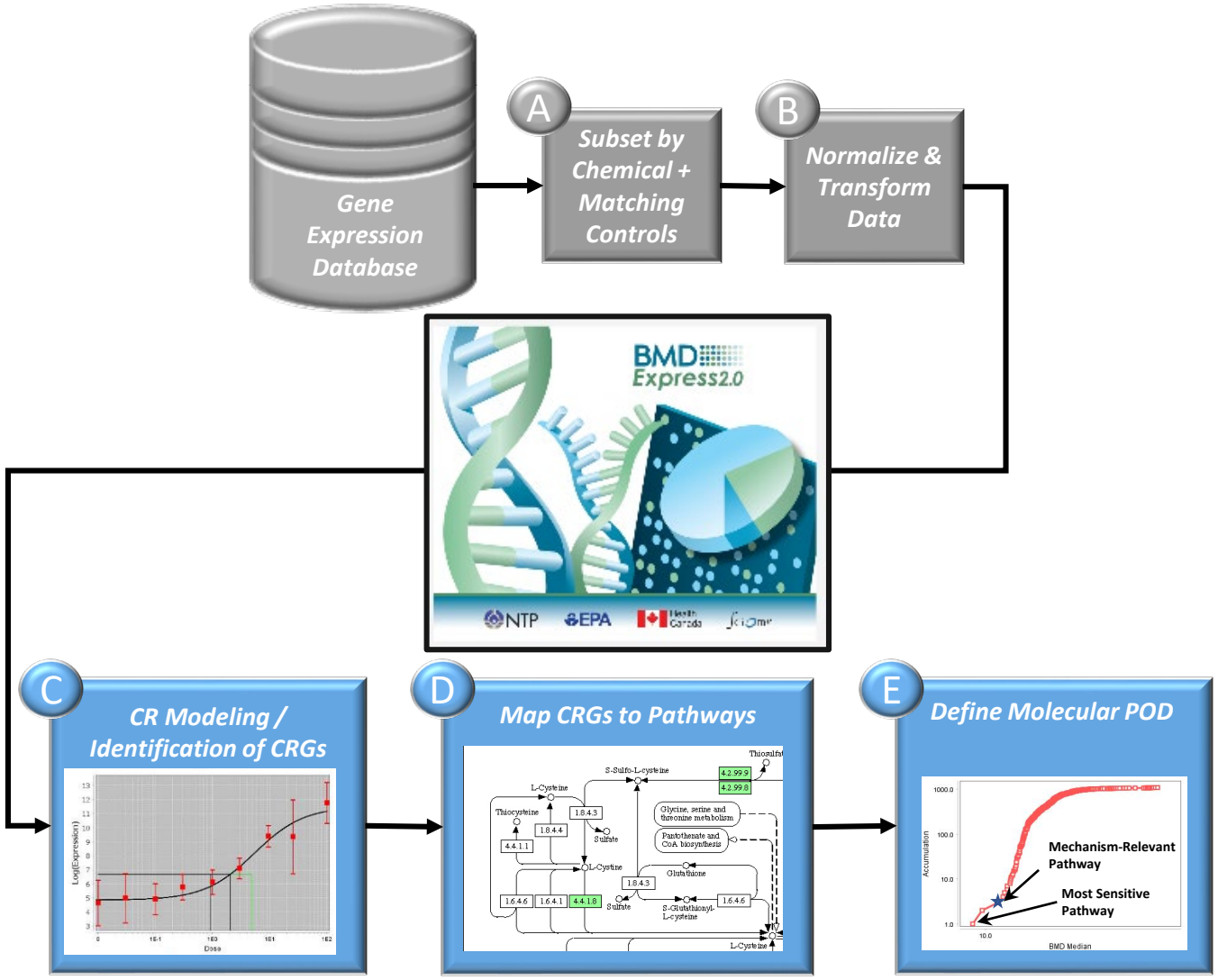
- All cells grown at ALI
- Apical side **washed** and given fresh media 2h prior to exposure
- HEPES buffered media to maintain pH in low CO_2 environment

Post-exposure

- VOC exposure for 2h
- Cells removed from CCES and samples collected 4h post-exposure

Slide courtesy of Adam Speen and Mark Higuchi

Concentration-Response Modeling (BMDExpress)

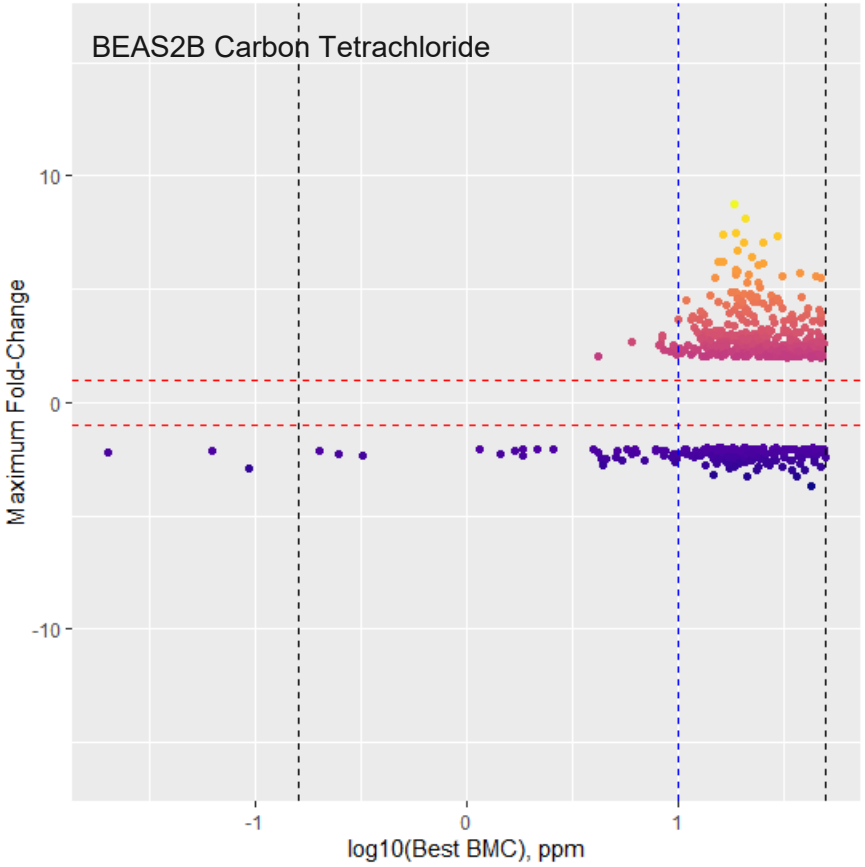


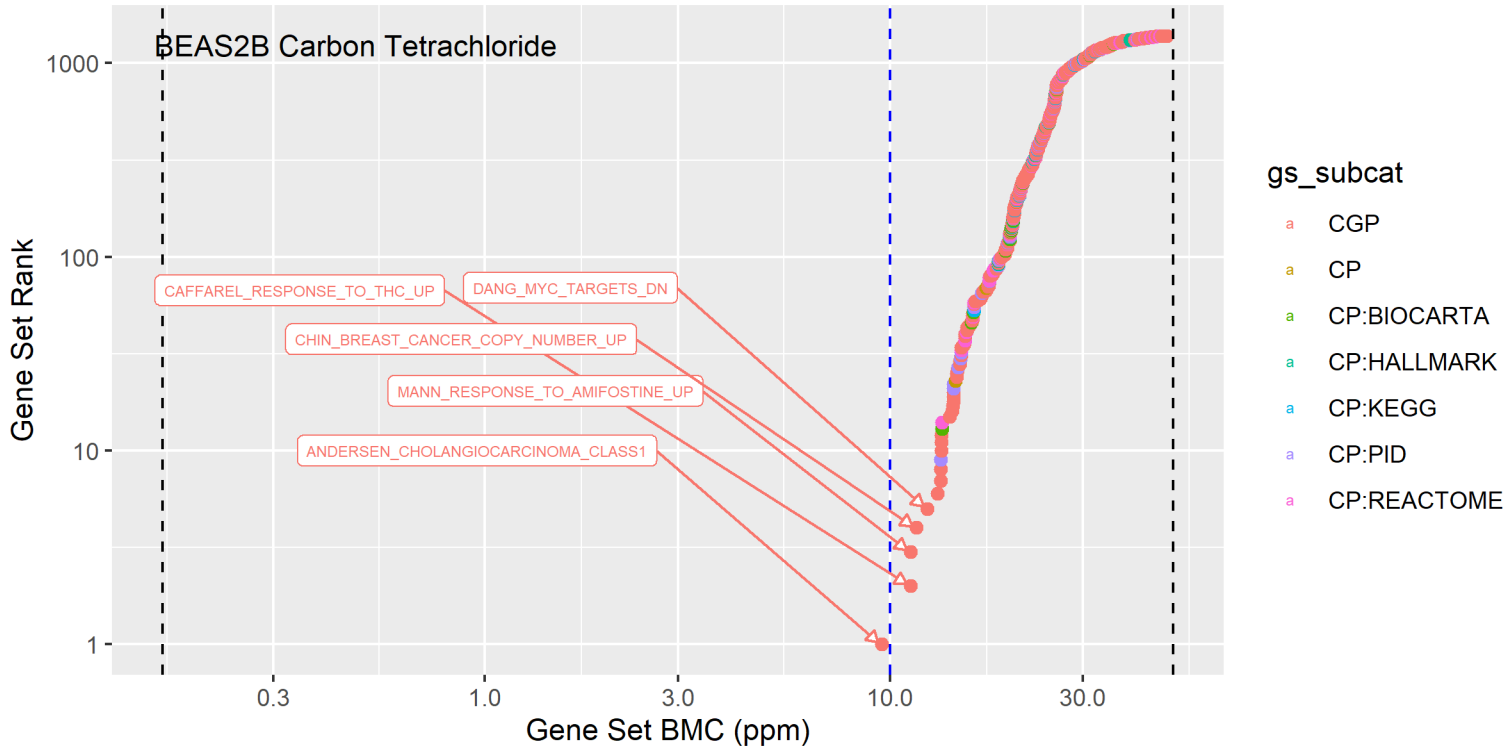
Adapted from Harrill et al. (2019)

Based on National Toxicology Program Approach to Genomic Dose-Response Modeling (NTP RR 5)

BMDExpress Parameter	Criteria
Pre-filter:	|FC| > 2 at any test concentration
Models	Hill, Power, Linear, Poly2, Exponential 2|3|4|5
BMR Factor:	1.349*SD of controls (10%)
Best Model Selection:	Lowest AIC
Hill Model Flagging:	'k' < 1/3 Lowest Positive Dose Exclude Flagged Hill Models from Best Model Selection
Conc-Response Hit Criteria	(0.1*lowest conc. < BMC < highest conc.) BMC fit p-value > 0.1 BMCL / BMCU < 40
Pathway Analysis:	≥ 3 Concentration-responsive genes ≥ 5% Gene Set Coverage
Gene Set Collections:	Molecular Signatures Database (v7)

Example of BMDExpress Modeling Output





Black Dashed Lines = Min. and Max. Test Concentrations
Blue Dashed Line = TLV (ppm)

Comparison of Molecular PODs from Volatiles Testing to Industrial Hygiene Standards

	ACGIH TLV-TWA (ppm)	BEAS-2B HTTr POD (ppm)	HBEC HTTr POD (ppm)
Acrolein	0.1	0.58	--
Formaldehyde	0.3	NA	--
1,3-Butadiene	10	13.98	--
Acetaldehyde	25	NA	--
1-Bromopropane	0.1 *	2.25	NA
Carbon Tetrachloride	10	9.56	NA
Trichloroethylene	50	44.8	28.1
Dichloromethane	100	142.13	266.7

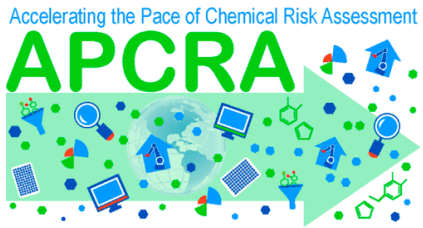
In 5 of 6 cases where a POD could be determined, that value was very close to the ACGIH TLV-TWA value.

The exception was 1-bromopropane

* The ACGIH TLV TWA for 1-bromopropane was updated to 0.1 ppm in 2012. Prior to that the TLV-TWA for 1-bromopropane was 10 ppm.

HTTr Screening Study Design

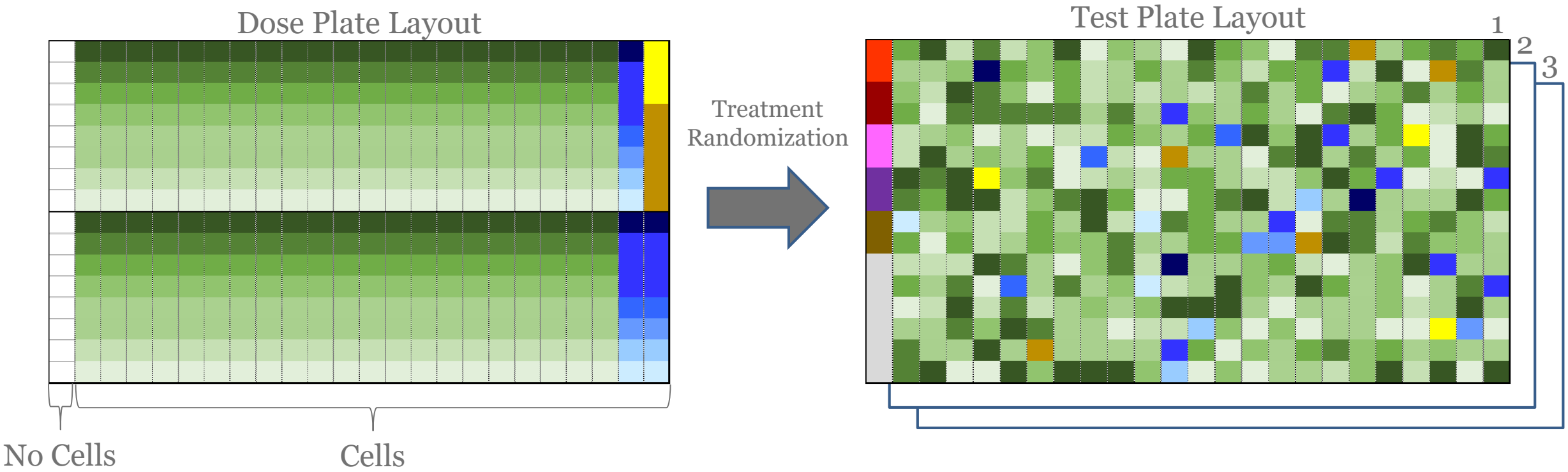
Parameter	Multiplier	Notes
Cell Type(s)	1	U-2 OS
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	1,218	Selected from ToxCast Collection Includes 462 APCRA case study chemicals
Time Points:	1	24 hours
Assay Formats:	2	High Throughput Transcriptomics (TempO-Seq) High Throughput Phenotypic Profiling (Cell Painting)
Concentrations:	8	3.5 $\log_{10}$ units; ~half-$\log_{10}$ spacing
Biological Replicates:	3	--



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

- International collaboration of regulatory scientists focused on developing case studies for evaluating the use of New Approach Methodologies (NAMs) in chemical risk assessment.
- ECHA Workshop (2017) case study focuses on **deriving quantitative estimates of risk based on NAM-derived potency information and computational exposure estimates**

Plate Layout



- Test Chemicals
- Reference Chemicals (Multi Conc)
- Vehicle (DMSO)
- Reference Chemical (Single Conc)
- Reference RNA, Sample A
- Reference RNA, Sample B
- Reference Lysate, Sample A
- Reference Lysate, Sample B
- Lysis Buffer Blank
- Reference Samples (Vendor)

Concentration-Response Modeling of Signature Scores

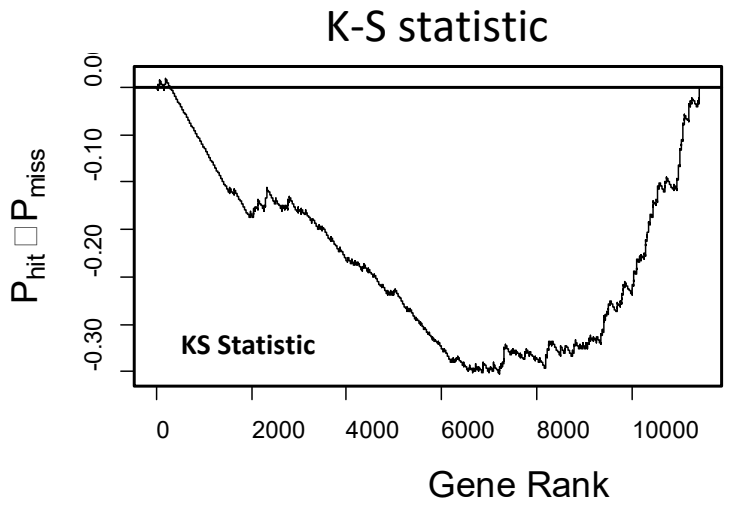
Step 1: Inputs

Experimental Data: Chemical_Conc × Gene matrix of log_2 (fold-change) (l2fc) values.

Signature Collections: MSigDB (*Liberzon et al. 2015*), BioPlanet (*Huang et al. 2019*), CMAP (*Subramanian et al. 2005*)

Step 2: Pathway Scoring

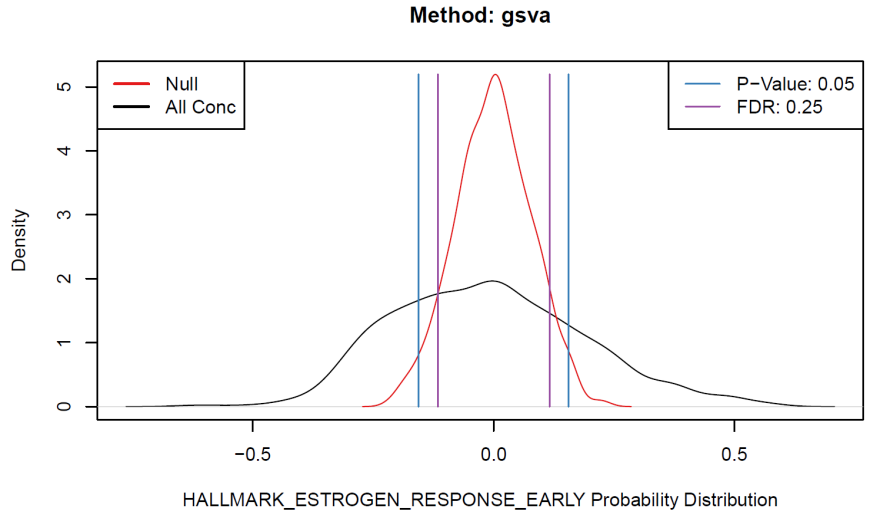
Scores based on single sample GSEA method (Barbie et al. 2009)



Chemical_Conc × Pathway matrix of scores.

Step 3: Cut-off Estimation via NULL Modeling

- For each gene, **resample** l2fc based on the cross-sample gene distribution → breaks gene correlation
- Calculate **pathway scores for "null" data**
  - One null distribution (n = 1000 scores) / pathway

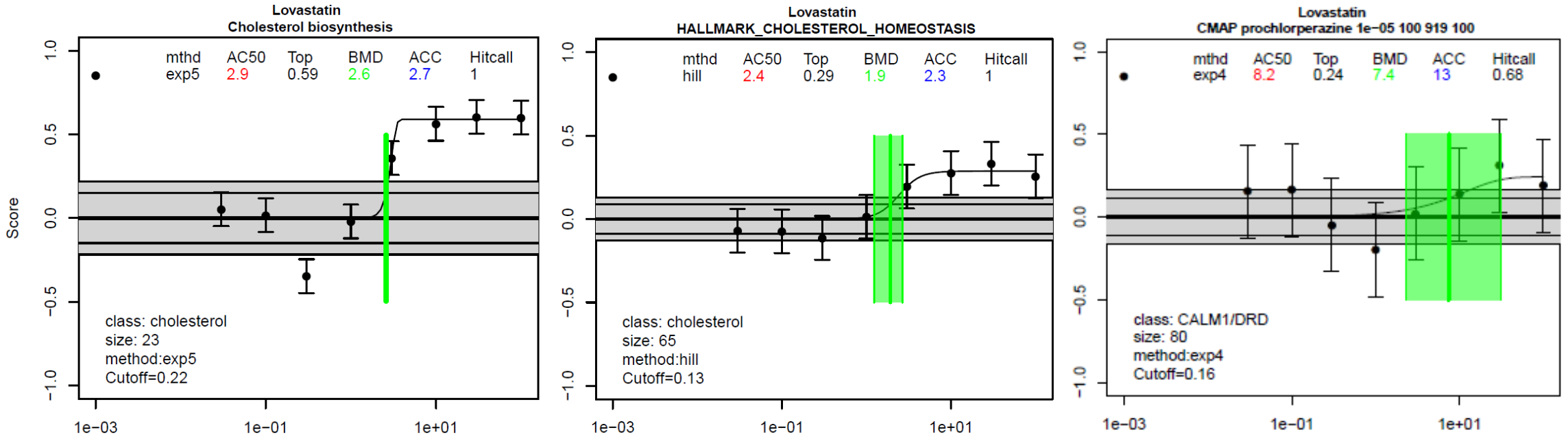


Analysis by Thomas Sheffield and Richard Judson

Concentration Response Modeling Example

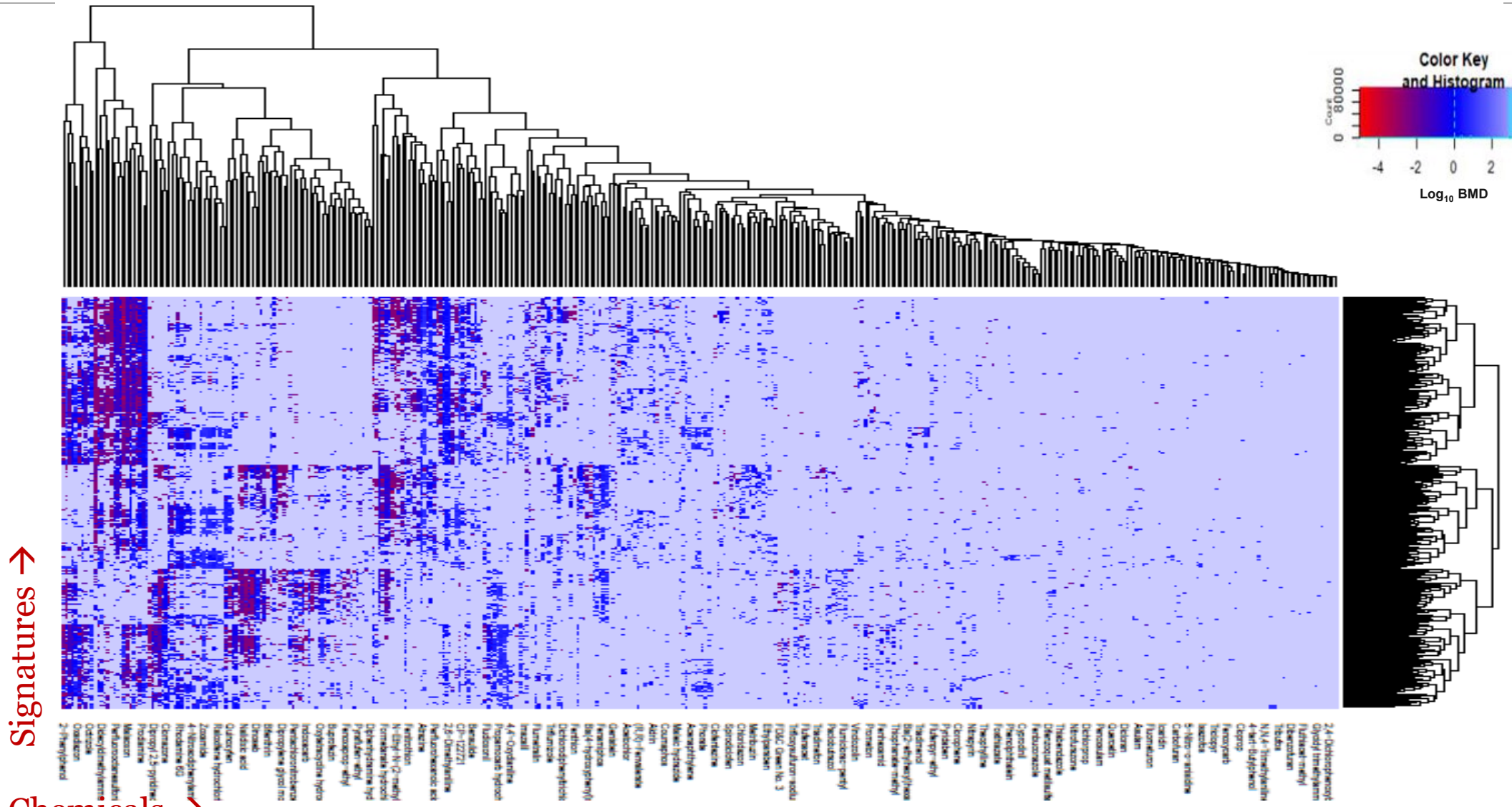
Step 4: CR Modeling

Concentration response modeling of signature scores using tcplfit2 (Sheffield et al. *submitted*)

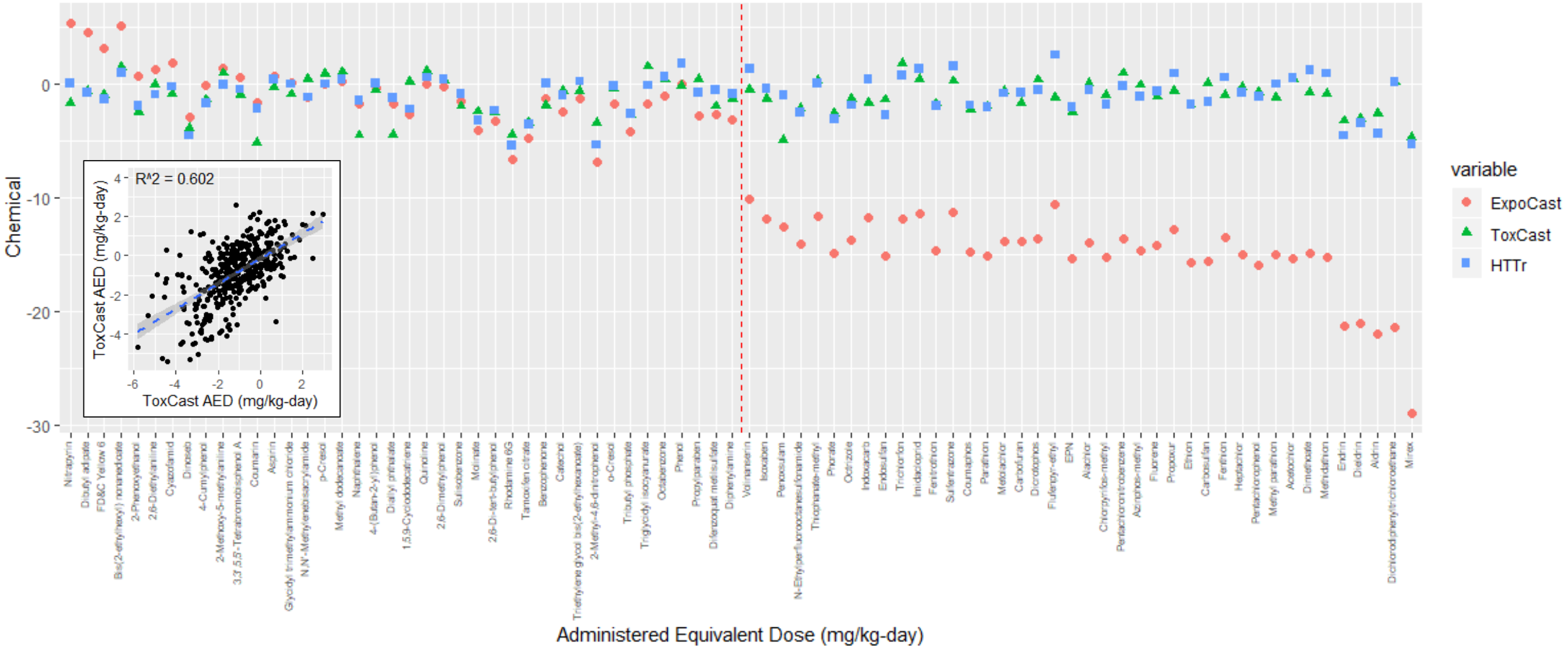


- Takes into account coordinated changes in gene expression that may not be identified using gene level fitting approaches.
- All curve forms from BMDExpress, plus constant model.
- Provides continuous hit calls for identifying high confidence and low confidence hits.

Concentration-Response Modeling Summary



Bioactivity-to-Exposure Ratio Analysis



Summary and Conclusions

- **Volatiles:** "Acute" *in vitro* screening of volatile chemicals using air liquid interface and HTTr yielded potency values approximating occupational exposure limits.

- **APCRA Chemicals:** Concentration-response modeling of HTTr signature scores in U-2 OS cells yielded molecular PODs that were positively correlated with molecular PODs from ToxCast.

- **Bioactivity Exposure Ratio:** *In vitro* to *in vivo* extrapolation of molecular PODs facilitated comparison to predicted human exposure estimates and ranking based on bioactivity exposure ratios.

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