Comparing and Interpreting Tox21 Data Analysis Approaches

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Conflict of Interest Statement

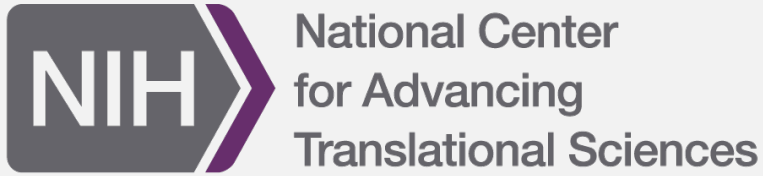
The author declares no conflict of interest.

The views expressed are those of the authors and do not necessarily reflect the views or policies of the US EPA

The Tox21 Federal Partnership

https://tox21.gov

Inception in 2008



Translation

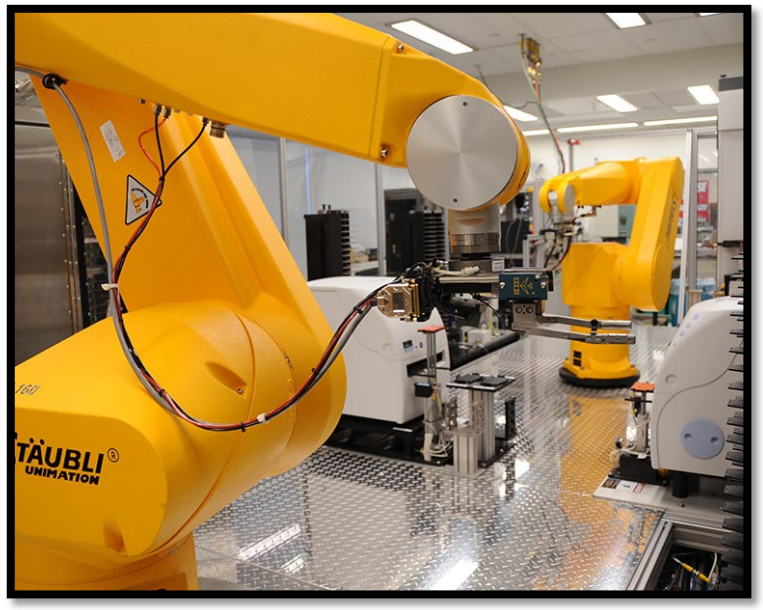
Develop methods & models toward prioritization and risk assessment

- Hazard potential
- Mechanism identification
- Compound-disease association
- Predictive models of *in vivo* outcomes

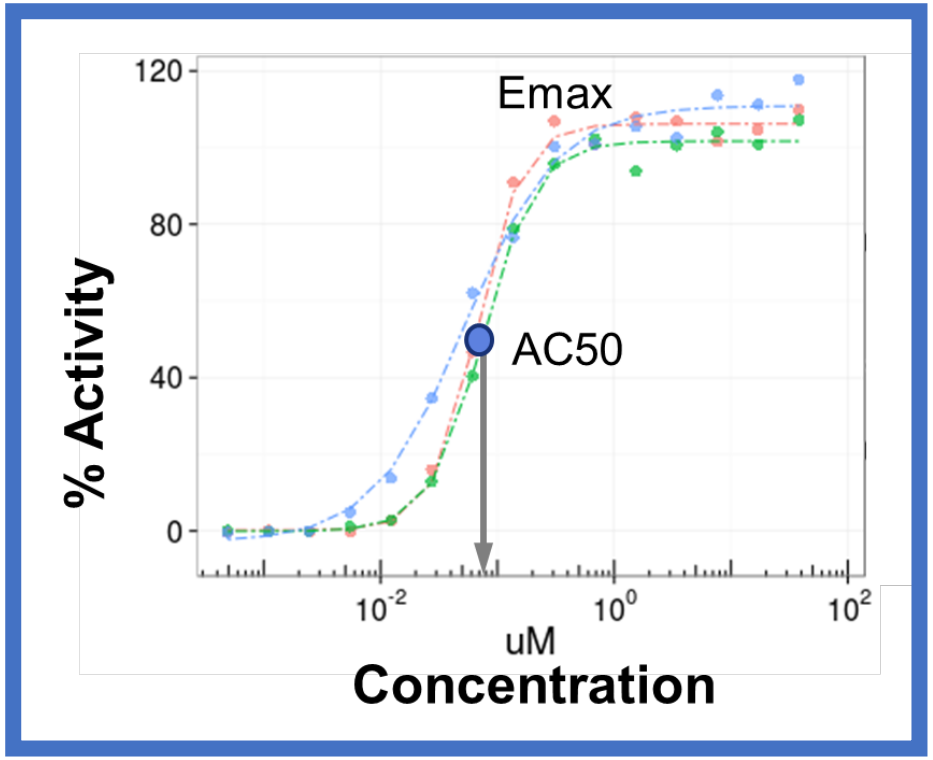
High-Throughput Data Generation

Quantitative high-throughput screening (qHTS)

>8,000 chemicals in >70 assays



>100 million concentration-response data points



Data Analysis

1. Requires consideration of concentration-response modeling
2. Derive values relevant for toxicological interpretation:
   - Potency
   - Efficacy

- Other considerations:
  - Identifying technological or assay issues
  - Integrating multiple readouts for some assays (e.g., cytotoxicity)

Understanding & Comparing Pipeline Processes

- Tox21 Informatics Workgroup
  - Nisha Sipes*
  - Jui-Hua Hsieh
  - Keith Shockley
  - Ruili Huang
  - Matt Martin
  - Richard Judson
  - Keith Houck
  - Huixiao Hong

Representatives from the Tox21 Partner agencies convened to focus on Tox21 Informatics needs.

Comparing hit calling approaches and better understanding similarities/differences was among their goals.

*Analyses results presented herein were generously provided by Nisha Sipes

Publicly Available Community Resource

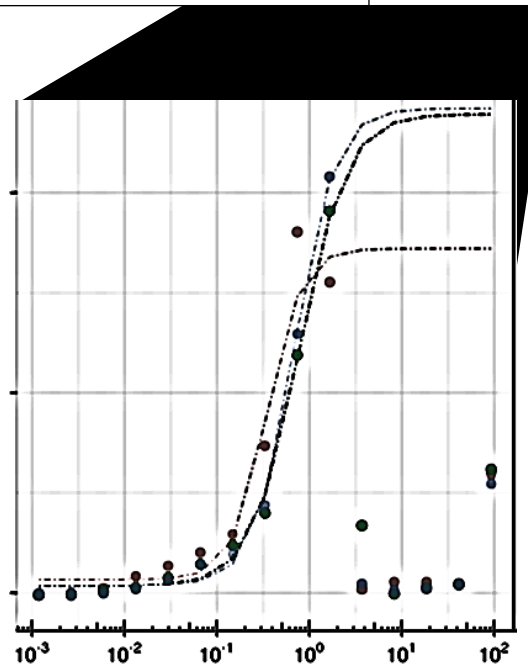
Four analysis pipelines for Tox21 data

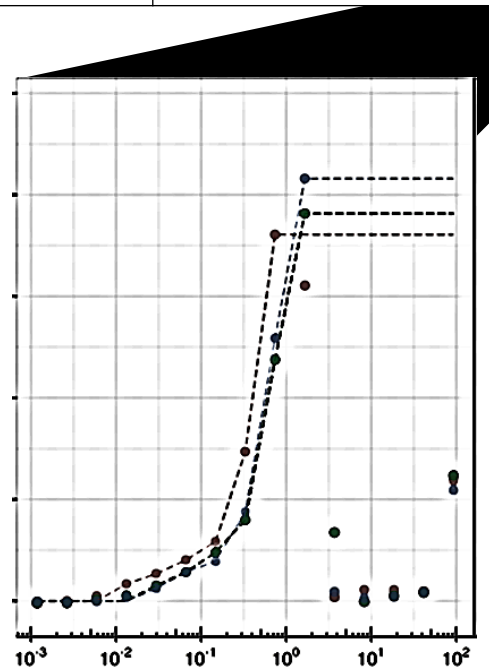
Name of Method	Institute	Public Access
3Stage	NIH/NIEHS	Shockley KR. 2012. *Environ Health Perspect* 120:1107–15.
CurveClass	NIH/NCATS	tripod.nih.gov & PubChem
CurvepwAUC	NIH/NIEHS/DNTP	https://ntp.niehs.nih.gov/whatwestudy/tox21/index.html
TCPL	US EPA/CCTE	https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data

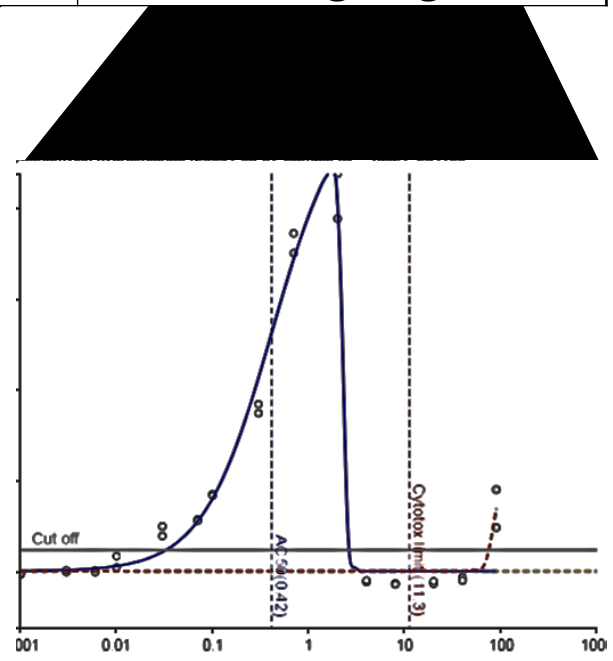
1. **3Stage**: Shockley KR (2012) Environ Health Perspect
2. **CurveClass**: Inglese J et al (2006) PNAS; Huang R et al (2011) EHP; Huang R et al (2014) Sci Rep; Huang R et al (2016) Methods in Moledular Biology 1473.1
3. **CurvepwAUC**: Hsieh JH et al (2015) J Biomol Screen; Hsieh JH (2016) *High-Throughput Screening Assays in Toxicology*
4. **TCPL:** Filer DL et al (2017) Bioinformatics

Diverse ways to fit data

	3Stage	CurveClass	CurvepwAUC	TCPL
data collapsing method	majority vote + curve shape; mean or median	Curve Rank + reproducibility; mean	median	N/A
curve fitting model	constant or Hill model	Hill model	model free	constant, gain-loss, or Hill model
Add'l info	outlier detection	outlier detection	outlier detection	fitting flags

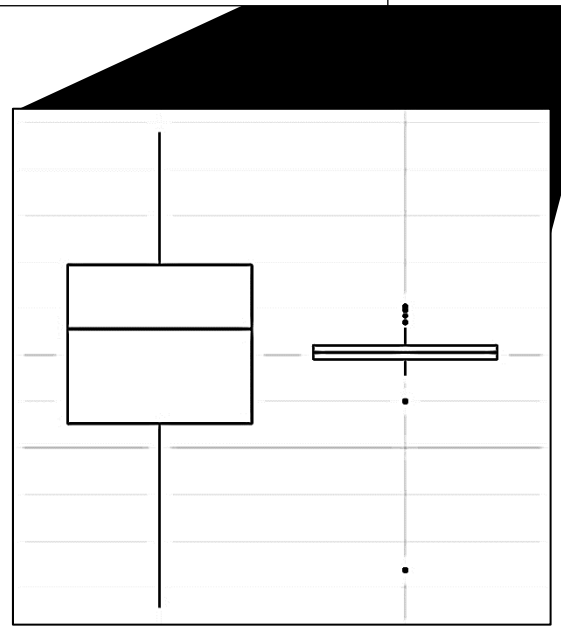


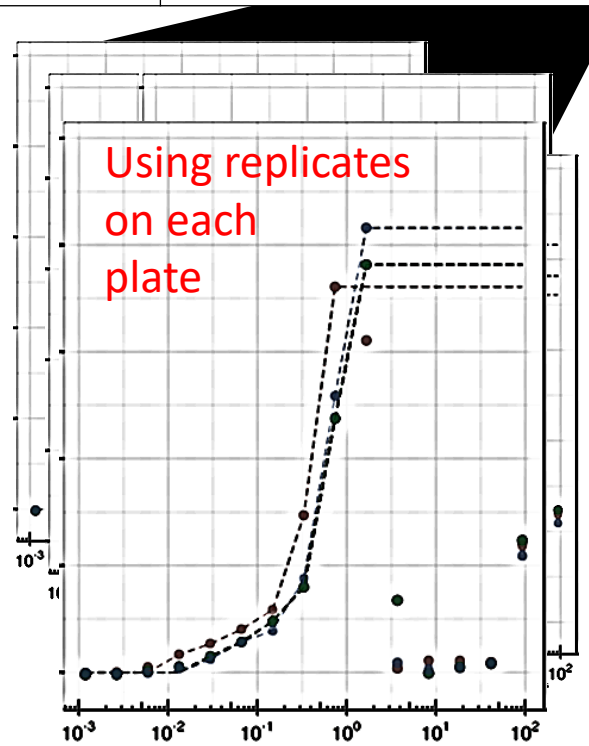


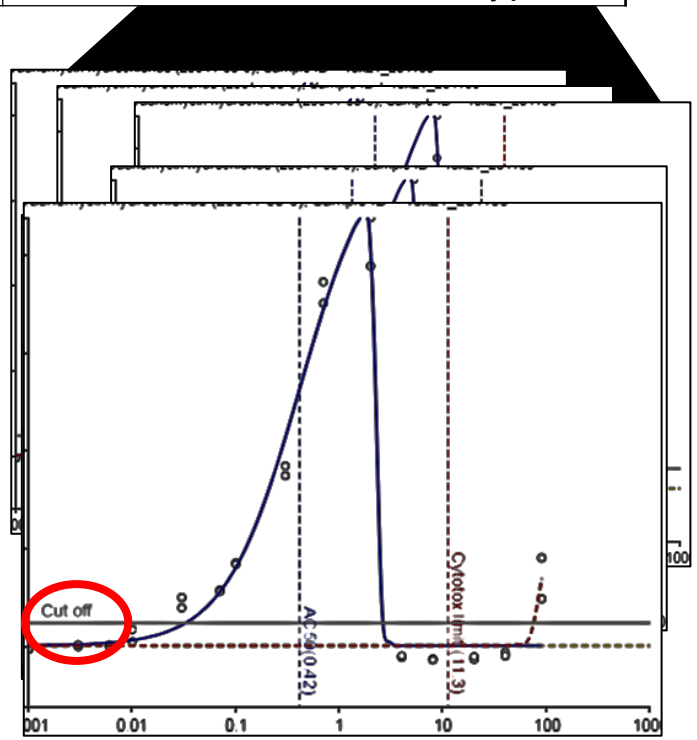


Response threshold varies

	3Stage	CurveClass	CurvepwAUC	TCPL
response threshold (THR)	3SD of normalized responses in DMSO plates	3SD of normalized responses in DMSO plates	Threshold to reduce POD variance using 5-45%	Custom or a multiple of BMAD: Baseline Median Abs Dev (MAD for DMSO/low concs across entire assay)







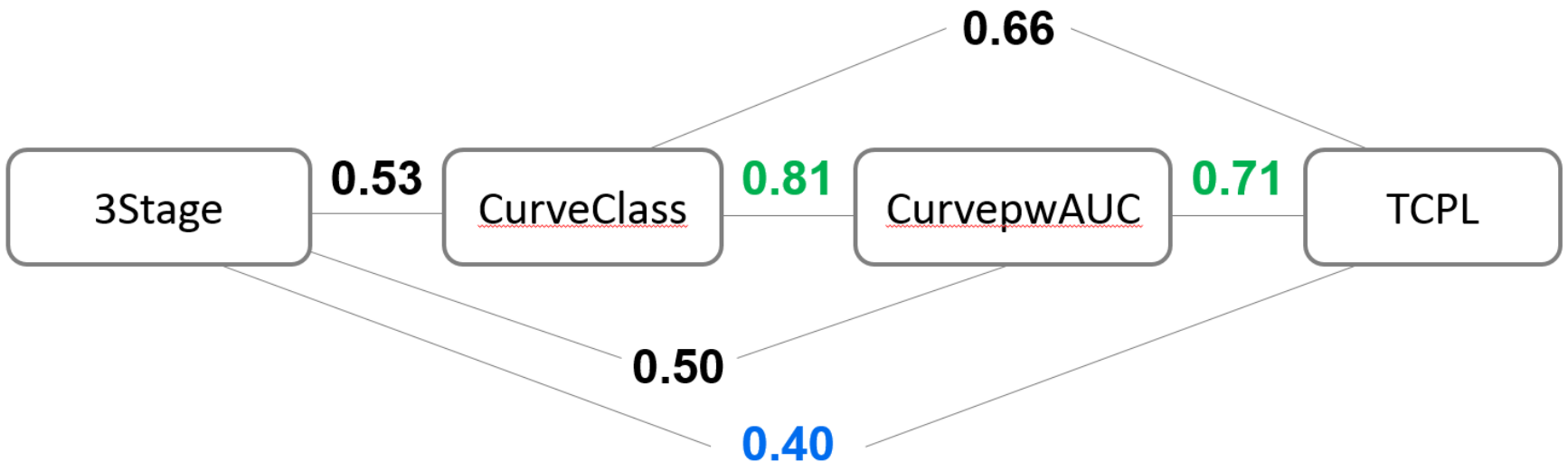
Summary of Activity Calls

	3Stage	CurveClass	CurvepwAUC	TCPL
Active	22,815 (3.4%)	38,886 (5.9%)	44,657 (6.7%)	64,147 (9.7%)
Inactive	564,504 (85.0%)	552,106 (83.1%)	580,851 (87.4%)	600,248 (90.3%)
Inconclusive	77,076 (11.6%)	73,403 (11.0%)	38,887 (5.9%)	NA

Pairwise Intraclass Correlation (ICC)

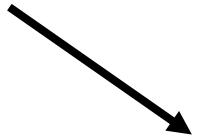
Summary of Activity Calls **(in %)**				
Call	**3Stage**	**Curve Class**	**Curvep wAUC**	**TCPL**
inactive	96.6	94.1	93.3	90.3
active	3.4	5.9	6.7	9.7

Note: for this analysis inconclusives were considered inactive



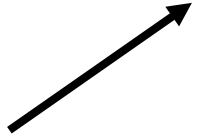
High agreement occurs often

3Stage	Curve Class	Curvep wAUC	TCPL	Counts
0	0	0	0	591699
0	0	0	1	19412
0	1	1	1	17101
1	1	1	1	15152
0	0	1	1	6378
0	1	0	1	2534
1	0	0	0	2352
0	0	1	0	2313
1	0	0	1	1600
1	0	1	1	1262
0	1	1	0	1164
0	1	0	0	979
1	1	1	0	794
1	1	0	1	708
1	0	1	0	493
1	1	0	0	454



Activity permutation count across pipelines

98% with ≥ 3 agreeing pipelines



0 = inactive or inconclusive call
1 = active call

Most discrepancies likely due to call adjustments

3Stage	Curve Class	Curvep wAUC	TCPL	Counts
0	0	0	0	591699
0	0	0	1	19412
0	1	1	1	17101
1	1	1	1	15152
0	0	1	1	6378
0	1	0	1	2534
1	0	0	0	2352
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0	1	0	0	979
1	1	1	0	794
1	1	0	1	708
1	0	1	0	493
1	1	0	0	454

3Stage	Curve Class	Curvep wAUC	TCPL	Counts
inconclusive	1	1	1	17070
0	1	1	1	31

>99%

Most discrepancies likely due to call adjustments

3Stage	Curve Class	Curvep wAUC	TCPL	Counts
0	0	0	0	591699
0	0	0	1	19412
0	1	1	1	17101
1	1	1	1	15152
0	0	1	1	6378
0	1	0	1	2534
1	0	0	0	2352
0	0	1	0	2313
1	0	0	1	1600
1	0	1	1	1262
0	1	1	0	1164
0	1	0	0	979
1	1	1	0	794
1	1	0	1	708
1	0	1	0	493
1	1	0	0	454

3Stage	Curve Class	Curvep wAUC	TCPL	Counts
inconclusive	inconclusive	inconclusive	1	16511
inconclusive	inconclusive	0	1	862
0	0	0	1	595
inconclusive	0	0	1	355
inconclusive	0	inconclusive	1	353
0	inconclusive	0	1	259
0	inconclusive	inconclusive	1	284
0	0	inconclusive	1	193

85%

Most discrepancies likely due to call adjustment

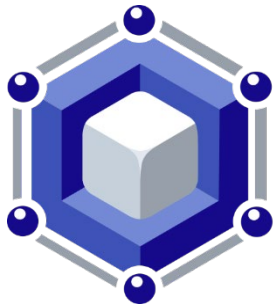
3Stage	Curve Class	Curvep wAUC	TCPL	Counts
0	0	0	0	591699
0	0	0	1	19412
0	1	1	1	17101
1	1	1	1	15152

3Stage	Curve Class	Curvep wAUC	TCPL	Counts	
inconclusive	inconclusive	inconclusive	1	16511	85%
inconclusive	inconclusive	0	1	862	

The Integrated Chemical Environment web tool (ICE) developed by NICEATM (NTP Interagency Center for the Evaluation of Alternative Test Methods) contains curated high-throughput screening data (cHTS):

- ICE cHTS data is curated tcpl data for both Tox21 and ToxCast.
  - Identifies noisy curves and integrates flags from tcpl outputs
  - Active tcpl calls rendered “Flag-OMIT” by curation
  - Flag-OMIT calls are highly correlated to the inconclusive calls from the other methods

- Using ICE cHTS data for tcpl outputs is anticipated to resolve many of the inconsistencies between inconclusive calls across 3Stage, CurveClass, and CurvepwAUC vs. actives in tcpl.



Integrated Chemical Environment

https://ice.ntp.niehs.nih.gov/

Summary and Conclusion

- Four methods have been developed for analyzing the Tox21 quantitative high-throughput concentration-response data
- Generally good agreement – especially for robust actives
- Working toward a consensus:
  - Majority of actives have at least three methods in agreement
  - Further curation of tcpl to address actives with flags (i.e., ICE cHTS) increases agreement with other methods

Abbreviations

- BMAD: Baseline median absolute deviation
- CCTE: Center for Computational Toxicology and Exposure
- CurvepwAUC: Curvep weighted area under the curve
- MAD: Median absolute deviation
- EPA: United States Environmental Protection Agency
- HTS: High-throughput screening (qHTS; quantitative HTS)
- ICC: Intraclass correlation
- NCATS: National Center for Advancing Translational Sciences
- NIEHS: National Institutes of Environmental Health Sciences
- NIH: National Institutes of Health
- NTP: National Toxicology Program
- POD: Point of departure
- SD: Standard deviation
- TCPL: ToxCast Pipeline

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