Comparing the strengths and limitations of concentration-response modeling pipelines for developmental neurotoxicity (DNT) new approach methods (NAMs)

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International Neurotoxicology Association 18
Evaluating new approach methodologies for developmental neurotoxicity: Computational models to mechanisms of toxicity
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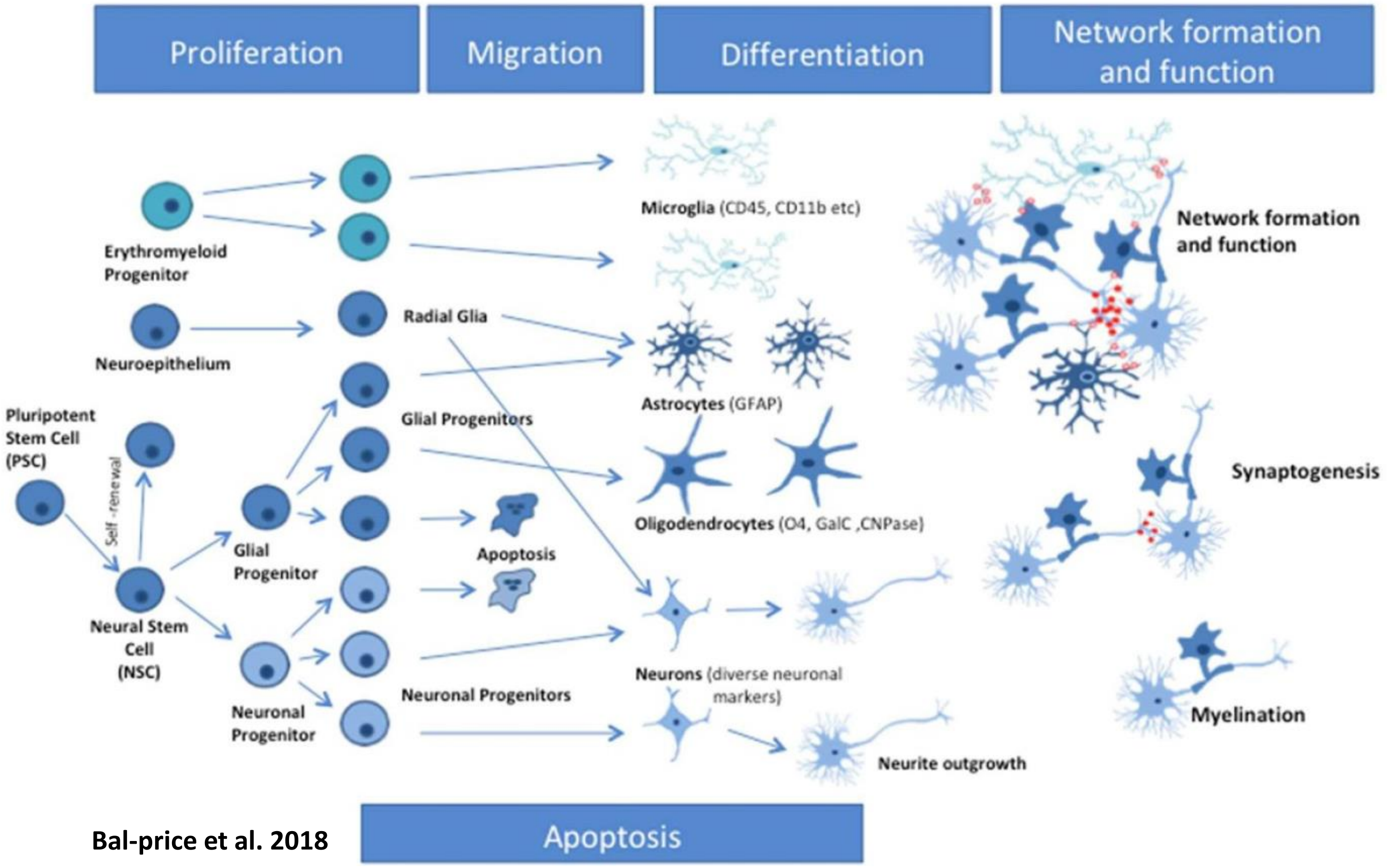
Neurodevelopmental processes in the DNT battery



Bal-price et al. 2018

Table 2. Proposed Assays for Evaluation As an *In Vitro* DNT Battery

Process	Assays	References
Proliferation	hNP1	Harrill *et al.* (2018)
	NPC1	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN1	Balmer *et al.* (2012)
Apoptosis	hNP1	Harrill *et al.* (2018)
Migration	NPC2	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN2	Nyffeler *et al.* (2017)
Neuron differentiation	NPC3	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Oligodendrocyte differentiation & maturation	NPC5/6	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Neurite outgrowth	iCell gluta	Harrill *et al.* (2018)
	UKN 4 & 5	Krug *et al.* (2013)
	NPC4	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Synaptogenesis	Rat primary synaptogenesis	Harrill *et al.* (2018)
Network formation	MEA-NFA	Brown *et al.* (2016) and Frank *et al.* (2018)

Sachana, M., et.al. 2019, Toxicological Sciences

Concentration-response modeling

❖ Concentration-response modeling can be used to derive a statistical point of departure (POD), such as a benchmark dose (BMD), for human health risk assessment.

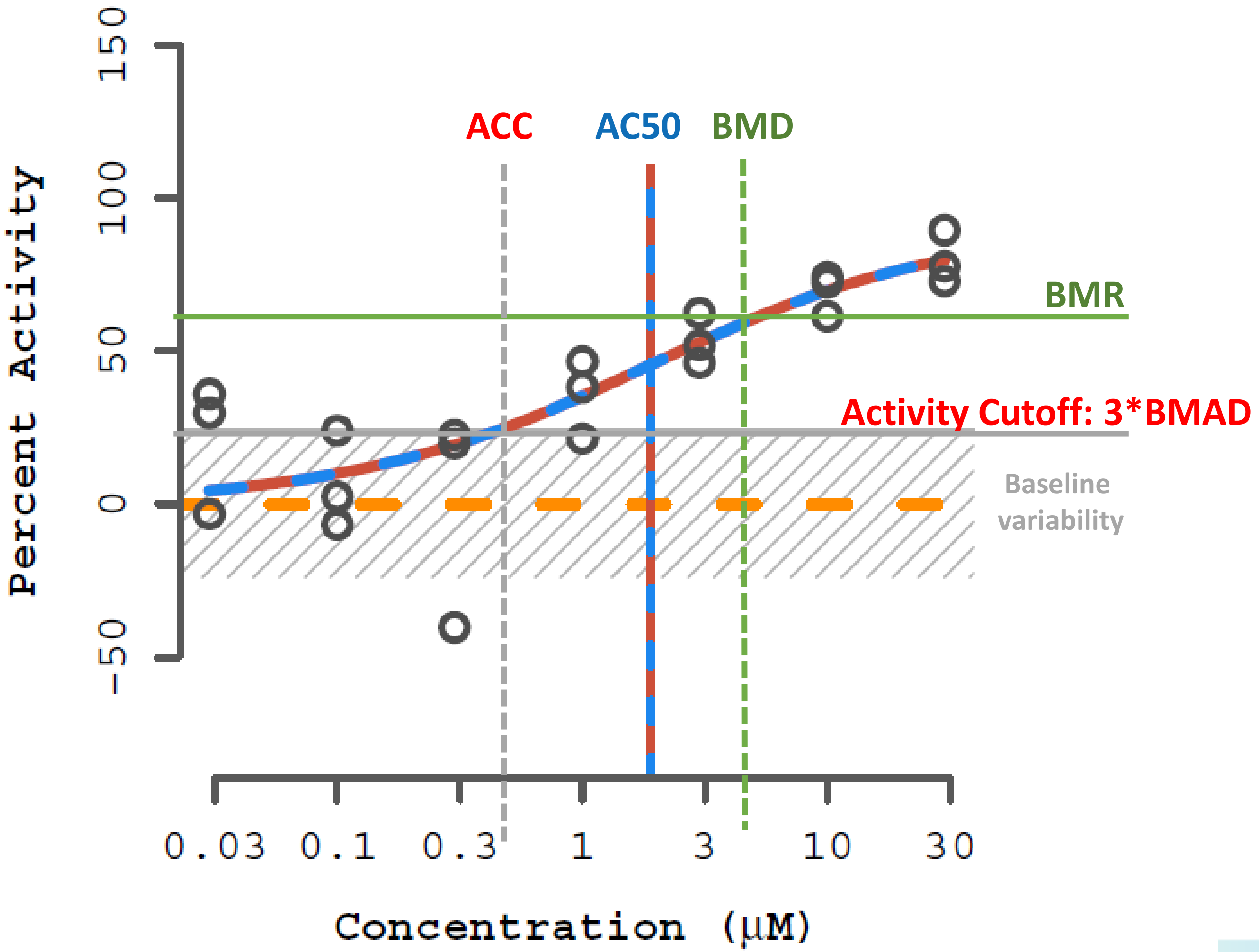
❖ BMD: a chemical dose or concentration that produces a predetermined change in the response rate of an adverse effect.

❖ POD values are dependent on the concentration-response modeling tool, as well as other user-defined variables, e.g.

- Normalization methods
- Model Selection
- Benchmark Response

❖ International efforts to evaluate DNT NAMs currently utilize at least three different concentration-response modeling pipelines.

BMR : benchmark response
BMD : benchmark dose
BMAD : Baseline median absolute deviation
ACC : concentration at activity cutoff
AC50 : concentration at 50% maximal activity



Concentration-response modeling pipelines for DNT

- **ToxCast Pipeline**: US EPA
- **CRStats**: Leibniz Research Institute for Environmental Medicine, University of Konstanz
- **DNT-DIVER**: National Institute of Environmental Health Science (NIEHS) Division of Translational Toxicology (DTT)
- **PROAST**: RIVM National Institute for Public Health and the Environment, European Food Safety Authority

JOURNAL ARTICLE

tcpl: the ToxCast pipeline for high-throughput screening data FREE

Dayne L Filer, Parth Kothiya, R Woodrow Setzer, Richard S Judson, Matthew T Martin

Bioinformatics, Volume 33, Issue 4, February 2017, Pages 618–620,

Developmental **N**euro**T**oxicity **D**ata **I**ntegration and **V**isualization **E**nabling **R**esource (**DNT-DIVER**)

RIVM Committed to *health and sustainability*

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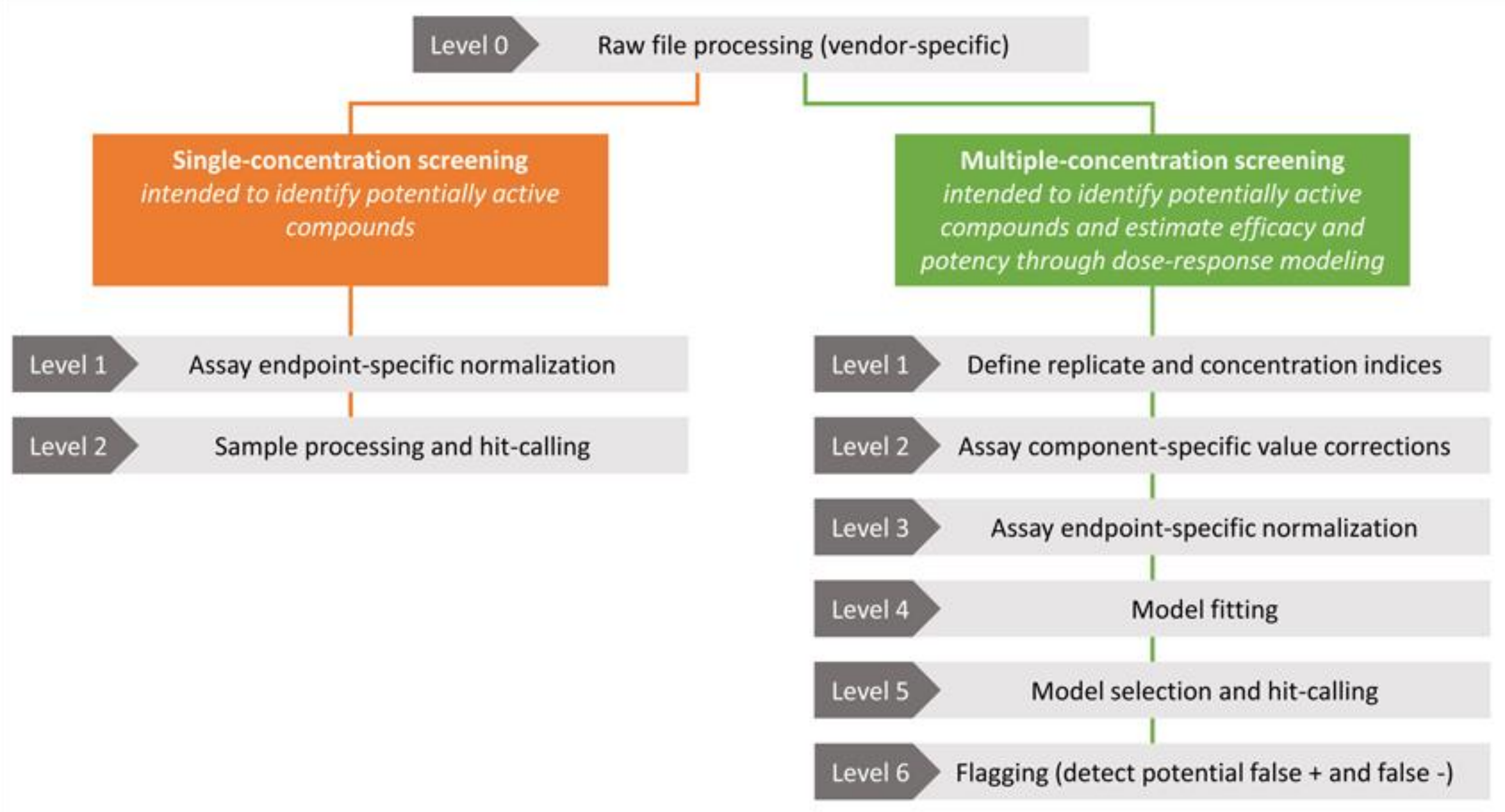
PROAST

GitHub ArifDoenmez / **CRStats** Public

ToxCast Pipeline data processing

https://github.com/USEPA/CompTox-ToxCast-tcpl.git

GitHub



ToxCast pipeline (tcpl) R package (version 2.0.3 [publicly available](https://www.epa.gov/chemical-research/toxcast-data-generation-toxcast-pipeline-tcpl)) (Filer et al. 2017)

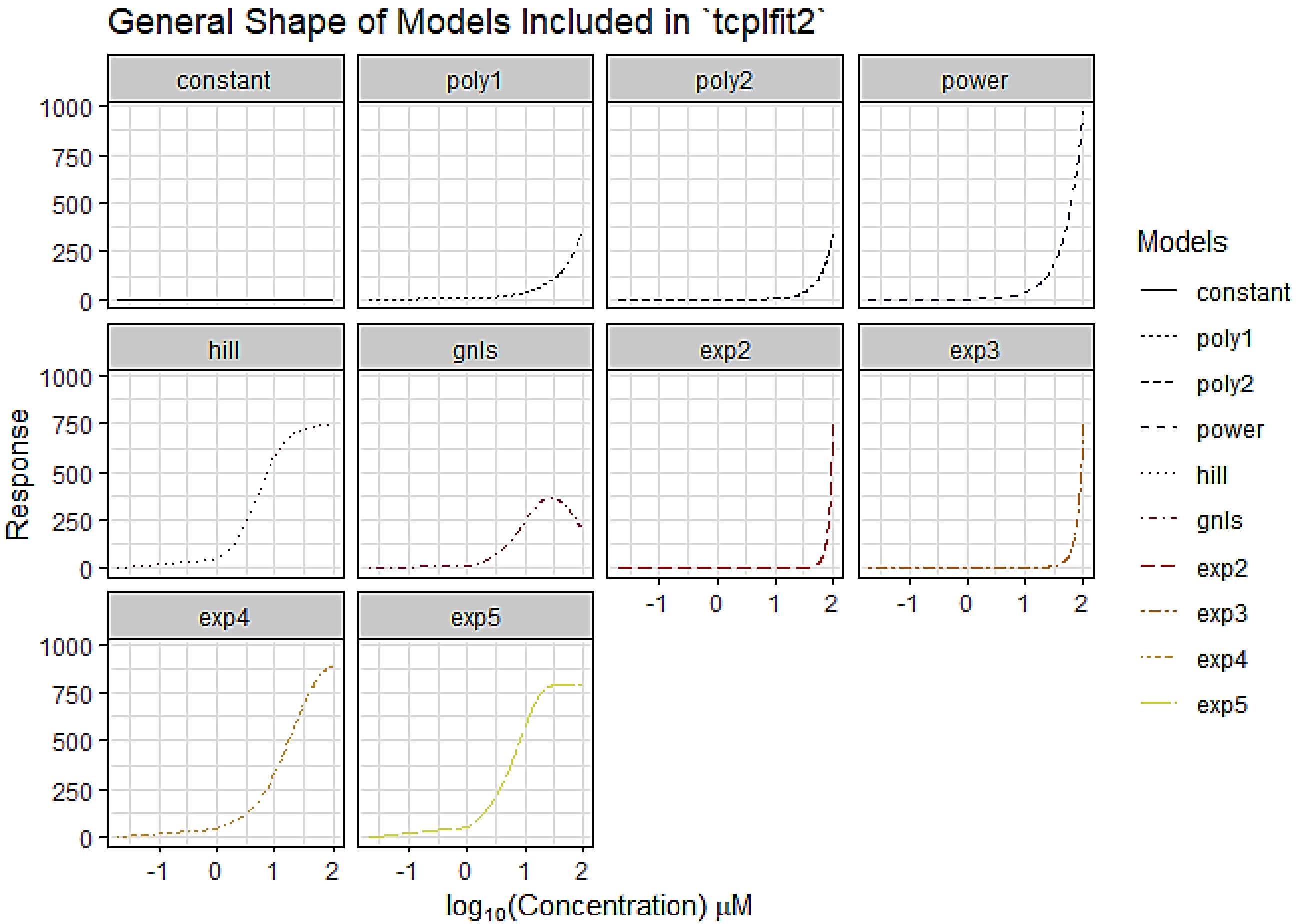
https://www.epa.gov/chemical-research/toxcast-data-generation-toxcast-pipeline-tcpl

ToxCast Pipeline data processing

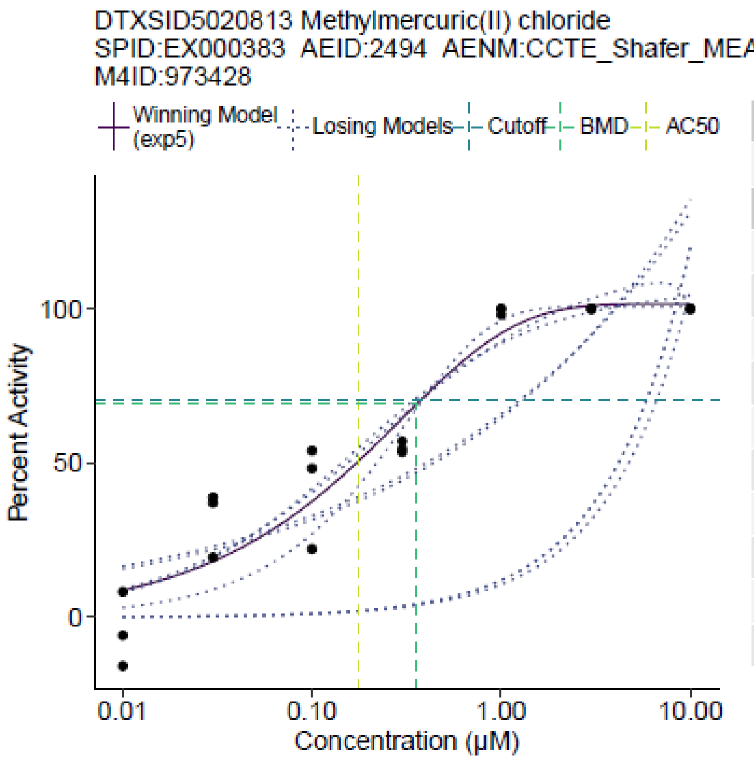
https://github.com/USEPA/CompTox-ToxCast-tcpl.git
R package: ‘tcpl’ v3.0

GitHub

Level 4: Model fitting



Level 5: Model Selection and hit calling



Hitcall	BMD	AC50
1.000	0.358	0.177

model	aic	rme
exp5	170.672	11.295
exp4	171.812	13.130
hill	173.180	11.731
gnls	175.749	11.435
pow	197.581	21.467
exp3	202.967	22.753
poly1	231.149	50.412
exp2	233.157	50.423
poly2	233.488	51.122
cnst	244.459	71.749

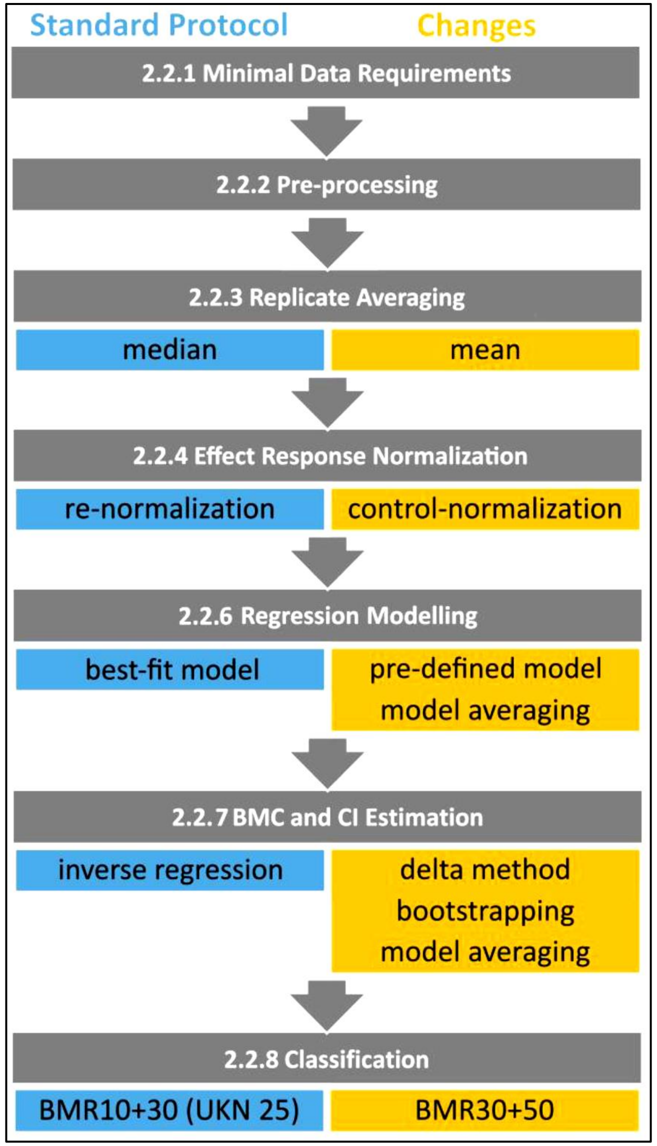
‘Continuous hit call’ > 0.9 defines a positive hit in ToxCast.

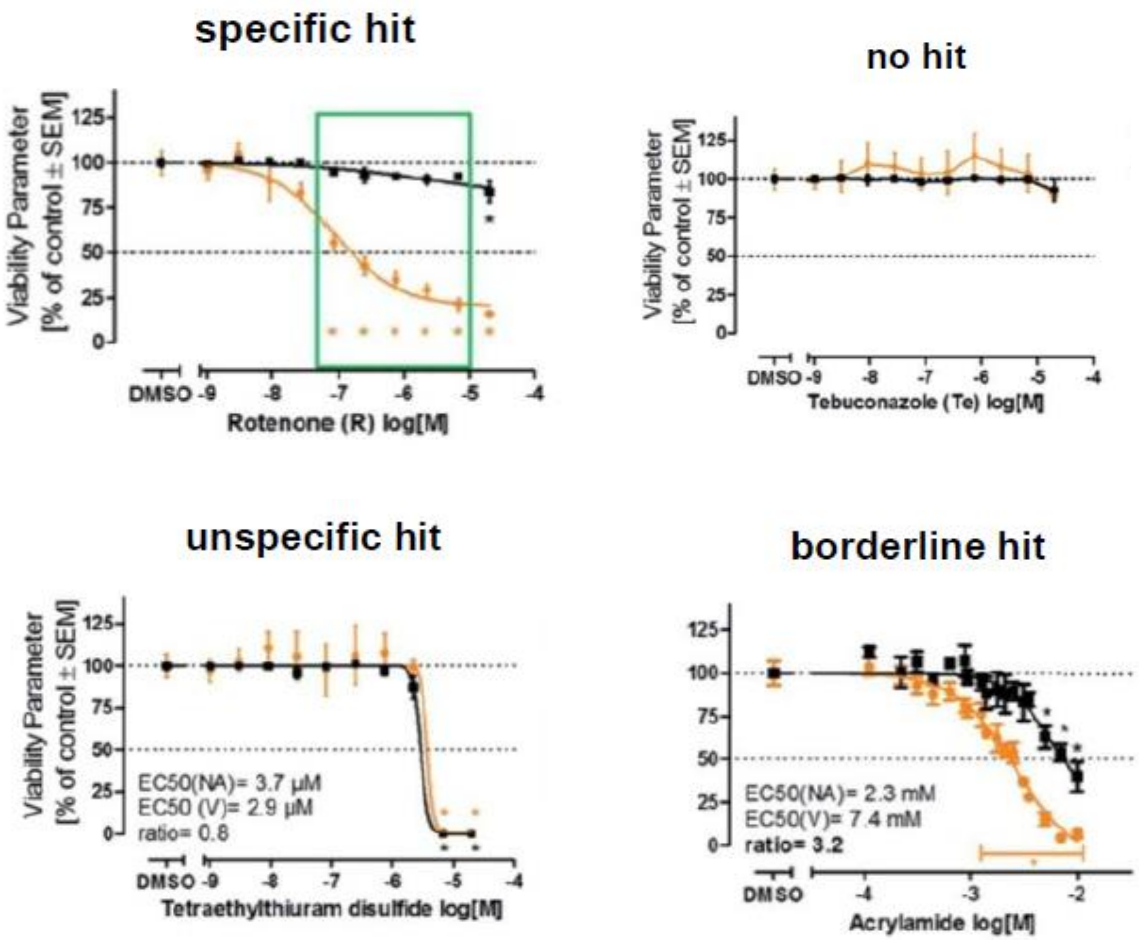
https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing.html

CRStats Pipeline data processing

https://github.com/ArifDoenmez/CRStats.git

GitHub





Classification of ‘specific hit’, ‘unspecific hit’, or ‘borderline’ defines a positive hit in CRStats.

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https://www.biorxiv.org/content/10.1101/2022.10.18.512648v1.full.pdf

Masjosthusmann and Blum, 2020, EFSA.

CRStats Pipeline data processing

https://github.com/ArifDoenmez/CRStats.git

GitHub

Table S2: Regression models

Model[2)]	*drc* syntax	Model equation[1)]
general logistic	logistic2()	$f(x) = c + \frac{d-c}{(1+\exp(b(\log(x)-\log(e))))^f}$
3-parameter log-logistic	LL.3()	$f(x) = 0 + \frac{d-0}{1+\exp\big(b(\log(x)-\log(e))\big)}$
4-parameter log-logistic	LL.4()	$f(x) = c + \frac{d-c}{1+\exp\big(b(\log(x)-\log(e))\big)}$
2-parameter exponential	EXD.2()	$f(x) = 0 + (d-0)(\exp\left(-\frac{x}{e}\right))$
3-parameter exponential	EXD.3()	$f(x) = c + (d-c)(\exp\left(-\frac{x}{e}\right))$
3-parameter Weibull	w1.3()	$\mathrm{f(x) = 0 + (d-0)exp(-exp\big(b(log(x)-e)\big))}$
4-parameter Weibull	w1.4()	$\mathrm{f(x) = c + (d-0)exp(-exp\big(b(log(x)-e)\big))}$

Model names and abbreviation from Analysis of Dose-Response Curves (Ritz et al. 2016).

https://www.biorxiv.org/content/10.1101/2022.10.18.512648v1.full.pdf

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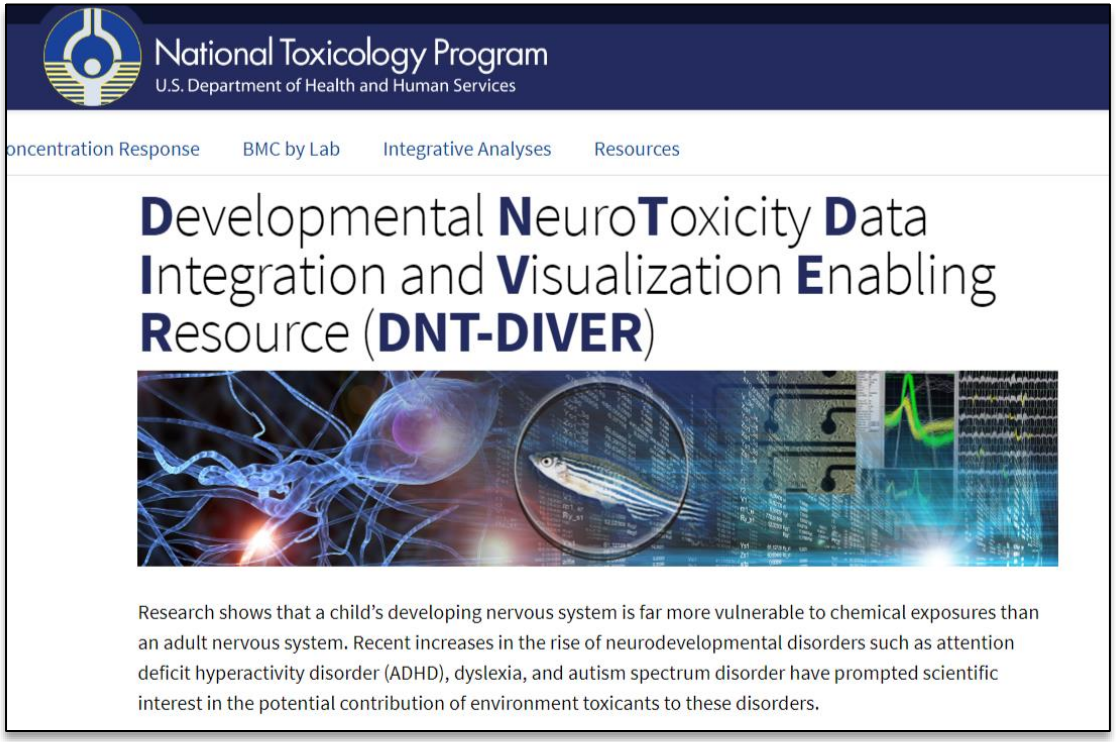
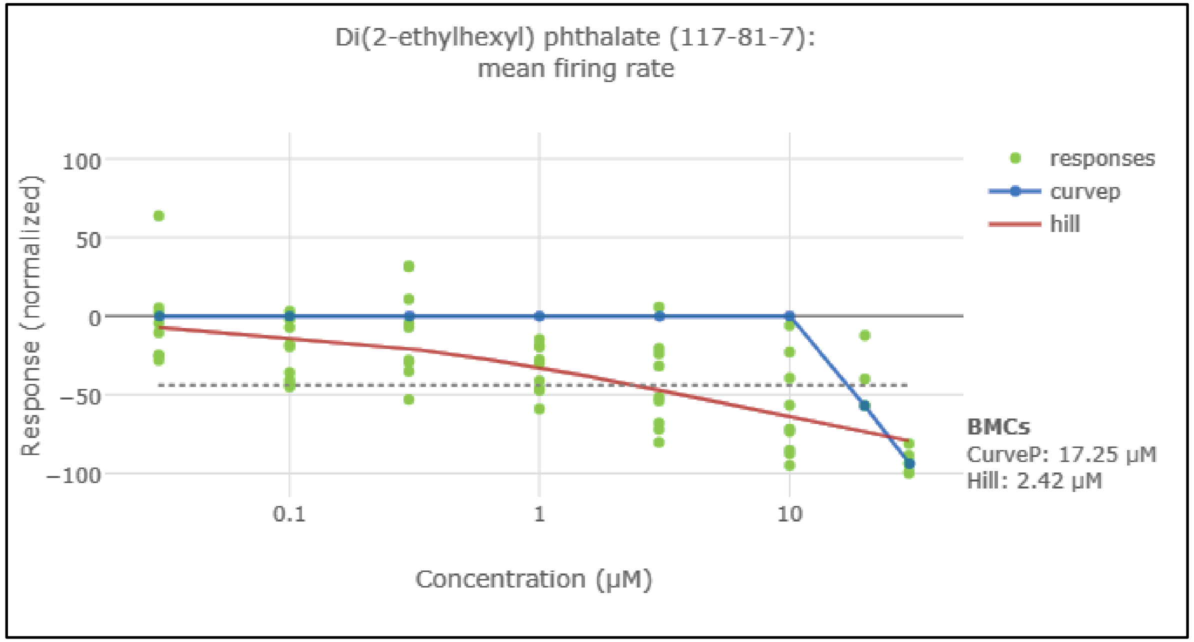
DNT-DIVER Pipeline data processing

https://sandbox.ntp.niehs.nih.gov/neurotox/

NIH National Institute of Environmental Health Sciences
Your Environment. Your Health.





'Hit confidence' of > 0.5 defines a positive hit in DNT-DIVER.

DNT-DIVER

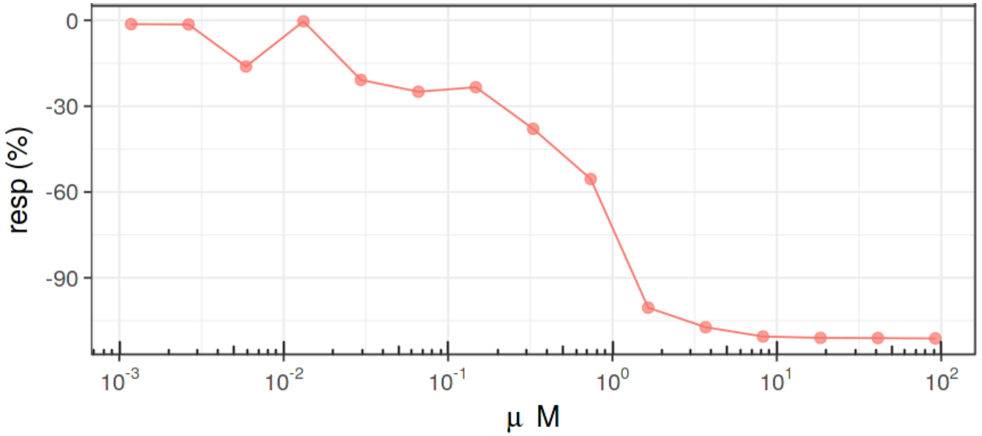
https://github.com/moggces/Rcurvep.git

GitHub

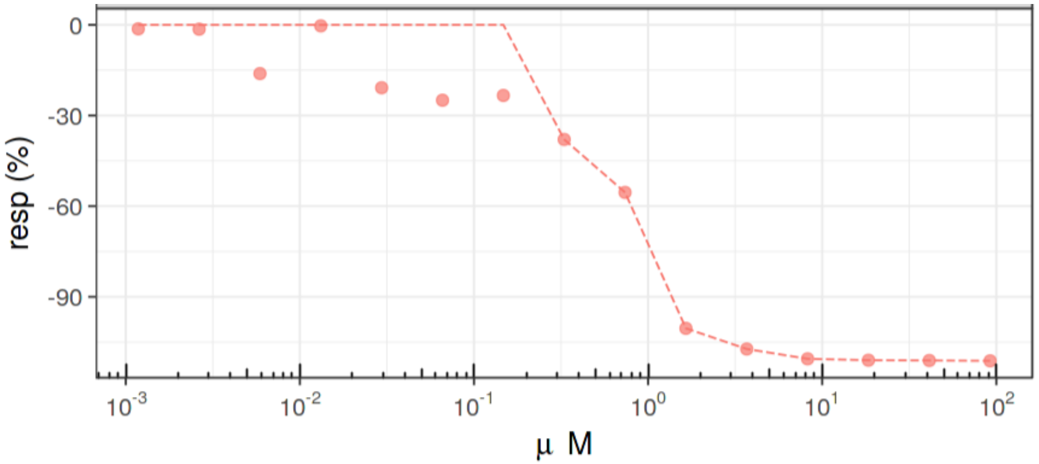
Curvep Model

Hill Model

Normalized Screening Data

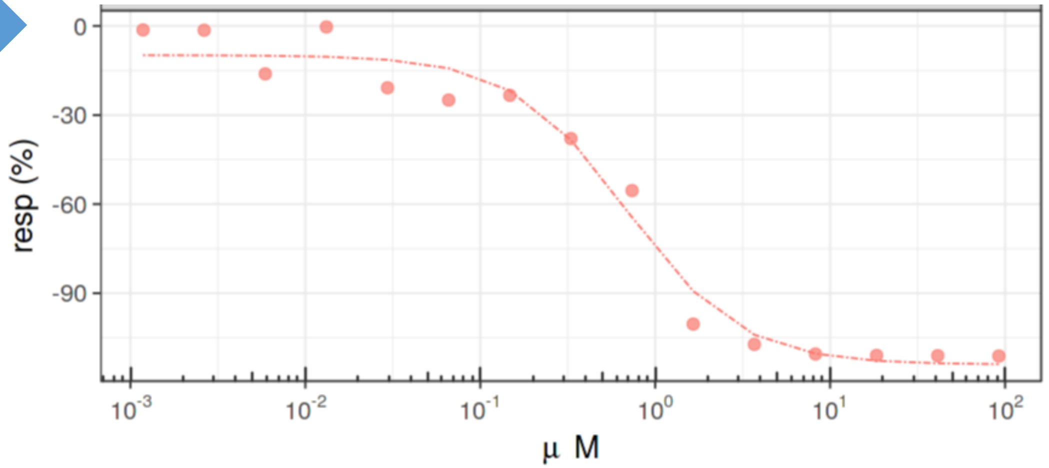


Curvep results



Noise filtering algorithm (Sedykh A, 2016)

Hill equation results



Parametric (based on ToxCast Hill model)

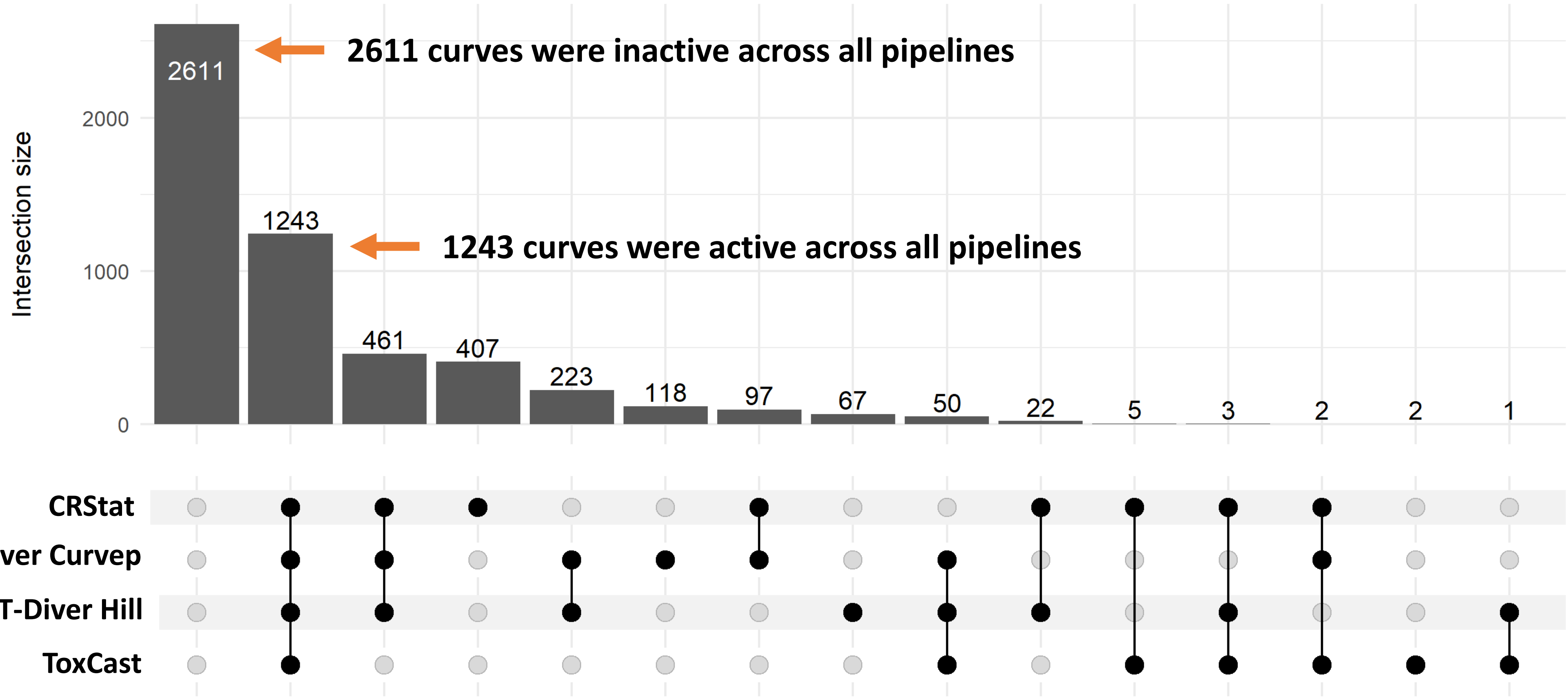
Slide courtesy Jui-hua Hsieh

Data Landscape

- ❖ 143 chemicals
- ❖ 7 DNT NAMs assays
- ❖ 44 DNT NAMs endpoints
- ❖ 5313 chemicals x endpoint concentration-response series
- ❖ 3 pipelines: ToxCast, CRStats, DNT-DIVER
- ❖ 2 classification models: classifying specific DNT activity (activity below cytotoxicity)

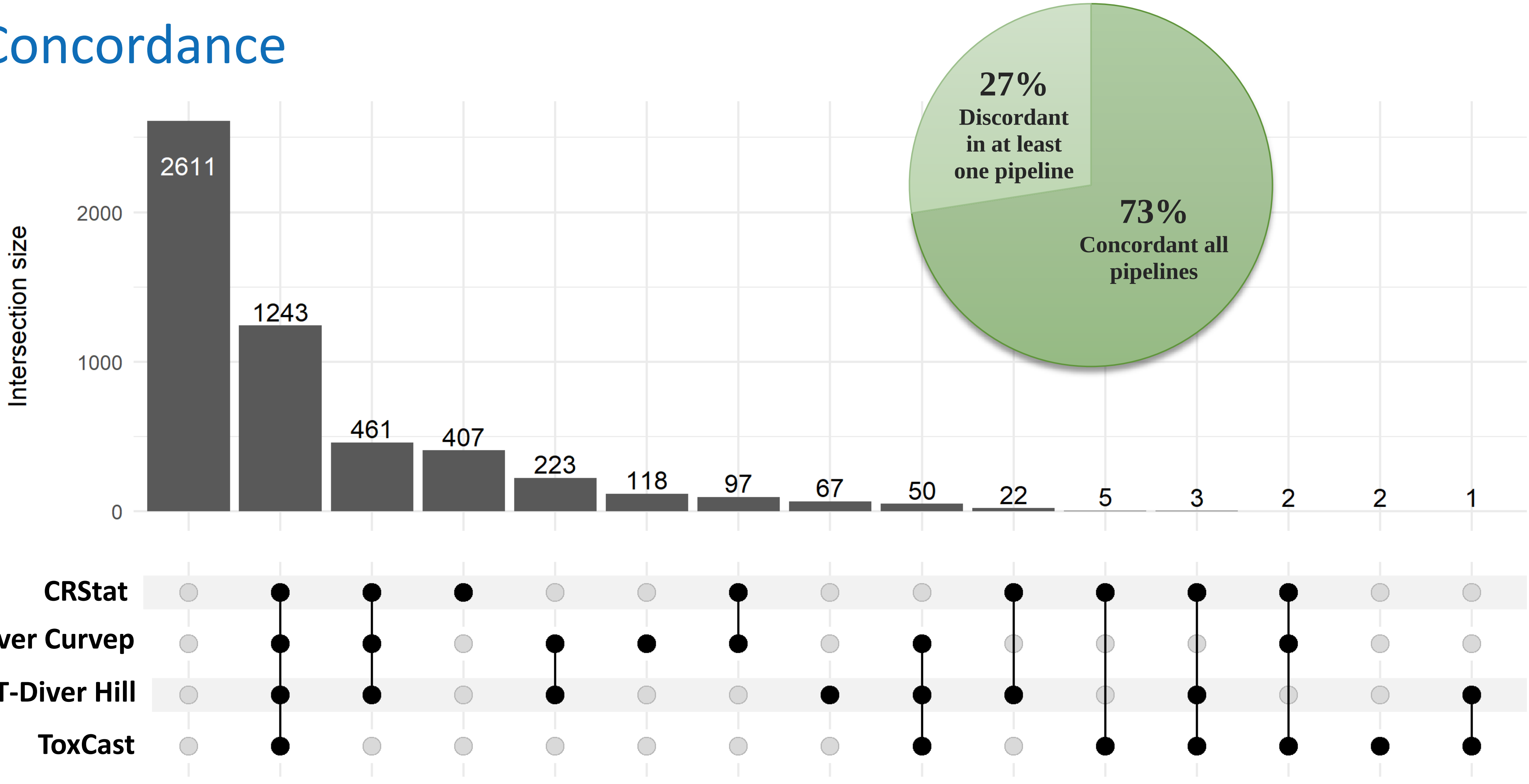
Neurodevelopmental Process	Species	Assay	N Chemicals Tested
Apoptosis	Human	Apoptosis, hNP1	126
Network formation & function	Rat	MEA NFA	143
NOG	Human	NOG, hN2	46
NOG	Rat	NOG, rat	130
NOG	Human	NOG, CDI	79
Proliferation	Human	Proliferation, hNP1	126
Synaptogenesis	Rat	Synaptogenesis, rat	130

Hit Call Concordance



Hitcall Concordance by Pipeline, Total 5313 chem x endpoint

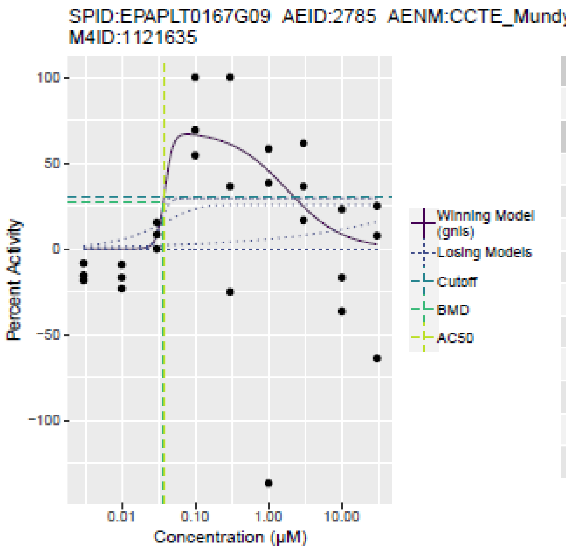
Hit Call Concordance



Hitcall Concordance by Pipeline, Total 5313 chem x endpoint

Example curves only active in one pipeline

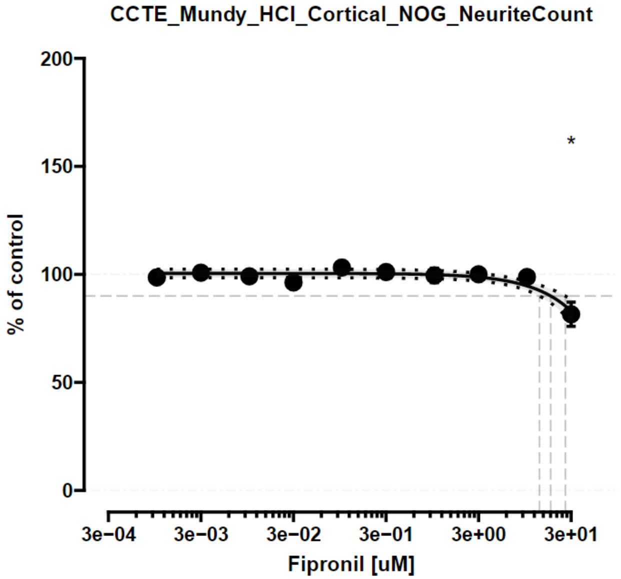
ToxCast (2)



Hitcall	BMD	AC50
0.977	0.037	0.038
model	**aic**	**rme**
gnls	281.144	45.170
exp4	287.309	48.138
exp5	287.734	47.965
hill	287.735	47.966
cnst	288.998	49.919
poly1	290.998	49.919
pow	292.218	49.837
poly2	292.998	49.919
exp2	292.998	49.919
exp3	294.998	49.919

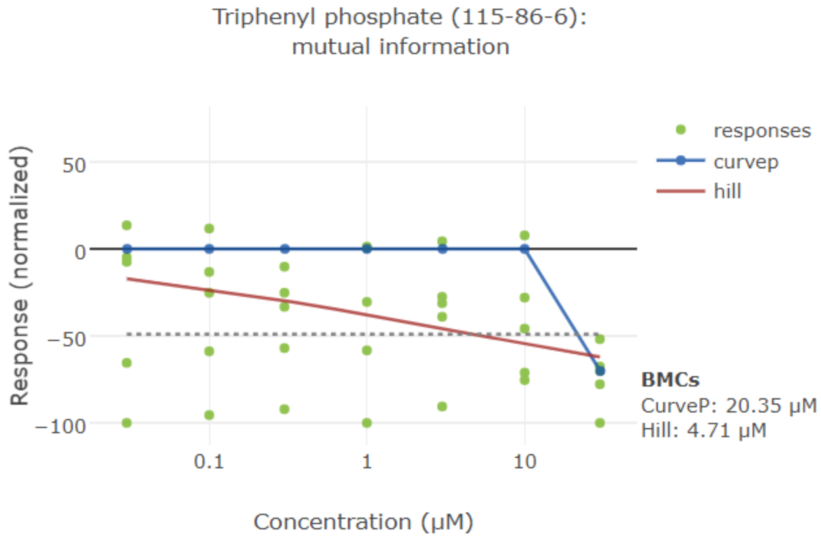
Trends:
- Fitting gain-loss model
- Noisy data

CRStats (407)



Trends:
- Activity at highest concentration exceeded the cutoff threshold in CRStats but not other pipelines
- Borderline activity

DNT-DIVER (408*)



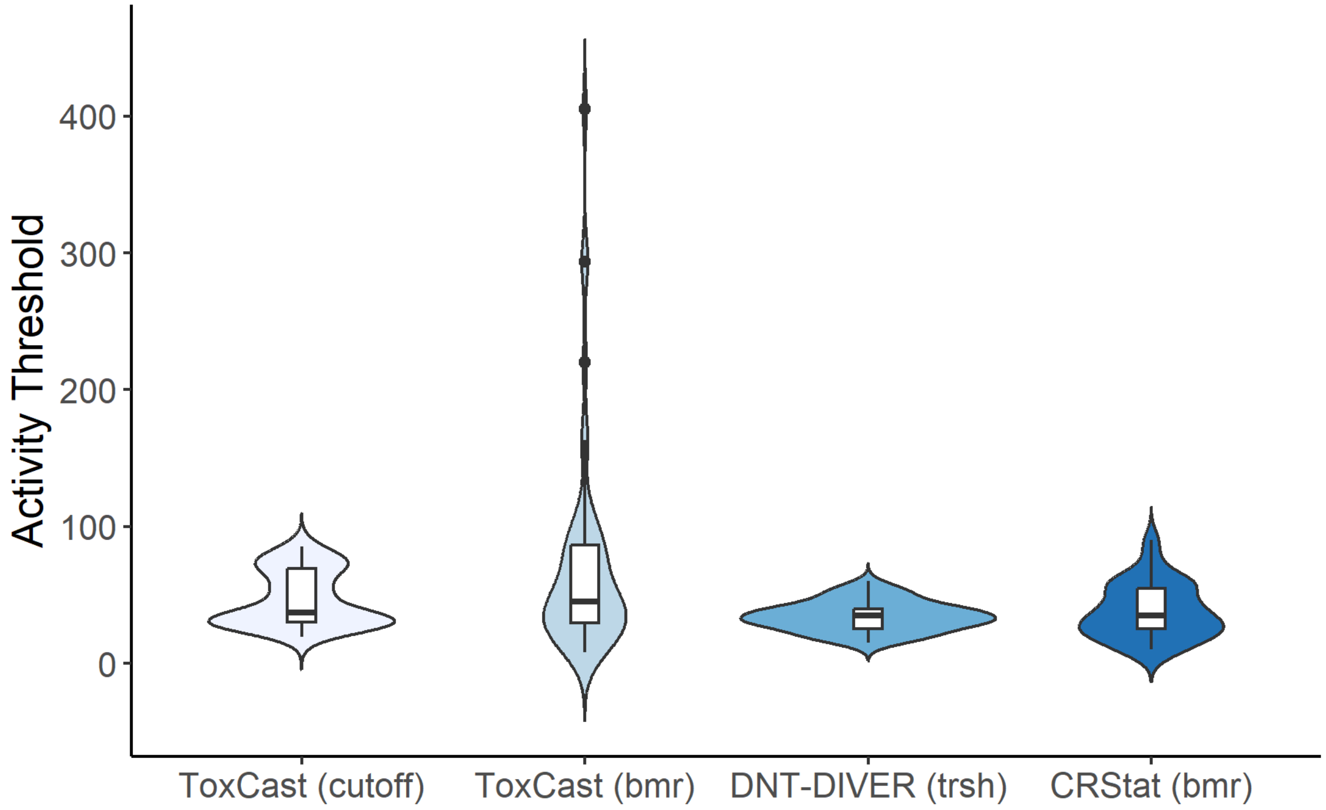
Trends:
- Activity at highest concentration exceeded the cutoff threshold in DNT-DIVER but not other pipelines
- Borderline activity
- Noisy data

*Combined curves that were hits in only Curvep or Hill or these two models combined.

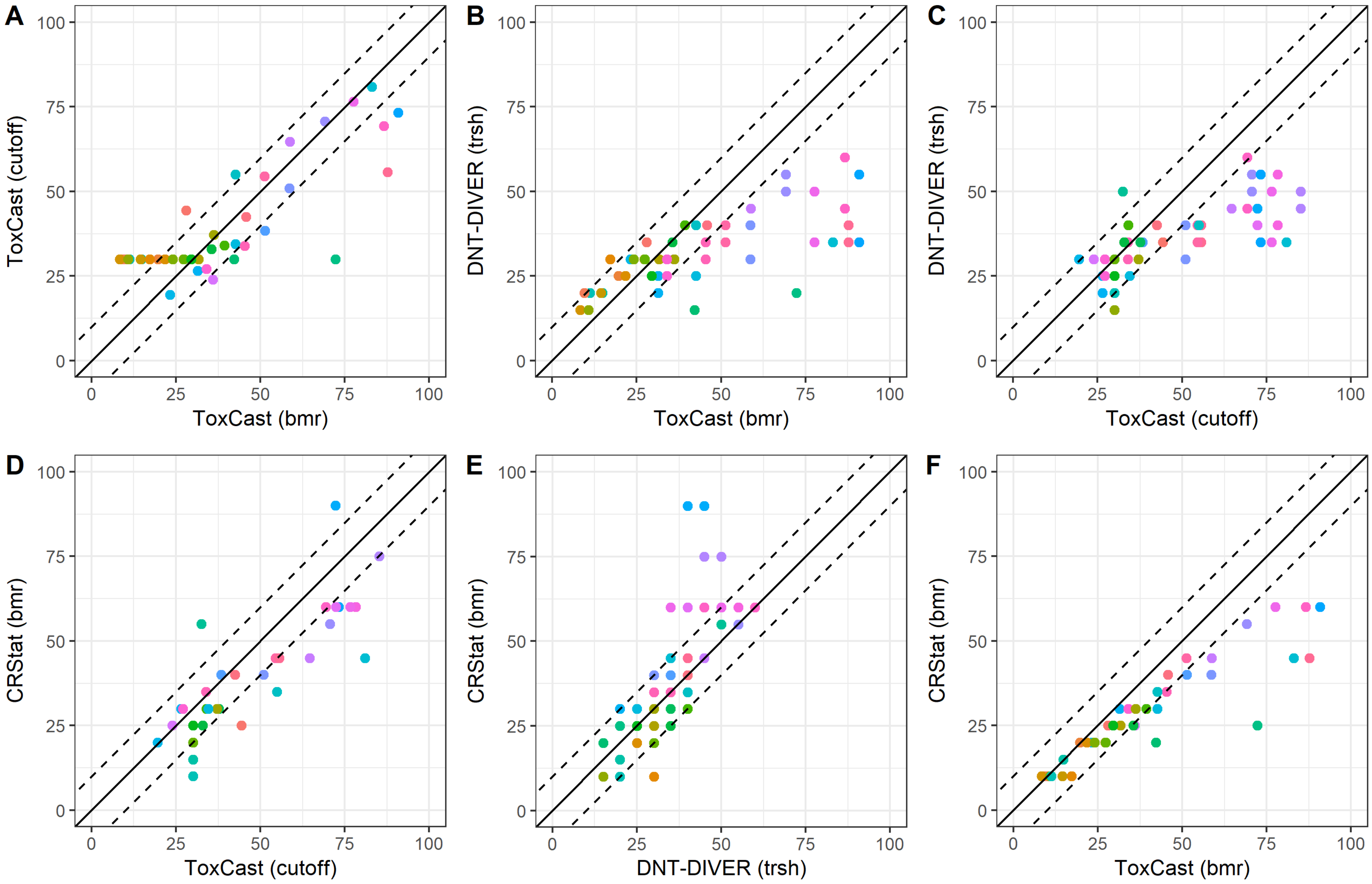
Activity Threshold (%) Comparison by Pipeline

POD: point of departure
BMR : benchmark response
BMD : benchmark dose
BMAD : Baseline median absolute deviation
SD : standard deviation
ACC : concentration at cutoff

	POD	Defining an activity threshold cutoff
ToxCast	BMD	BMR= 1 SD controls * 1.349
	ACC	Cutoff= 3 * BMAD
DNT-DIVER Curvep	BMD	BMR= Response threshold at which minimum variance in BMC is achieved from 1000 bootstrap curves
DNT-DIVER Hill	BMD	Same as above.
CRStats	BMD	BMR= User-defined as small ‘relevant’ change from controls



Activity Threshold (%) Comparison by Endpoint

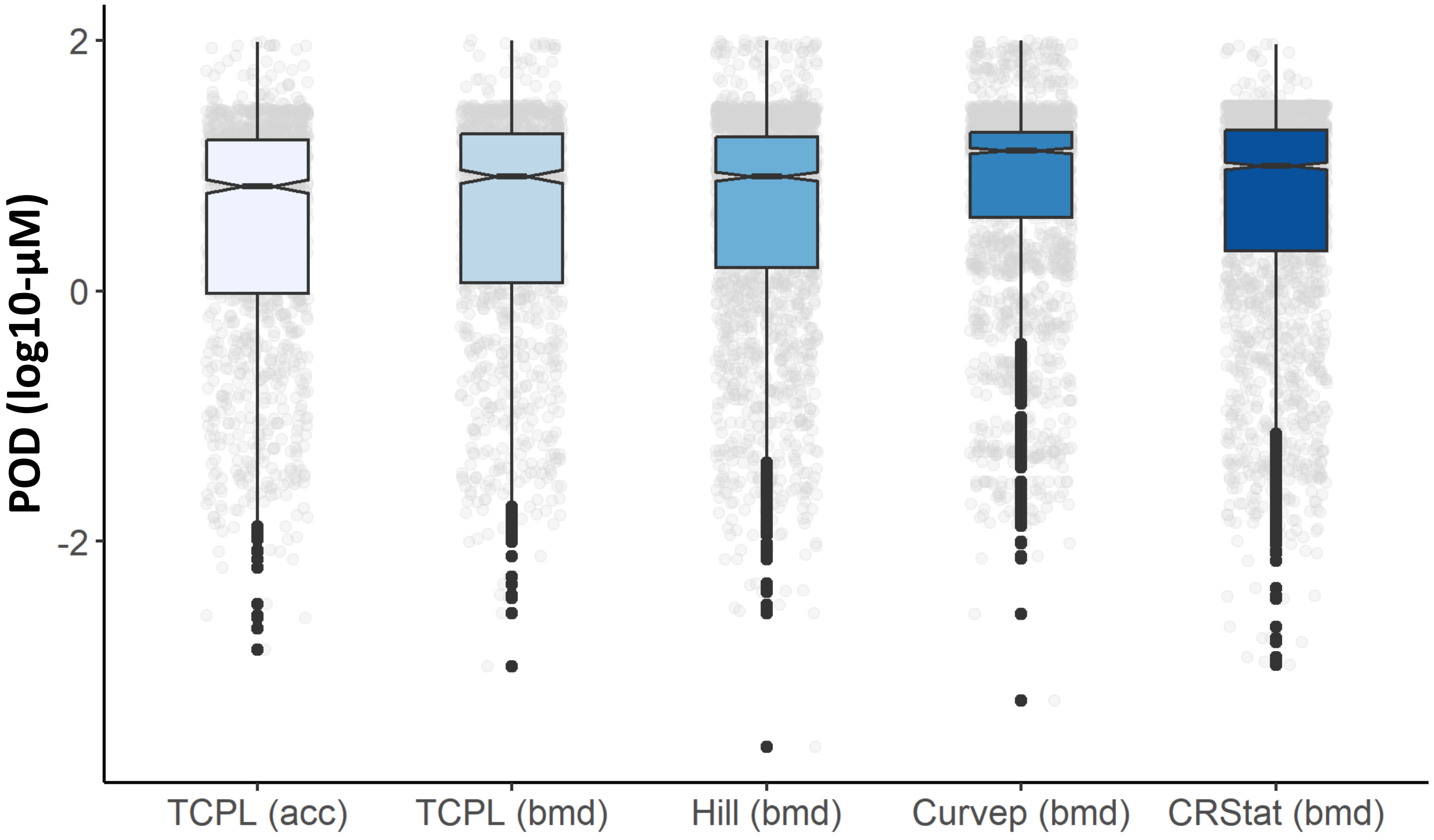


Note: Only showing comparisons between activity threshold cutoffs below 100%.

Legend:
Color indicates DNT endpoints

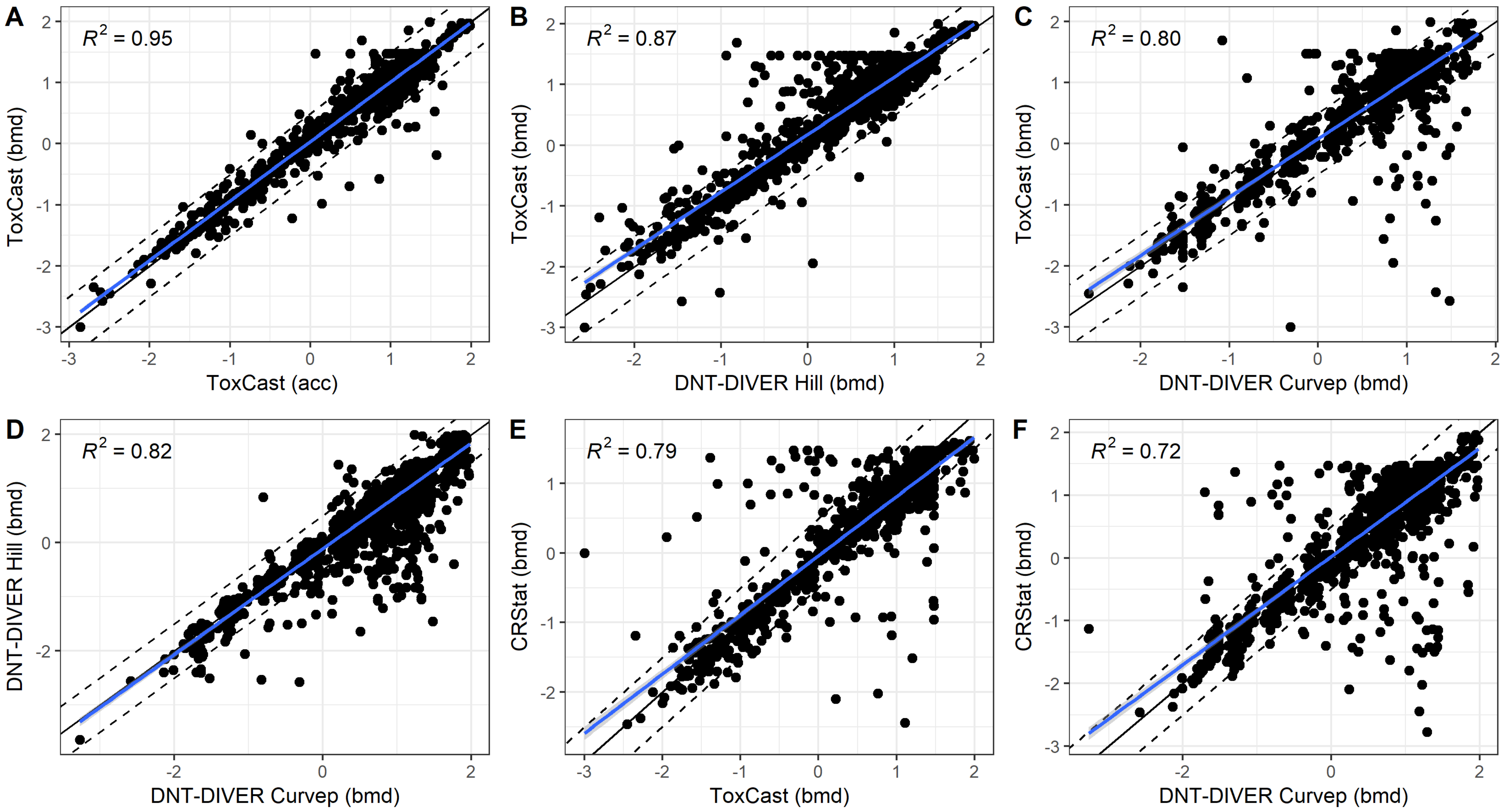
POD Comparison by Pipeline

POD: point of departure
BMR : benchmark response
BMD : benchmark dose
BMAD : Baseline median absolute deviation
SD : standard deviation
ACC : concentration at cutoff



Pipeline	Mean	SD
ToxCast (acc)	0.51	0.93
DNT-DIVER Hill (bmd)	0.58	0.92
ToxCast (bmd)	0.60	0.88
CRStat (bmd)	0.67	0.9
DNT-DIVER Curvep (bmd)	0.79	0.79

POD Comparison by Pipeline and Chemical x Endpoint level



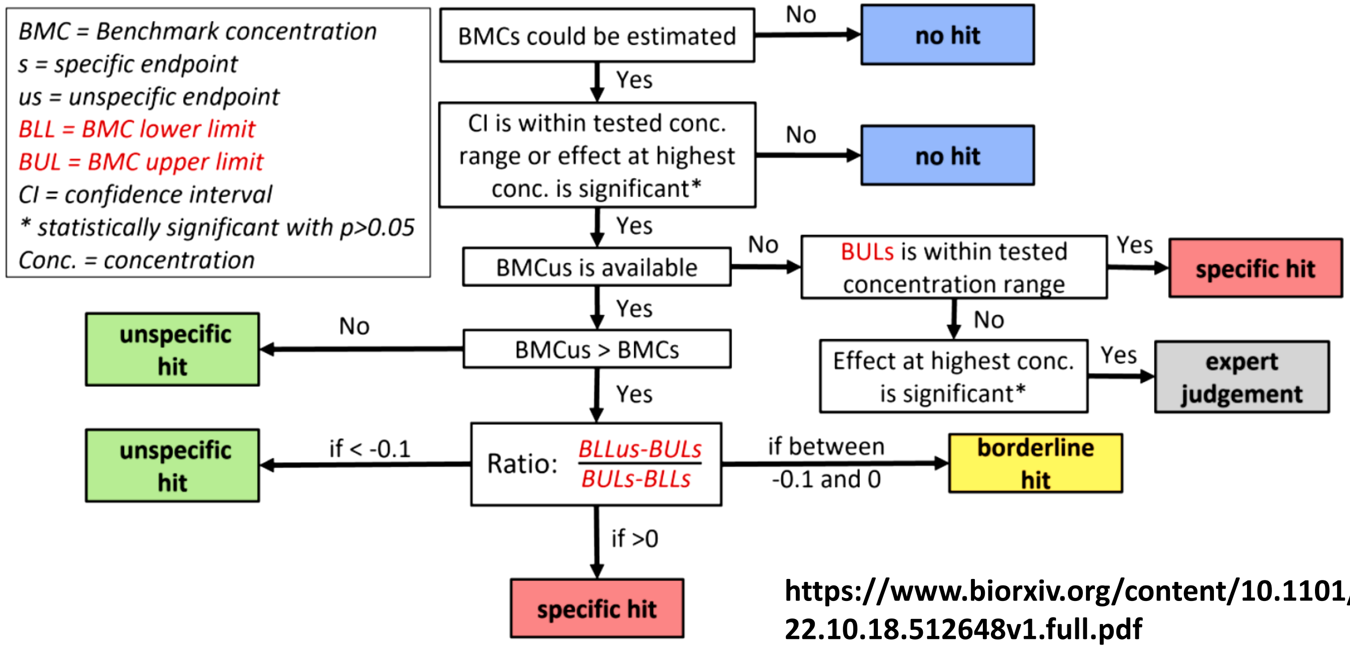
Classification Methods

Classification: specific hit versus unspecific hit

Specific hit: activity occuring below the threshold of cytotoxicity

Unspecific hit: activity occuring above the threshold of cytotoxicity

Method 1- CRStats Decision Tree



https://www.biorxiv.org/content/10.1101/2022.10.18.512648v1.full.pdf

Method 2- Selectivity Score

$$Selectivity\ Score = Cytotoxicity\ potency\ value\ (log10\ AC50\ \mu M) - Endpoint\ potency\ ((log10\ AC50\ \mu M)$$

Selectivity Score > 0.3 likely indicates a specific hit

https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0054

Classification Results on a ‘Chemical x Endpoint’ Level

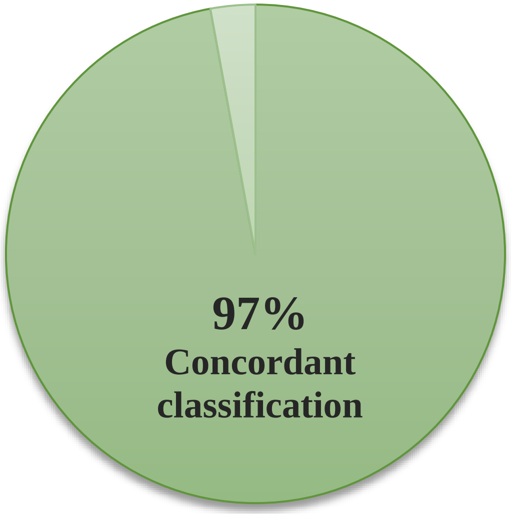
Data: DNT NAMs data pipelined in the same pipeline (CRStats) comprising 143 chemicals total.

		Method 2: Selectivity Score		
		specific hit	unspecific hit	no hit
Method 1: CRStats	specific hit	310	31	0
	unspecific hit	31	781	0
	no hit	0	0	1391
	borderline	9	3	0

Classification: specific hit versus unspecific hit

Specific hit: activity occuring below the threshold of cytotoxicity

Unspecific hit: activity occuring above the threshold of cytotoxicity



81% of ‘specific hits’ were concordant between the two methods
(310/381 total ‘specific hits’ classified by either pipeline).

100% of ‘no hits’ were concordant between the two methods
(1391/1391 total ‘no hits’ classified by either pipeline).

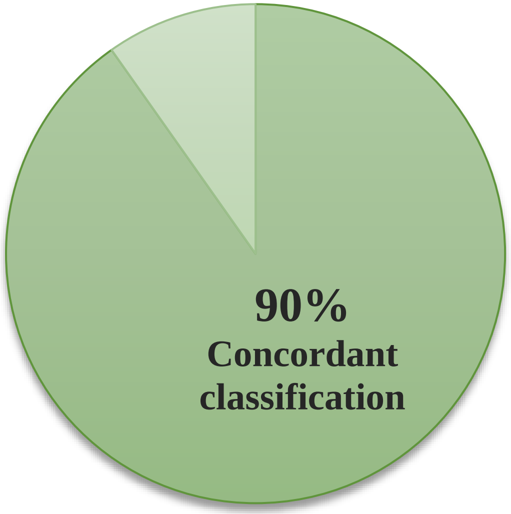
Classification Results on a Chemical Level (143 chemicals total)

		Method 2: Selectivity Score		
		specific hit	unspecific hit	no hit
Method 1: CRStats	specific hit	76	5	0
	unspecific hit	7	40	0
	no hit	0	2	13

Classification: specific hit versus unspecific hit

Specific hit: activity occuring below the threshold of cytotoxicity

Unspecific hit: activity occuring above the threshold of cytotoxicity



86% of 'specific hit' chemicals were concordant between the two methods

(76/88 total 'specific hits' chemicals classified by either pipeline).

87% of 'no hit' chemicals were concordant between the two methods

(13/15 total 'no hits' chemicals classified by either pipeline).

Conclusions

- Of the 5313 curves of DNT NAMs data, 73% demonstrated concordant hit calls across three different concentration-response pipelines (ToxCast, DNT-DIVER, and CRStats) indicating that **the pipeline can impact the interpretation of DNT NAMs activity**.

- Discordant curves between pipelines appeared to be associated with:
  - Borderline activity (maximal activity occuring near the activity cutoff threshold)
  - Noisy data
  - Differences in activity cutoff thresholds between pipelines

- ToxCast demonstrated the fewest hit calls that were active in a single pipeline suggesting that ToxCast has a higher threshold for hit calling compared to CRStats and DNT-DIVER.

- Despite notable differences in defining the activity threshold cutoff, each pipeline appeared to demonstrate a similar distribution of values, with a few exceptions from the ToxCast BMR (1SD*1.349)

- The ToxCast pipeline ACC (concentration at cutoff) demonstrated the lowest global POD (mean POD across all active curves) compared to other pipelines.

- Two classification methods demonstrated 90% concordance in classifying chemicals as DNT specific, nonspecific, or inactive. The 'Selectivity Score' method and CRStats method identified 7 and 5 'specific' chemicals, respectively, that did not agree between methods, suggesting that **the classification model can impact the interpretation of a DNT chemical**, despite being processed in the same pipeline.

Acknowledgments

Jui-hua Hsieh[1]
Arif Donmez[2]
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Office: 919-541-3834

Pipeline Resources:

https://github.com/ArifDoenmez/CRStats.git

https://github.com/USEPA/CompTox-ToxCast-tcpl.git

https://github.com/ArifDoenmez/CRStats.git