USING HIGH THROUGHPUT SCREENING FOR PREDICTIVE MODELING OF REPRODUCTIVE TOXICITY

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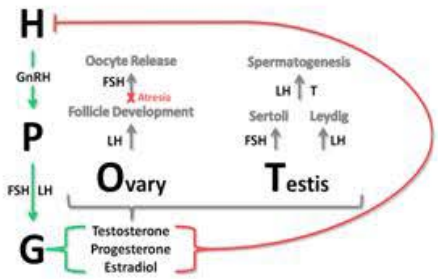
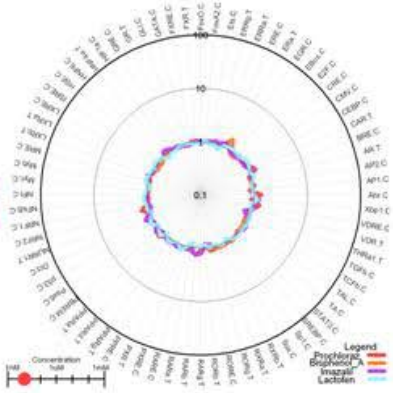
National Center for Computational Toxicology

Overview

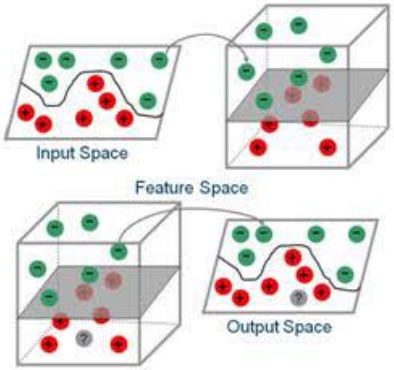
Reproductive Physiology & Impairment



Transcription Factor Profiling

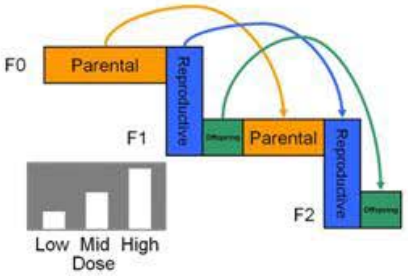


Classification Modeling of Toxicity

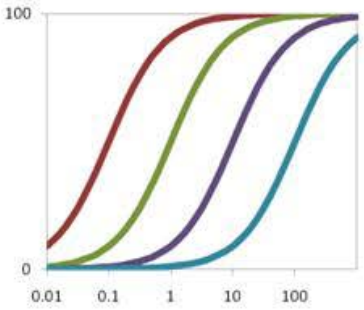


Forward Validation Study

Reproductive Toxicity Testing

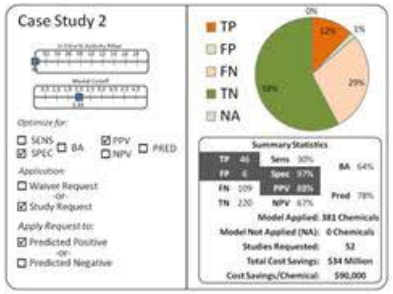


Quantitative High Throughput Screening



Predictive Model of Reproductive Toxicity

Case Studies in Testing Prioritization



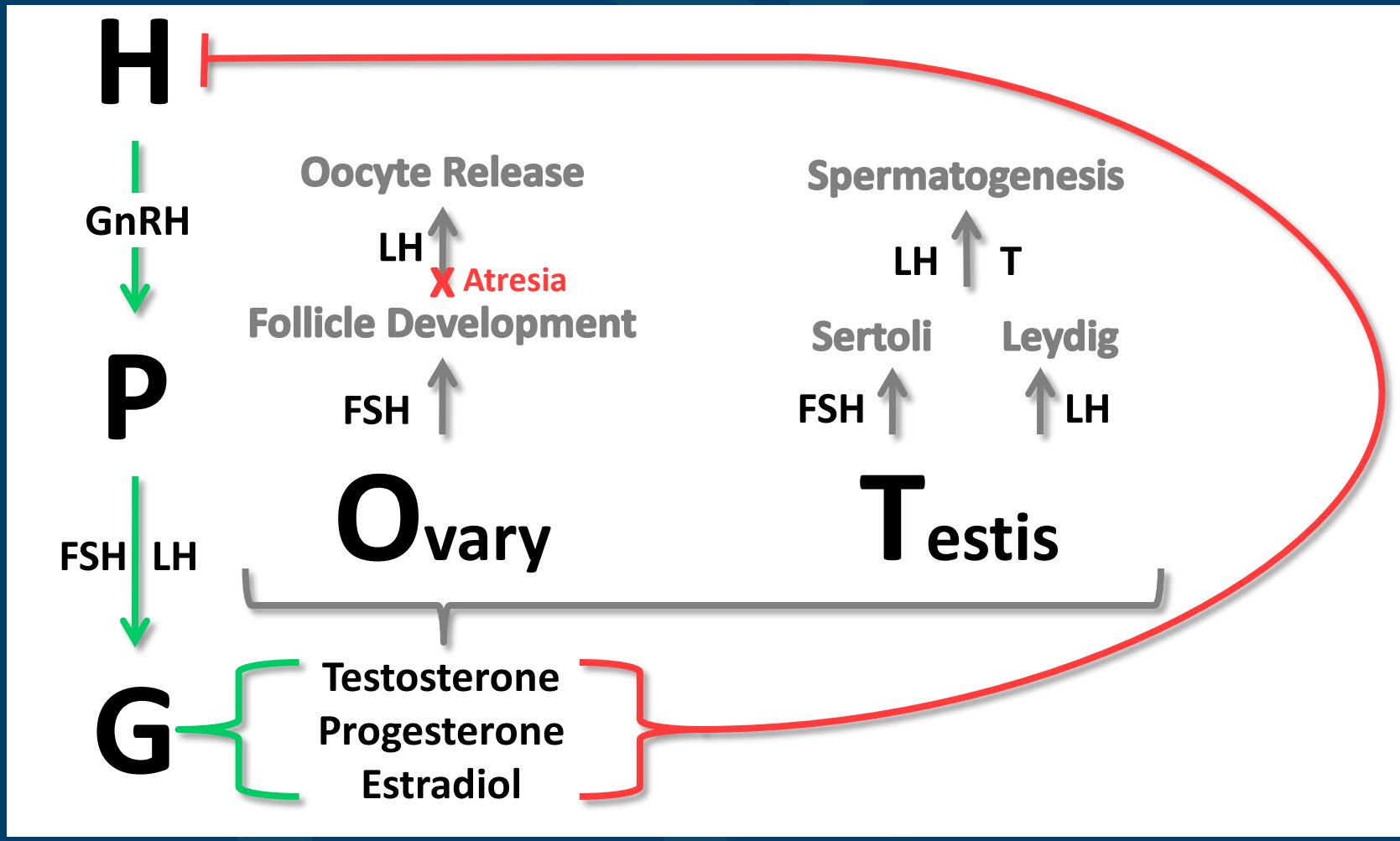
In Vivo | In Vitro | Modeling | Implementation

Overview of ToxRefDB & ToxCast

- ToxRefDB
  - Publically available relational database developed to capture and store traditional animal toxicity studies
  - Cancer, Reproductive and Developmental studies on 820 chemicals captured to date
  - Primary anchor for predictive modeling research
- ToxCast
  - High throughput data generating research program aimed at using bioactivity fingerprints to predict toxicity and apply to chemical testing decision making
  - Phase I: 309 data rich chemicals tested in over 600 assays and used to develop predictive models and serve as a proof-of-concept
  - Phase II: 700-1000 additional chemicals tested in potentially 1000 assays and used to validate, expand, and apply predictive models of toxicity

Reproductive Physiology & Impairment

Reproductive Physiology

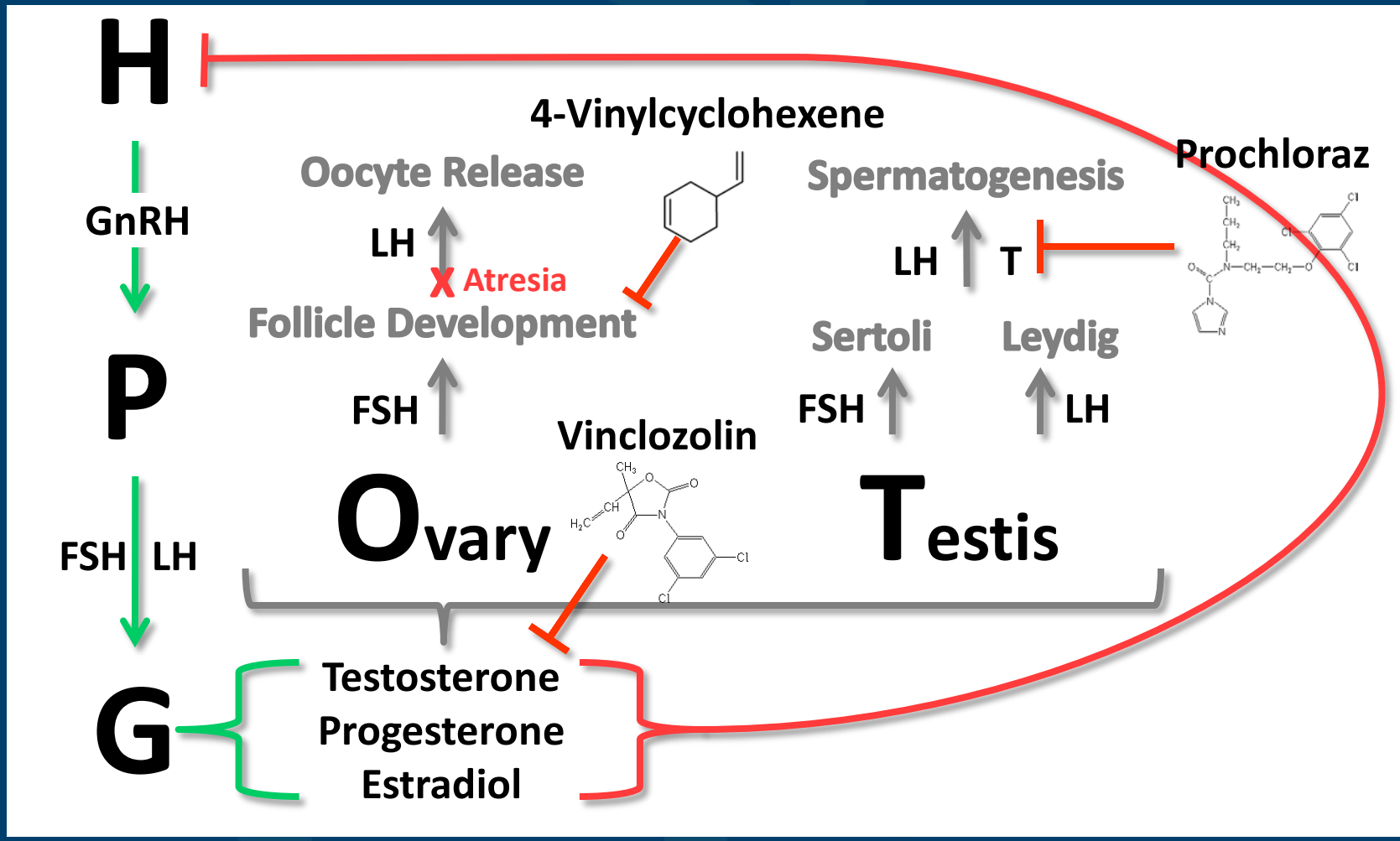


AXIS

H - Hypothalamus
P - Pituitary
G - Gonad

GnRH - Gonadotropin-releasing hormone
FSH - Follicle stimulating hormone
LH - Lutenizing hormone

Toxicology
~~Reproductive Physiology~~



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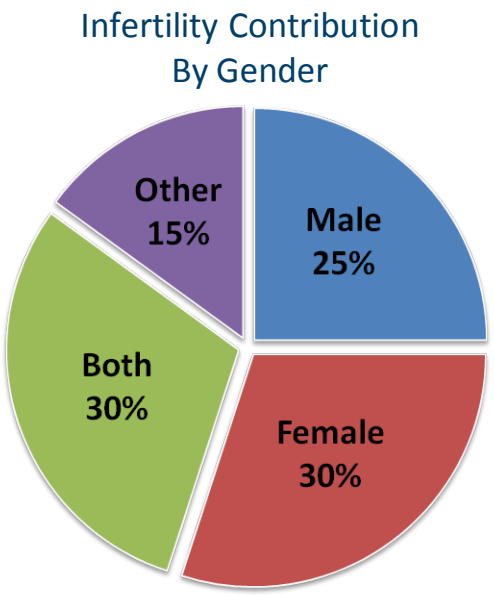
Human Reproductive Impairment & Toxicity

- 10-20% of couples sub-fertile or infertile
- Reproductive impairment
  - Male infertility
    - Generally sperm-related, but can be neuromuscular
  - Female infertility
    - Complex; many etiologies
  - Impaired fecundity (e.g., miscarriage)
    - 25% of pregnancies lost prior to clinical recognition



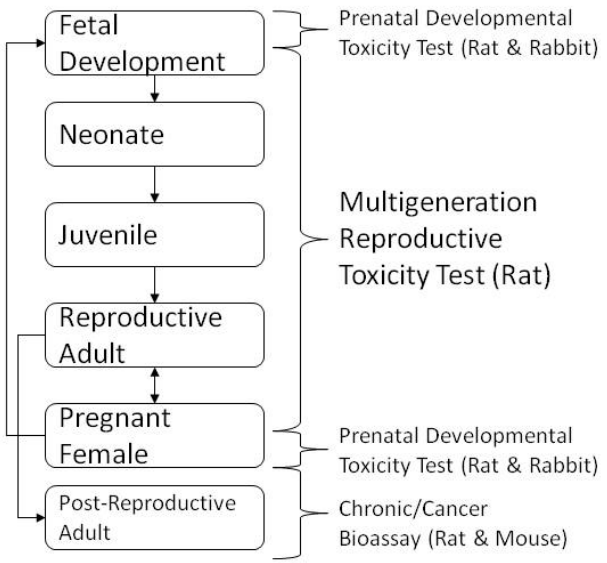
- Environmental contribution
  - Unknown, but links exist
    - Strong: Smoking, obesity, traffic exhaust, dioxins, combustion products
    - Suggestive: Pesticides, food additives, persistent pollutants, PCBs, PFAAs
  - Many study confounders
    - Age, lifestyle, disease background, sample size, privacy, endpoint selection

NO ROUTINE WAY TO ASSESS CHEMICALLY-INDUCED HUMAN REPRODUCTIVE TOXICITY POTENTIAL

Reproductive Toxicity Testing

Reproductive Toxicity Testing

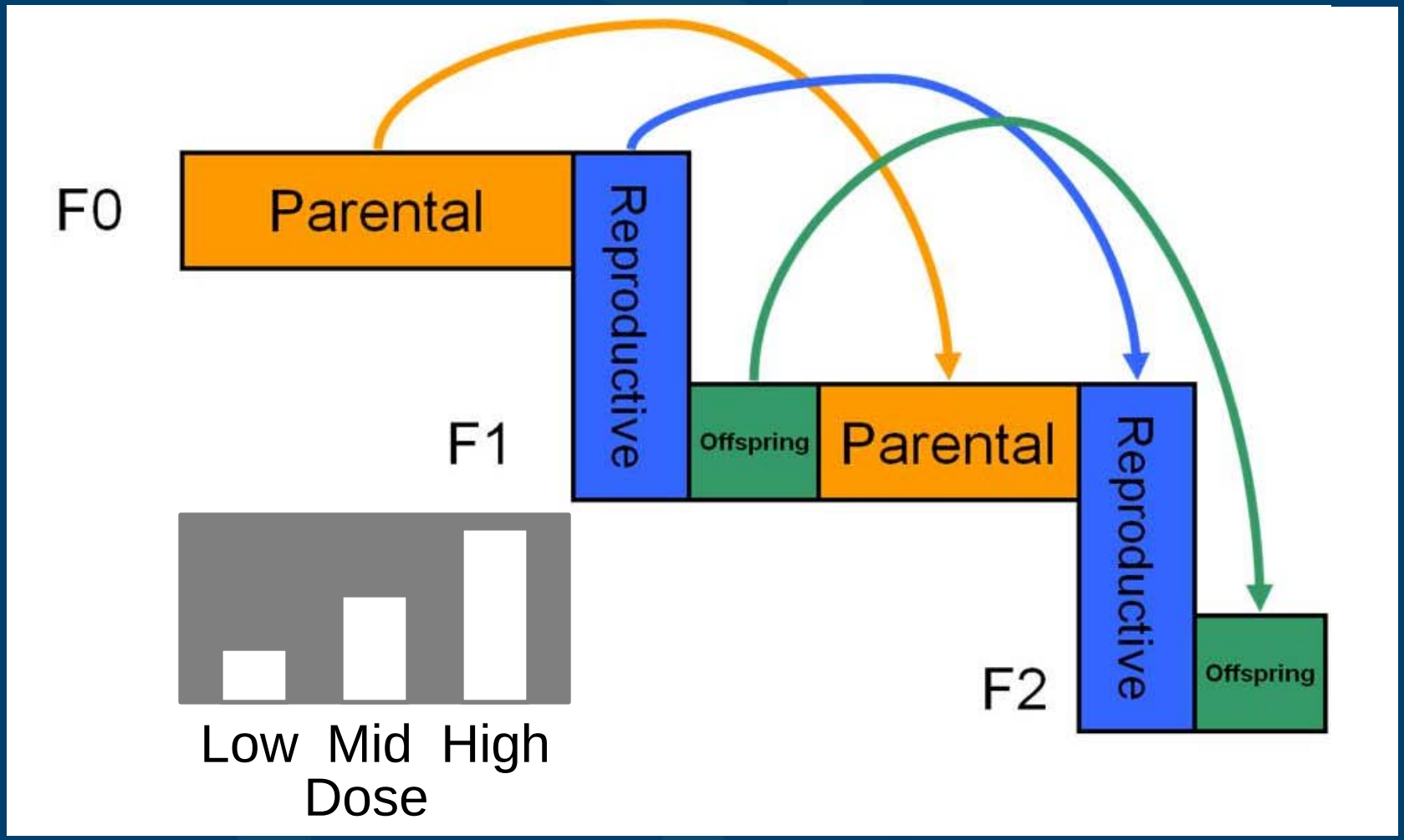
- Regulatory testing protocols
  - Environmental chemicals
    - **Multigeneration reproductive toxicity study (MGR)**
    - Continuous-breeding protocol
    - Extended one-generation reproductive toxicity study (EOGRTS)
  - Pharmaceuticals
    - Fertility study (Segment I)
    - Peri- & Post-natal toxicity study (Segment III)



- Additional reproductive test methods
  - Reproductive tissue culture systems
  - In vivo endocrine assays: Hershberger, uterotrophic, pubertal
  - In vitro endocrine assays: Receptor binding, transcriptional or steroidal
  - Computational models: Structure-based or In Vitro batteries

  - **NO CURRENT VIABLE ALTERNATIVE TO PRIORITIZE OR REPLACE MGR STUDY**

Multigeneration Reproductive Toxicity Study (MGR)



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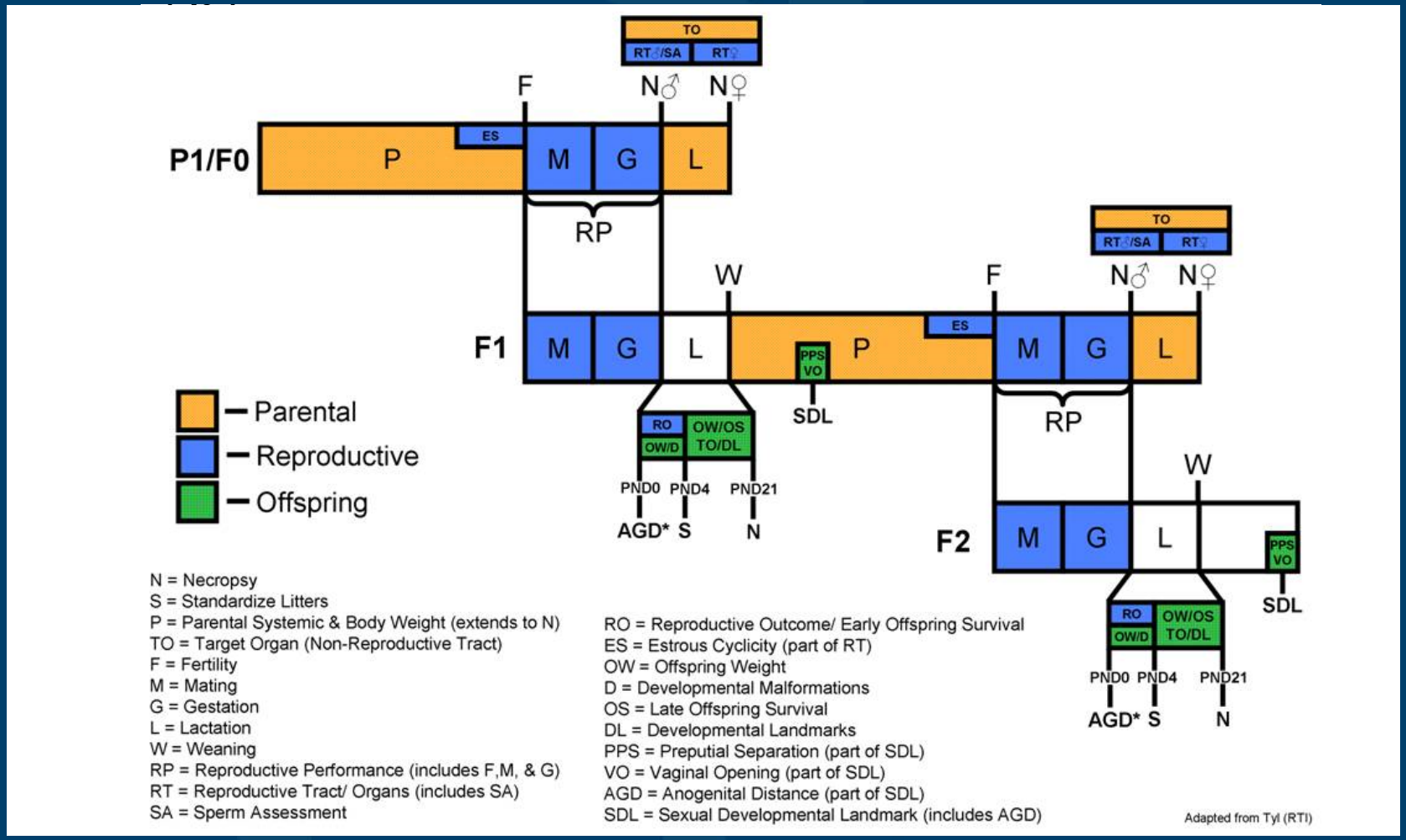
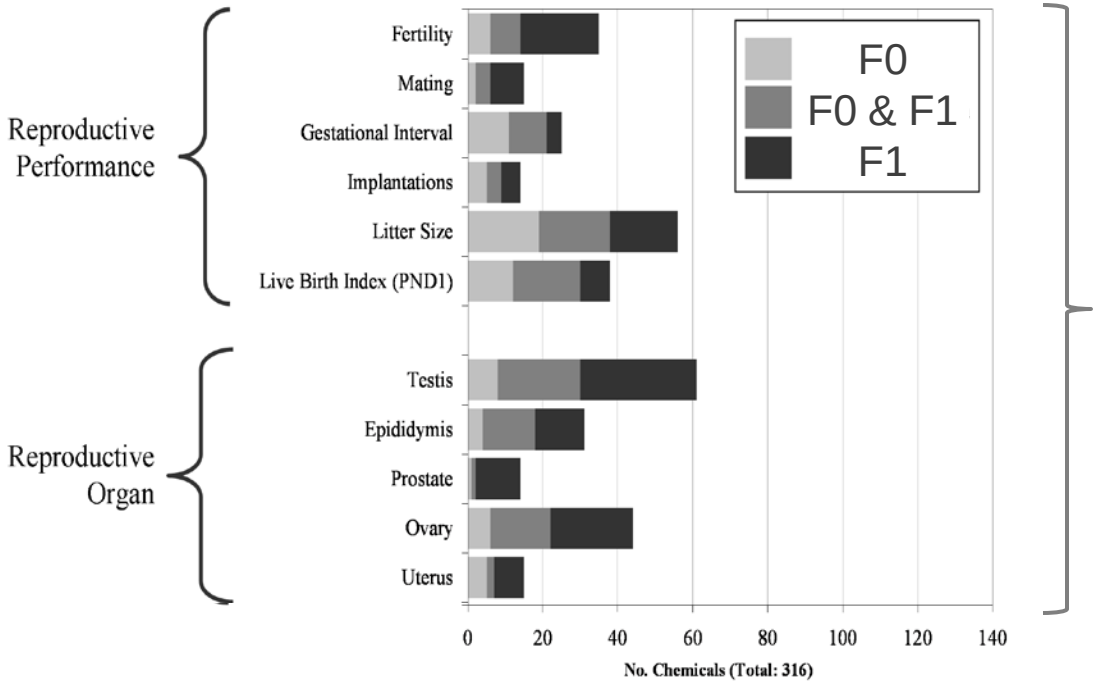


ToxRefDB
Capturing 30 years of Animal Toxicity Data

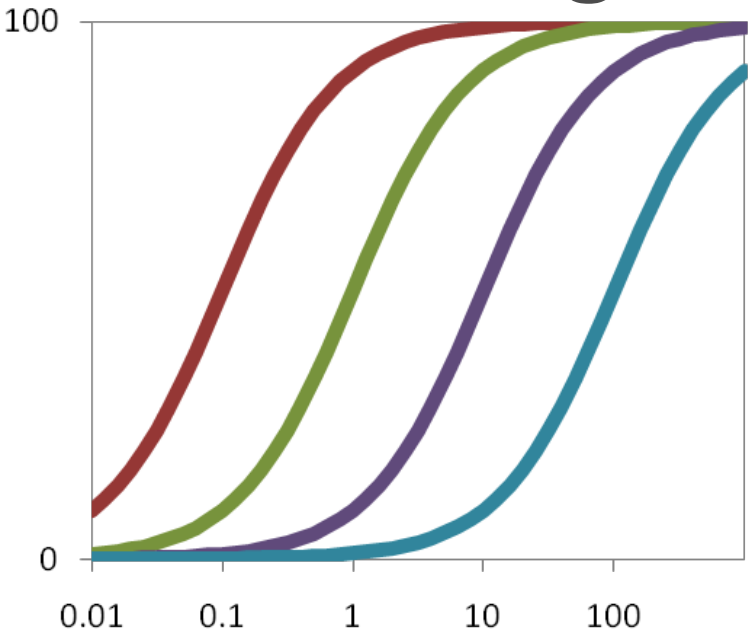
Multigeneration reproductive toxicity studies (MGR) in ToxRefDB

- As published in Martin et al. (2009): 316 chemicals entered
- As of July 2011: 416 chemicals entered
- 650 unique effects observed, 120 being unique reproductive effects
- Quantified universe of MGR studies & the inherent inefficiencies/deficiencies
- **Identified aggregated endpoint for predictive modeling**



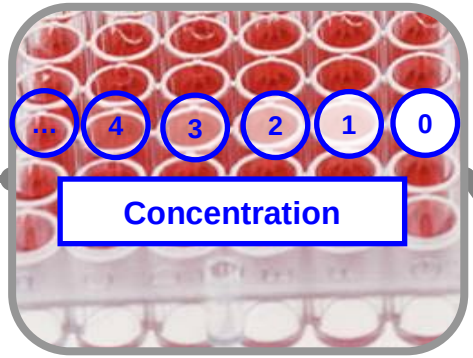
98 Chemicals w/ Reproductive LOAEL (rLOAEL)

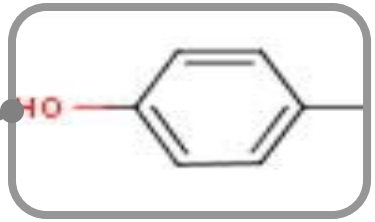
Quantitative High Throughput Screening



Quantitative High Throughput Screening

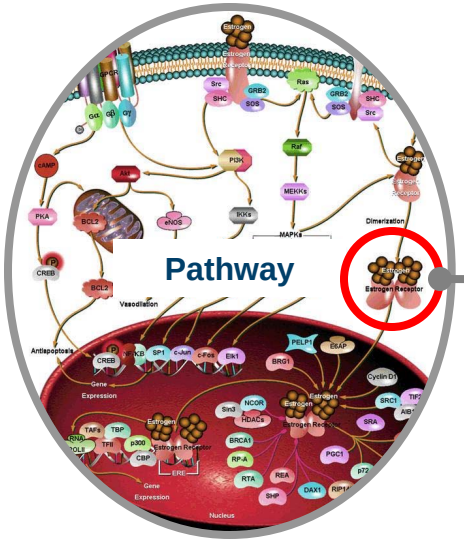
HTS Robotic Platform

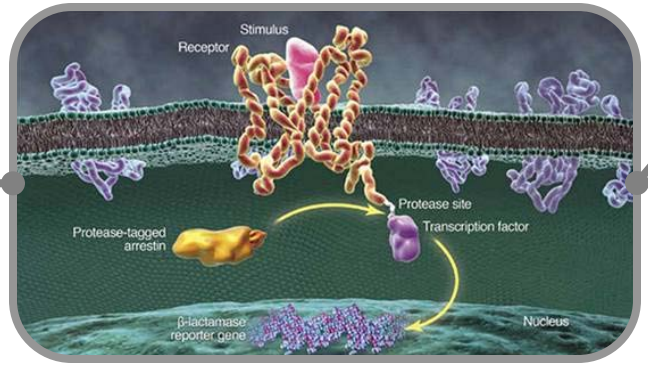




Chemical Exposure

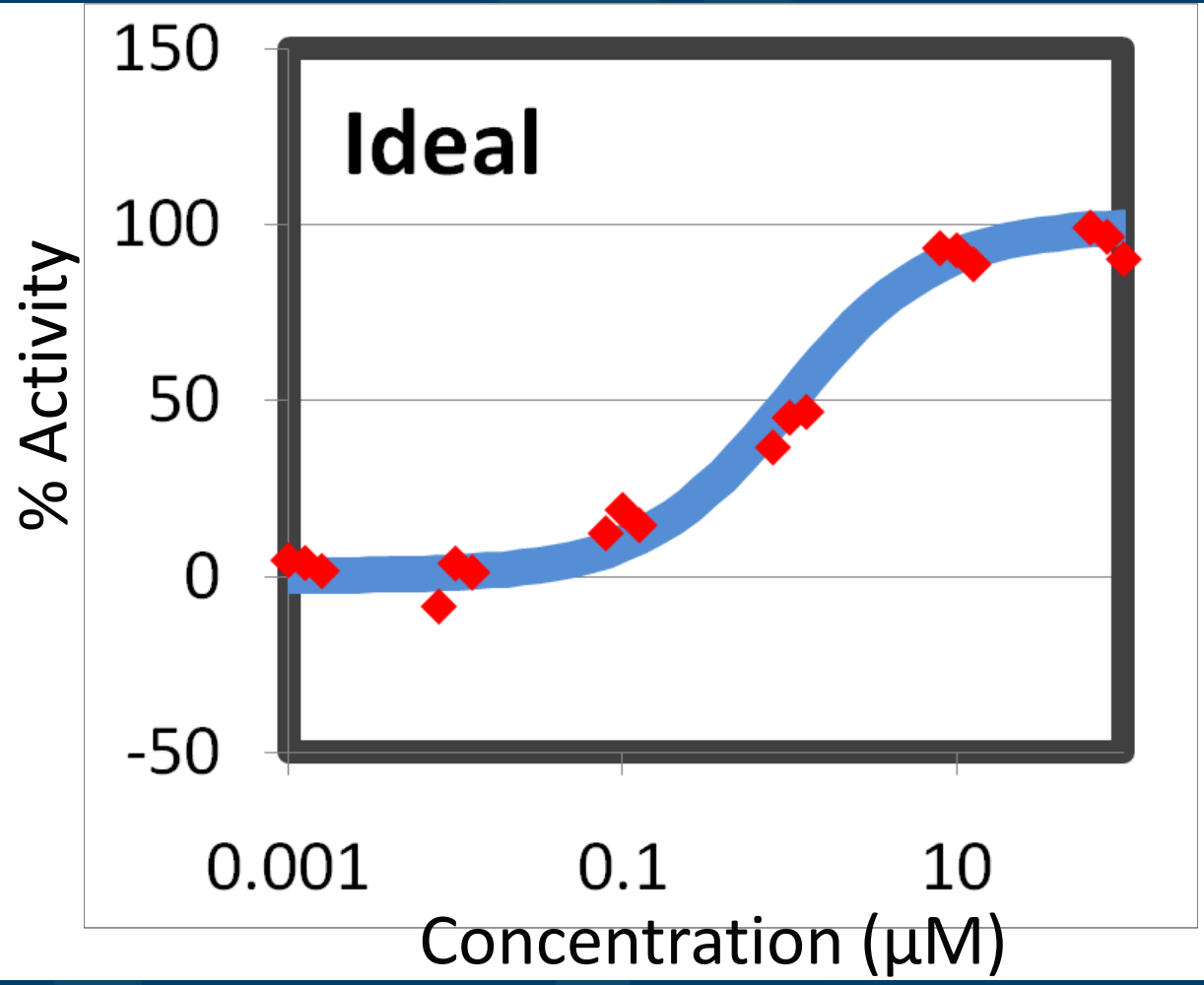
Cell Population





Assay Target Biology
(e.g., Estrogen Receptor)

ToxCast Data Analysis



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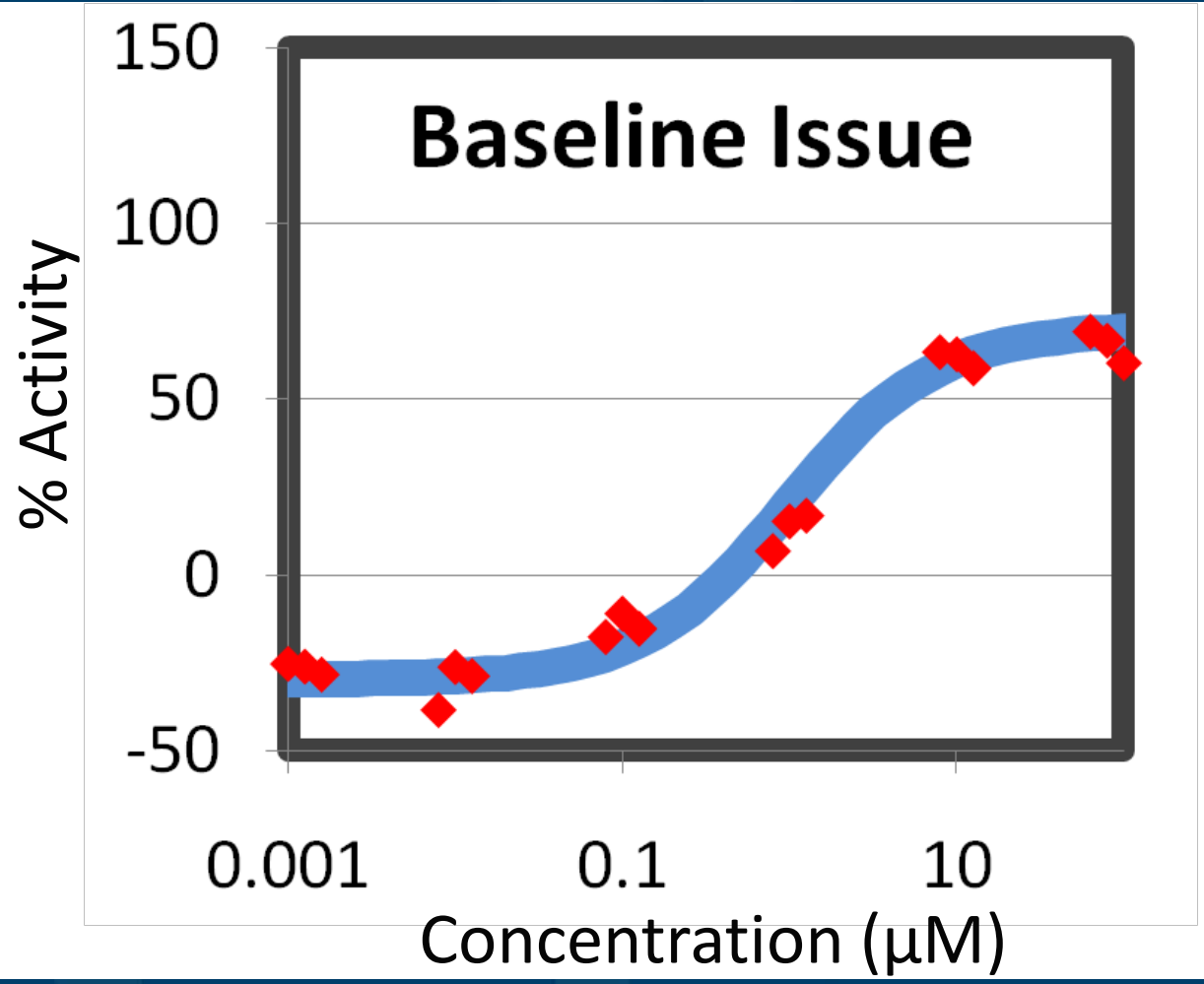

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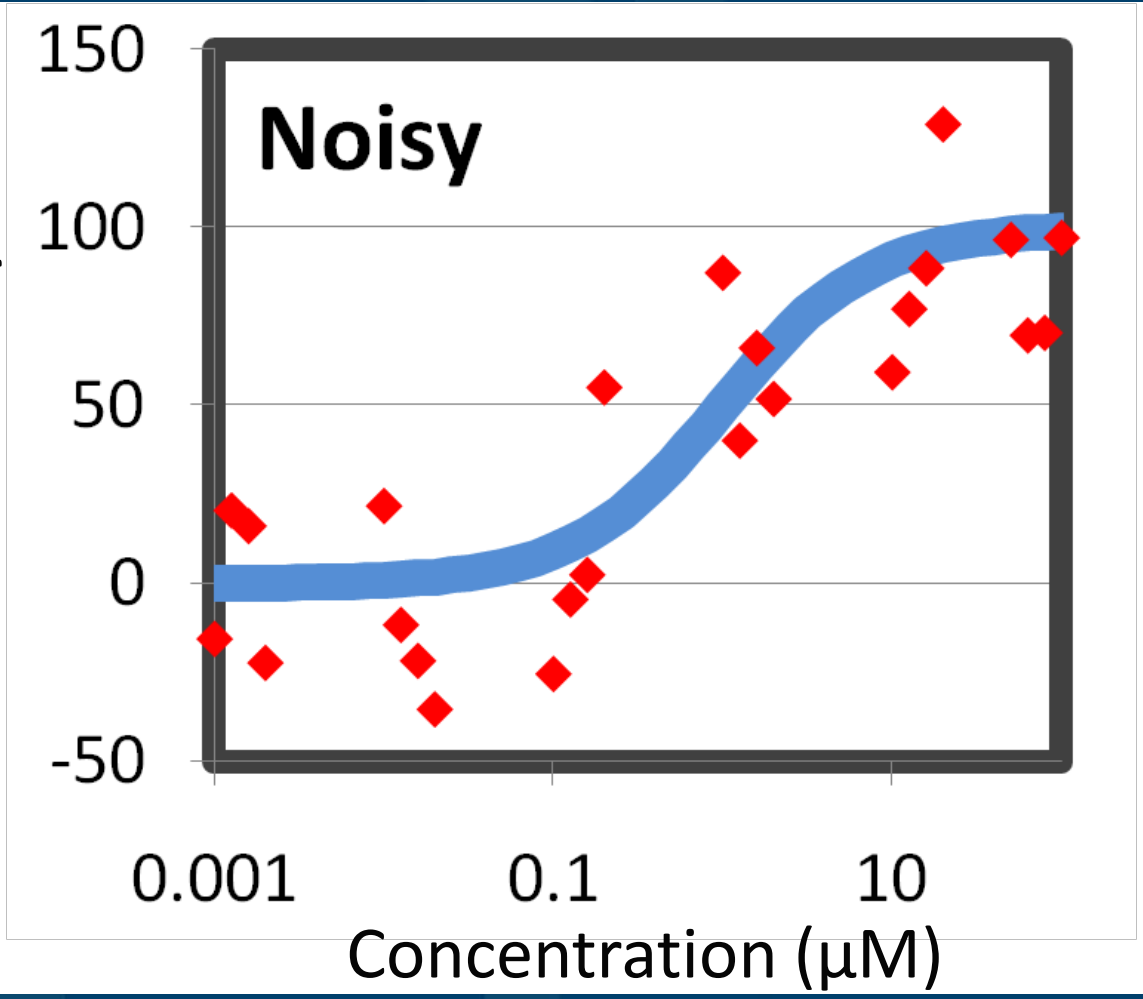

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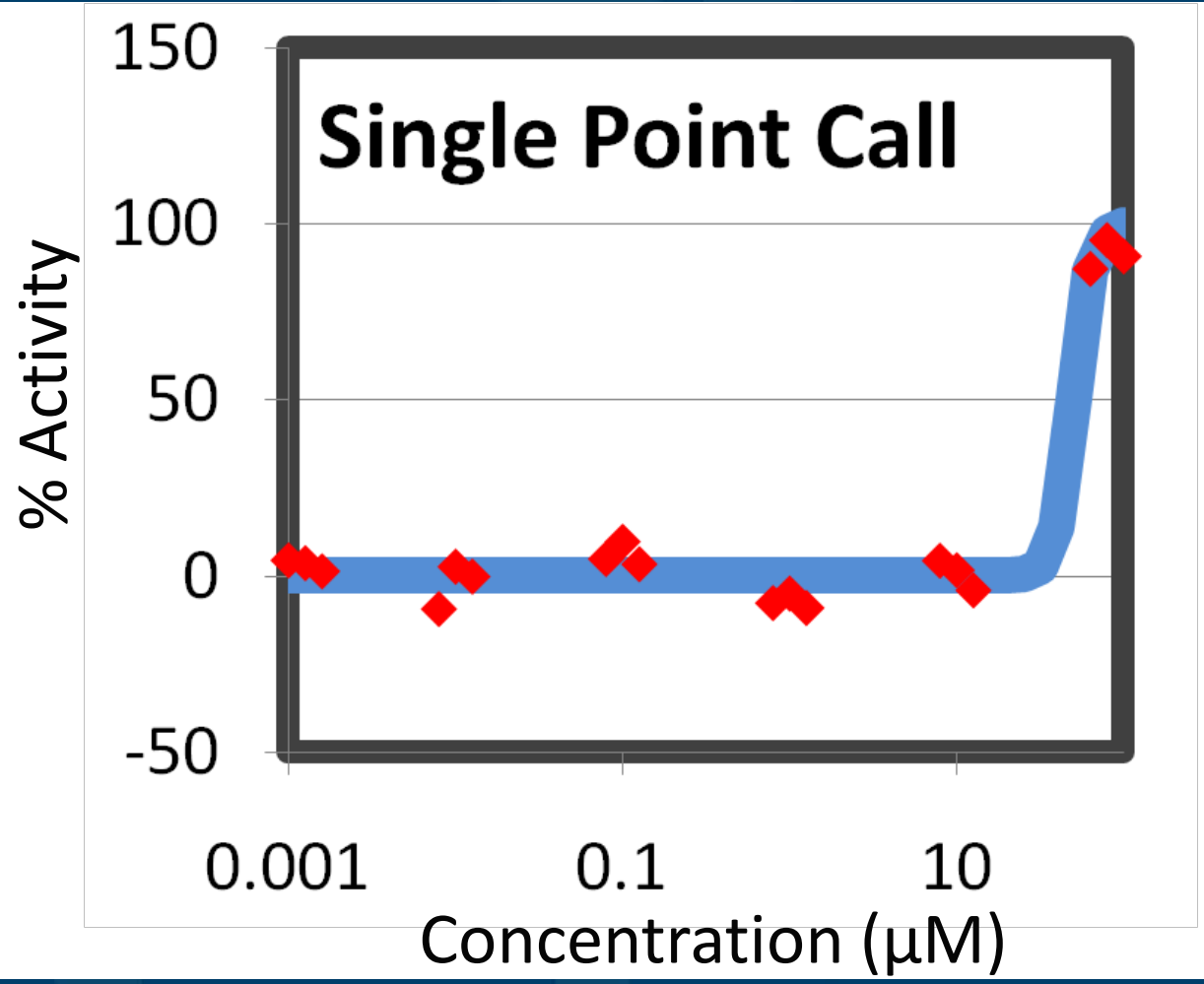

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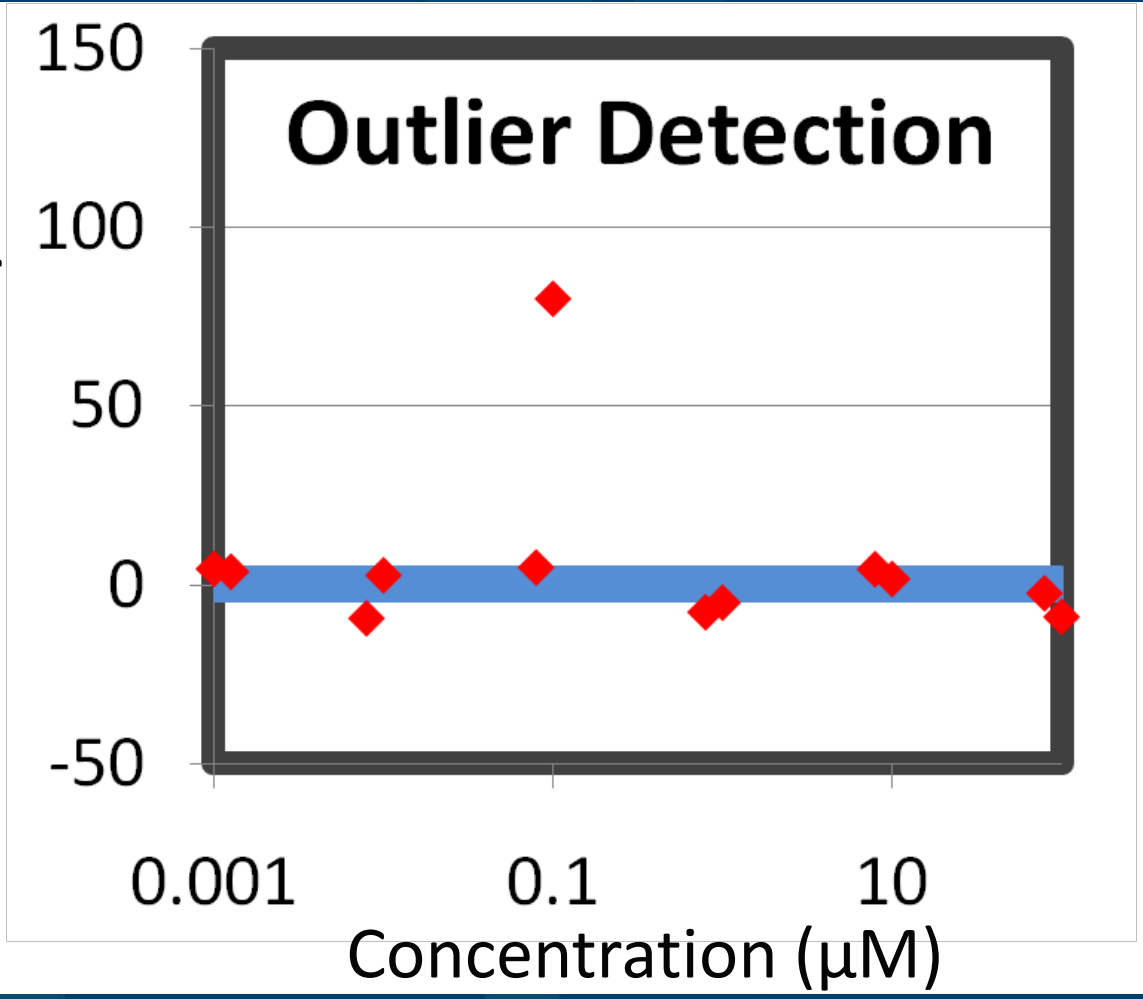

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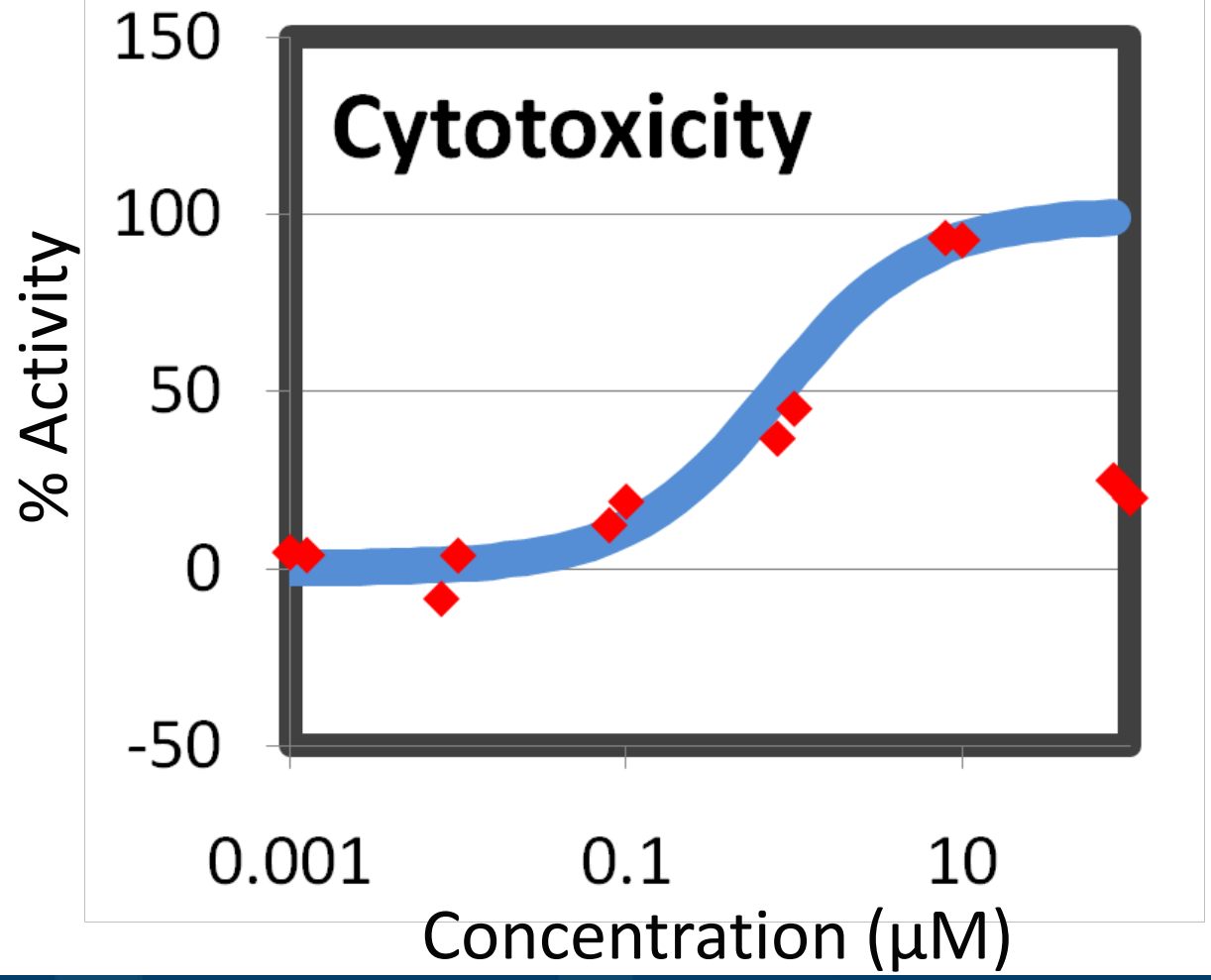

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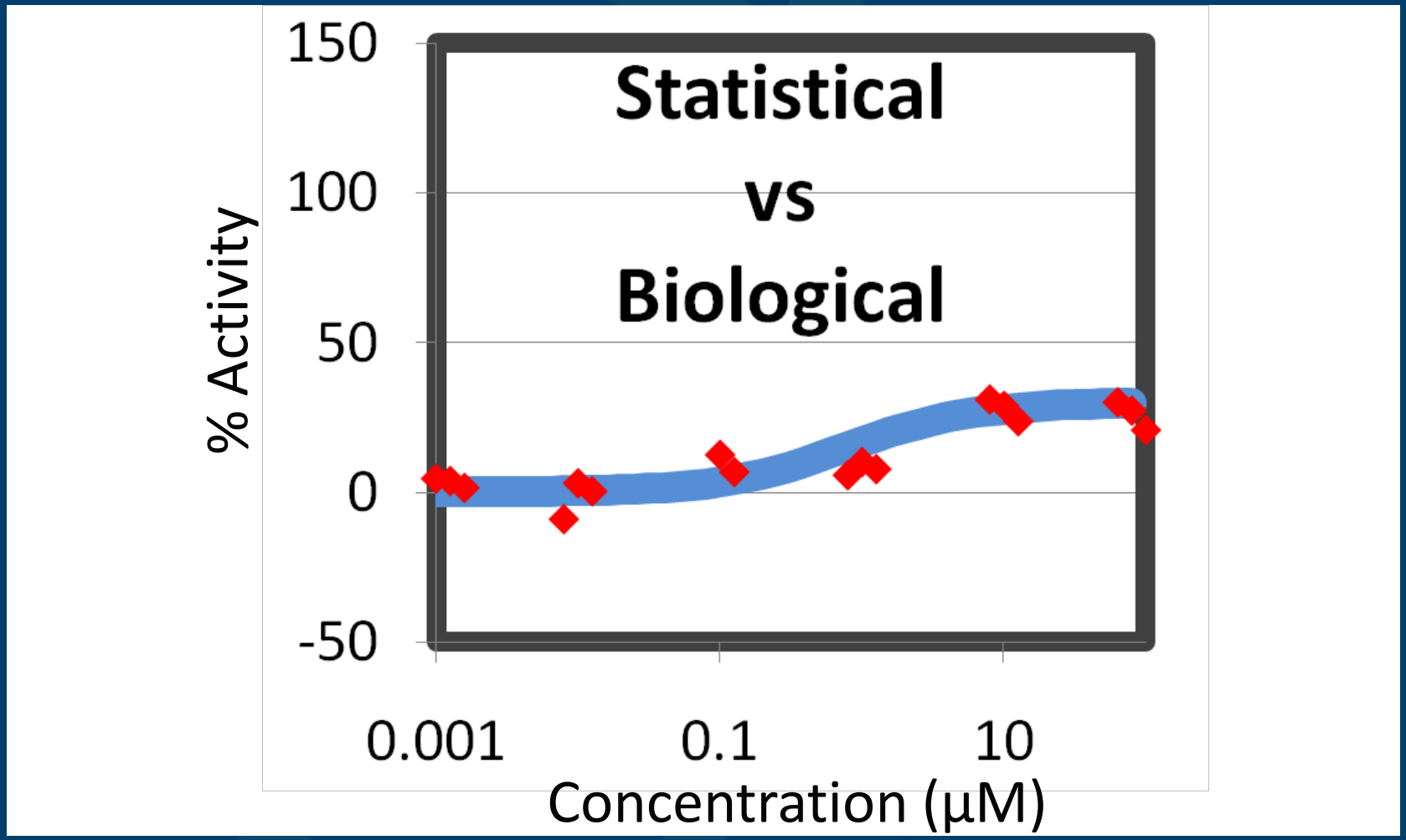
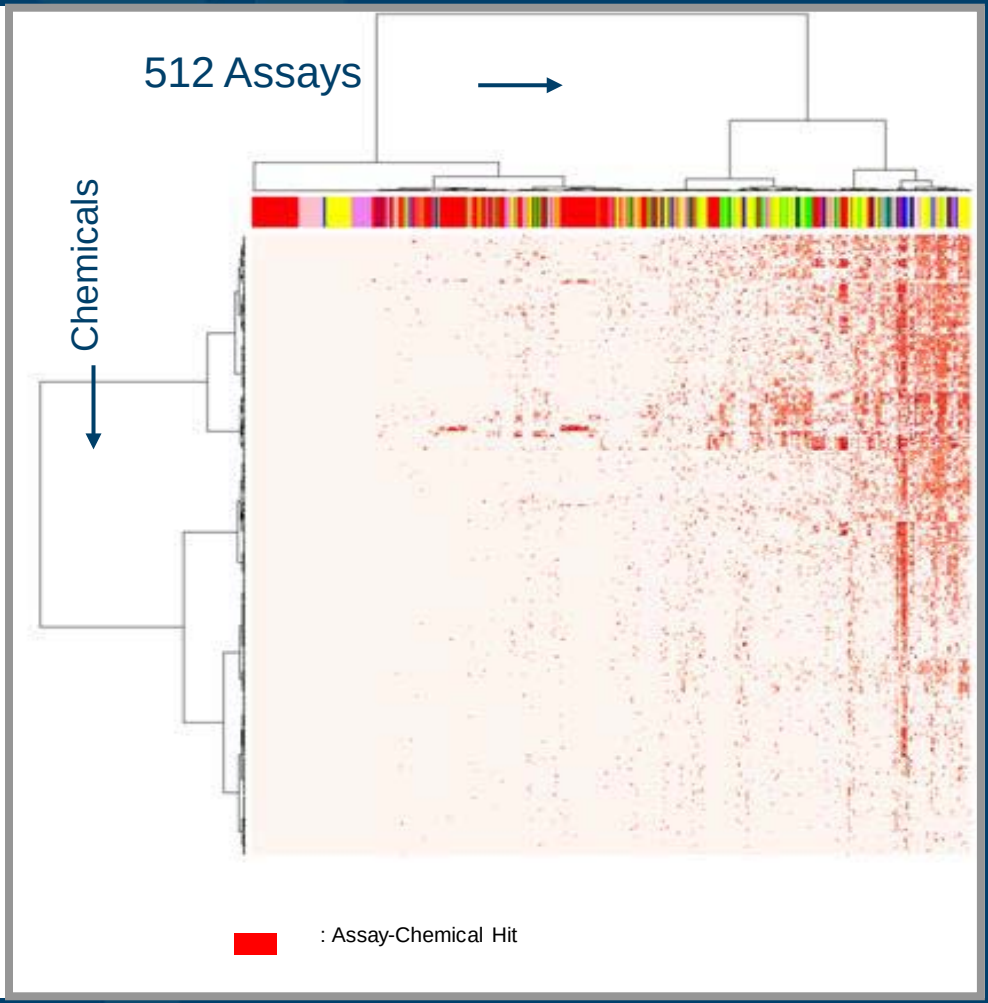


ToxCast Dataset Used In Modeling

- 8 Assay Technologies
- Commercially Available Cell-free & Cell-based Assays
- 5 Technologies Used In Modeling
- 512 Total Assays Used In Modeling
- Typically Run with Negative & Positive Control
- Run in Concentration Response Format
- >3 Million Data Points



Transcription Factor Profiling

Transcription Factor (TF) Profiling

– Complimentary readouts among 73 endpoints in 2 systems

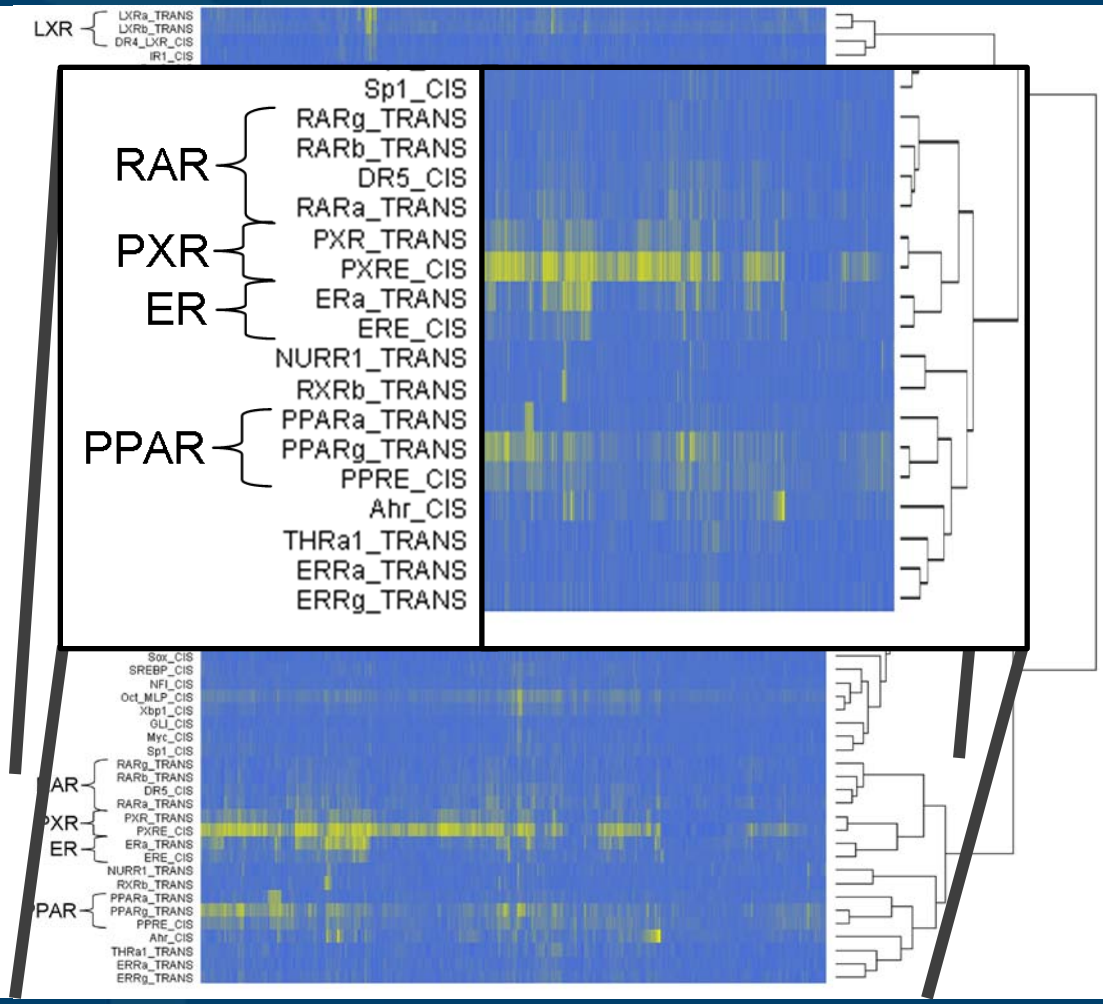
– CIS: Endogenous TF

– TRANS: Exogenous GAL4 reporter gene system

– Focus on nuclear receptors & oxidative stress pathways

– Reproducible data & highly sensitive

– Run on initial 309 chemicals and completed an additional ~700 chemicals



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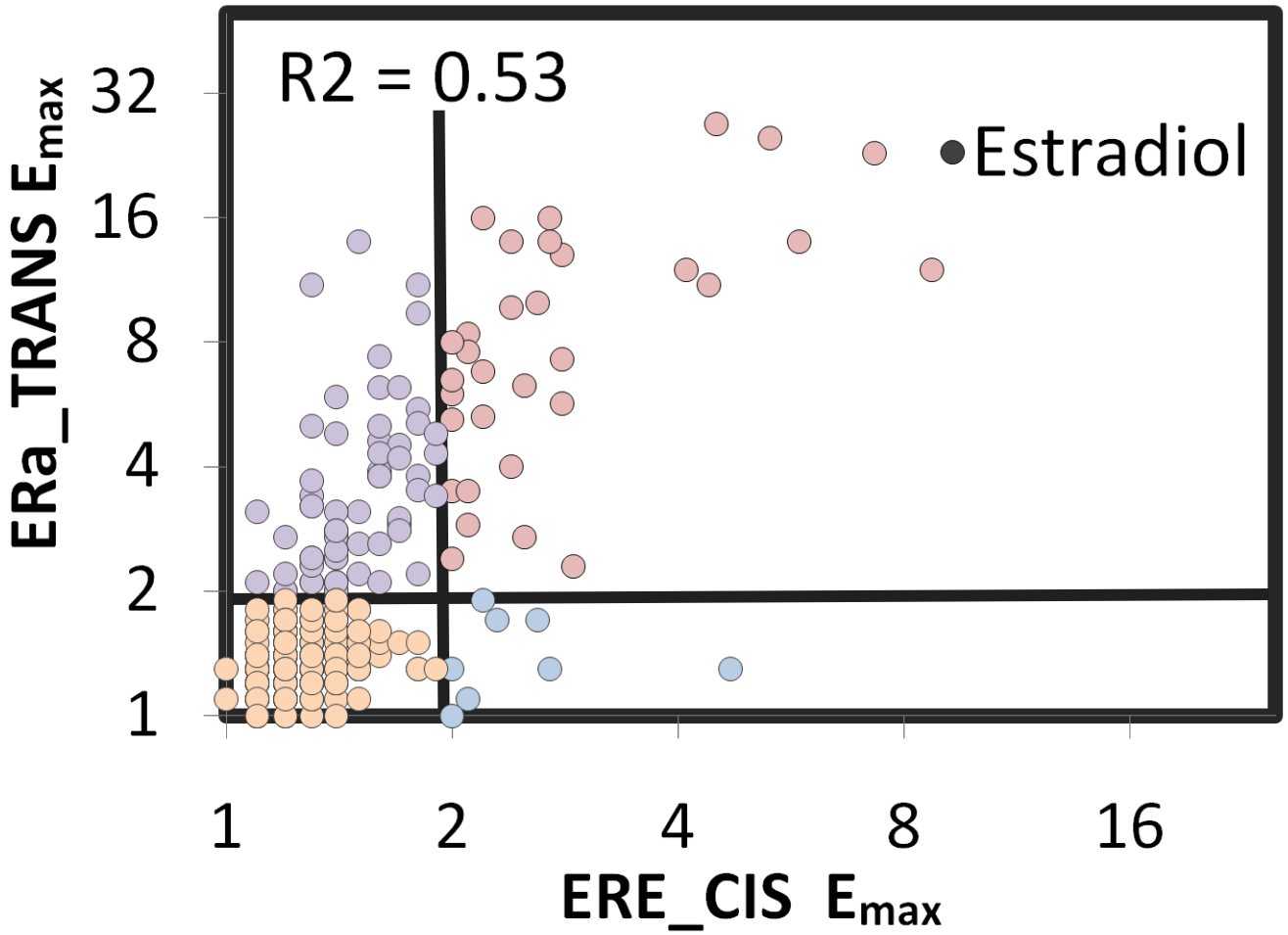

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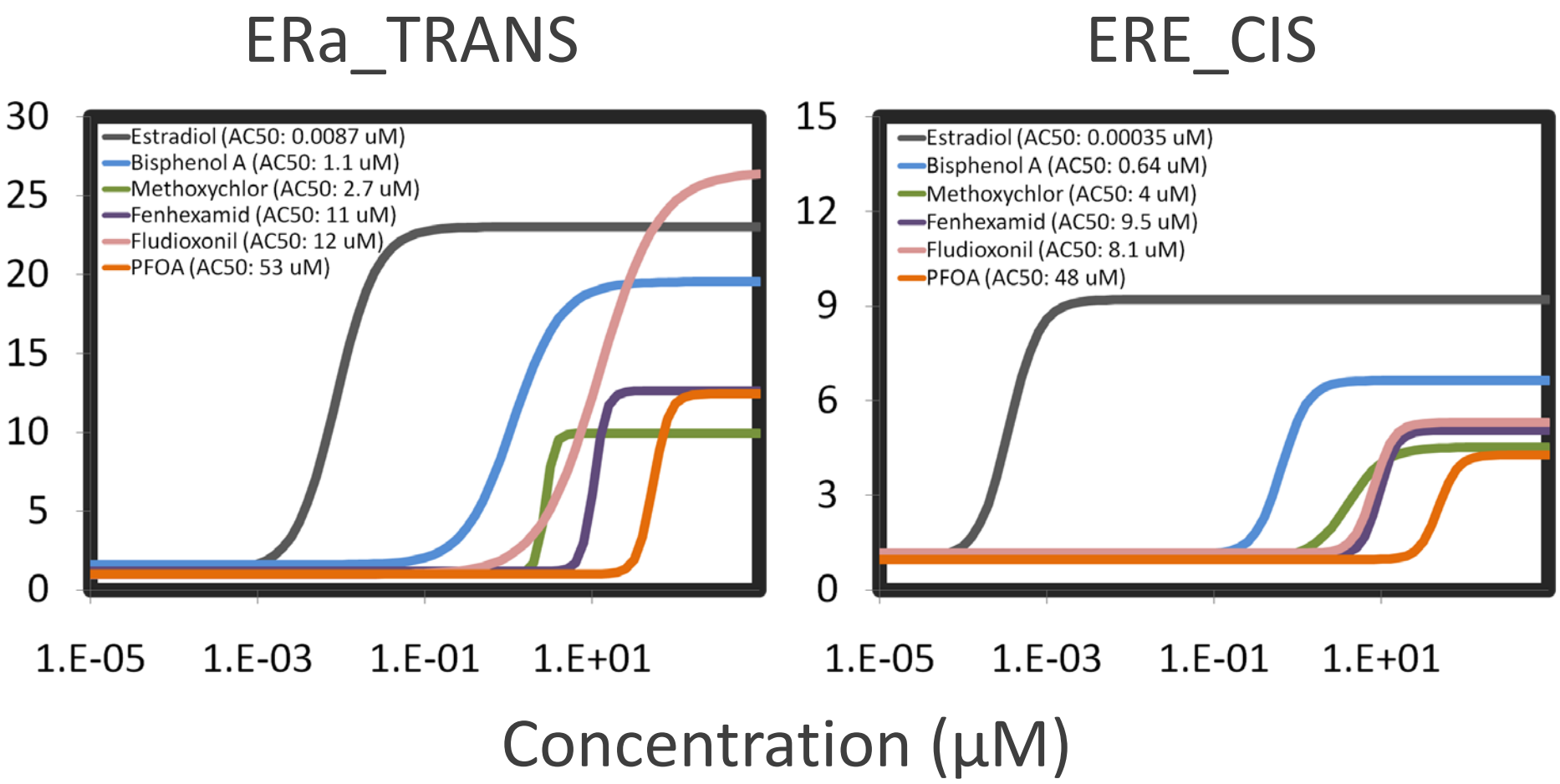

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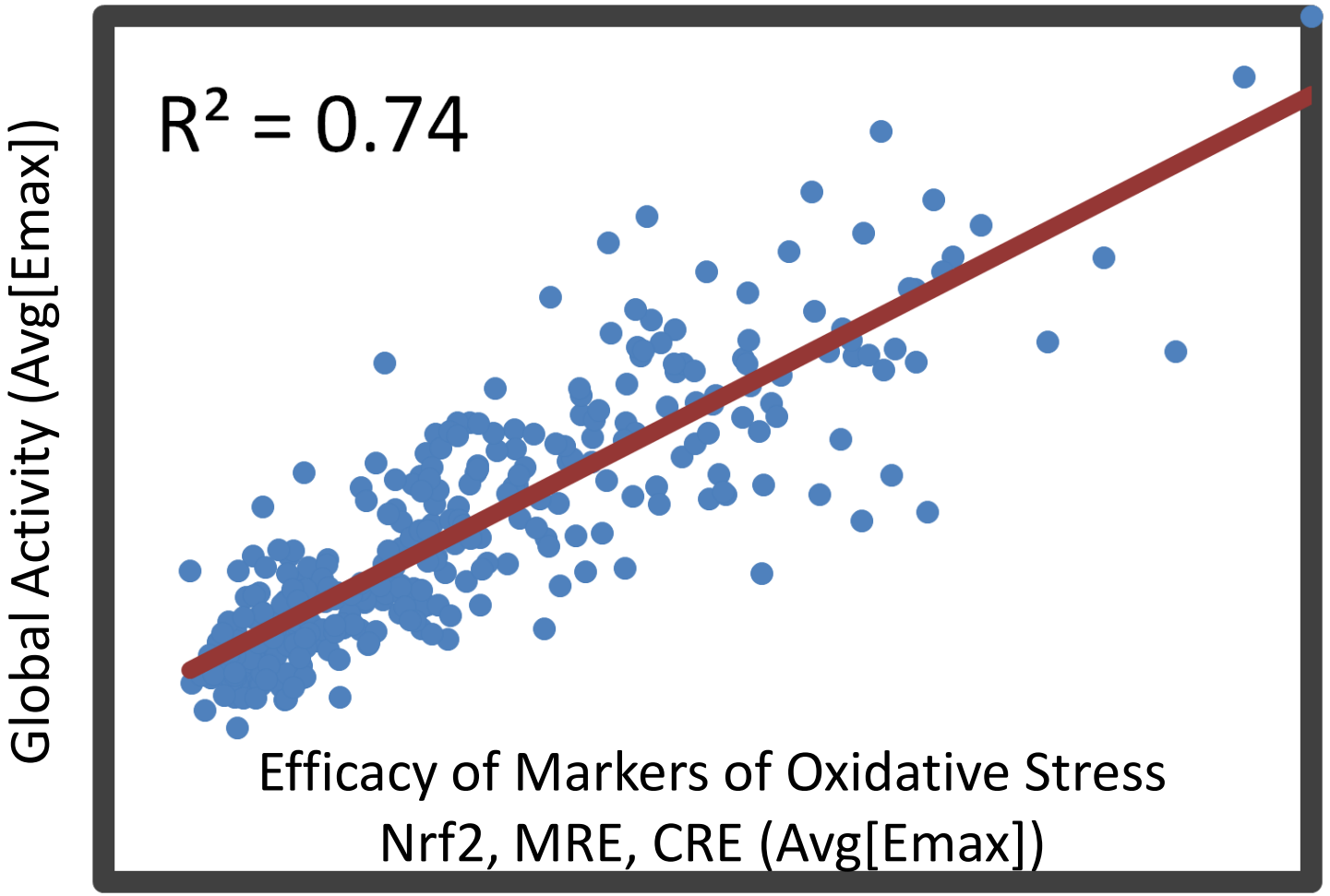

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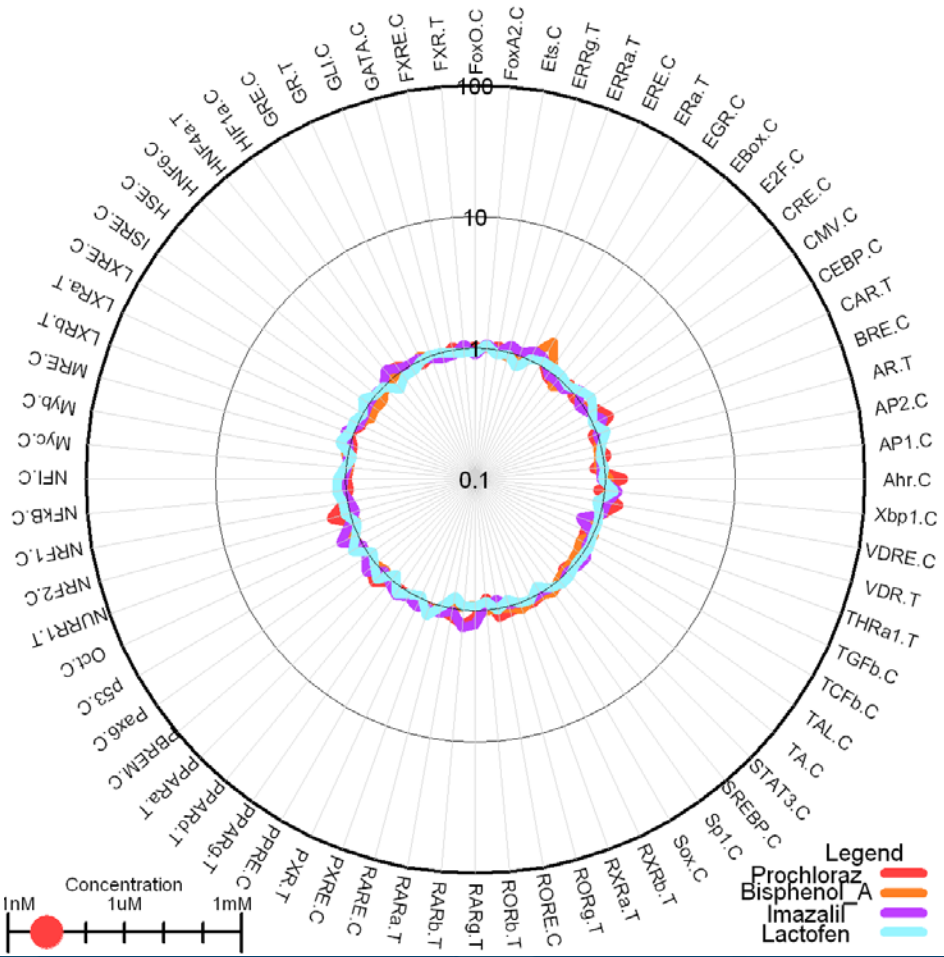
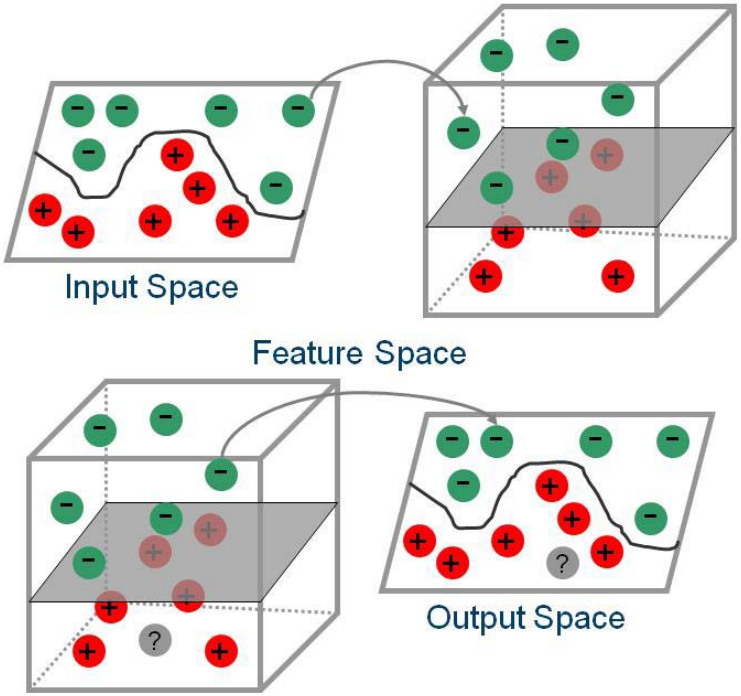
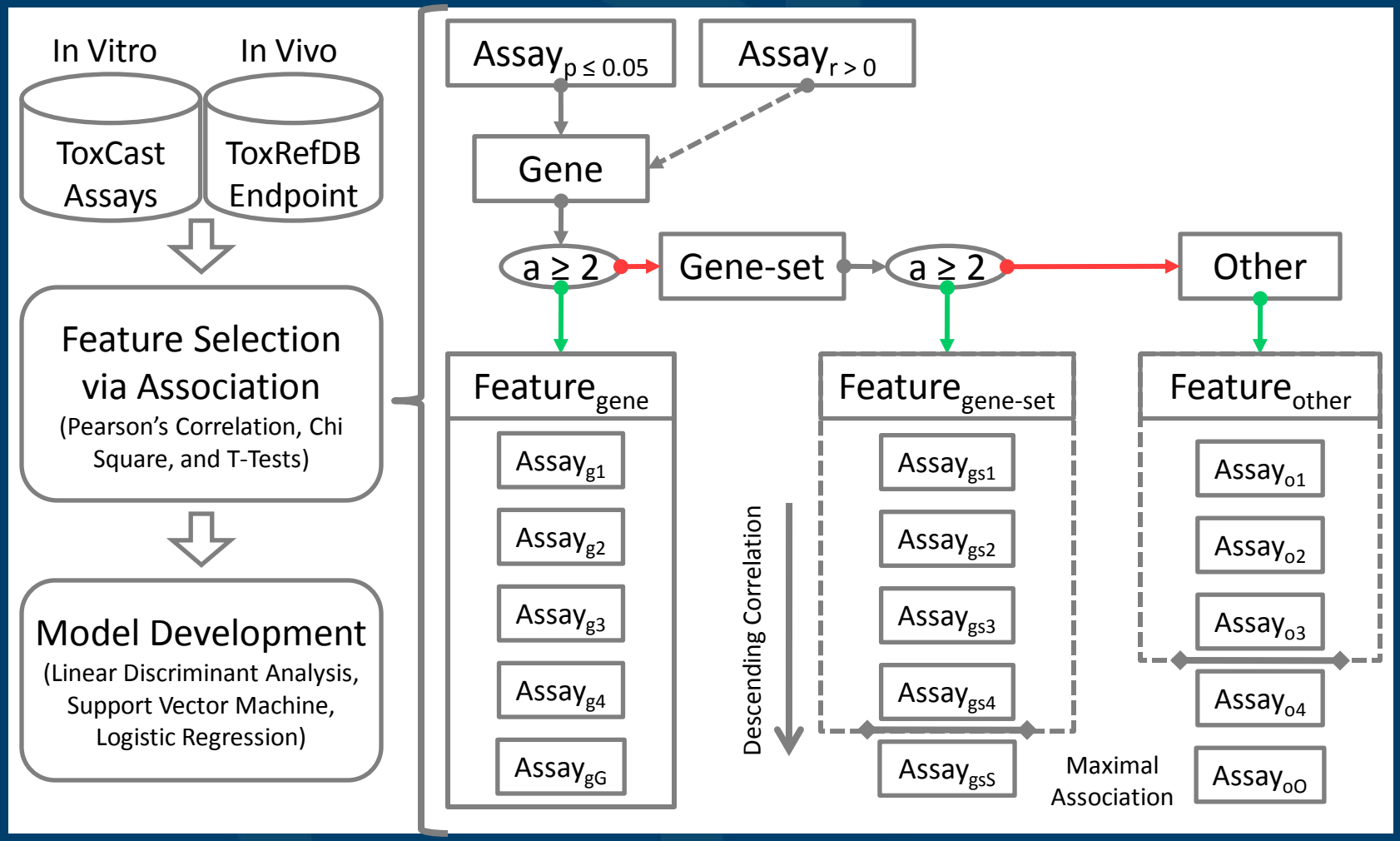


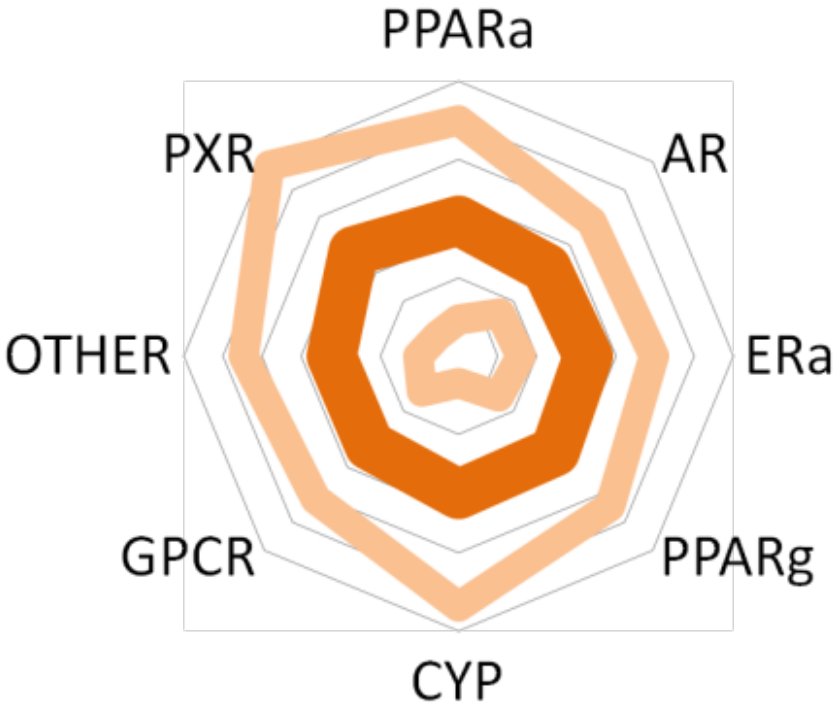
Classification Modeling Of Toxicity



Feature Selection, Aggregation & Reduction



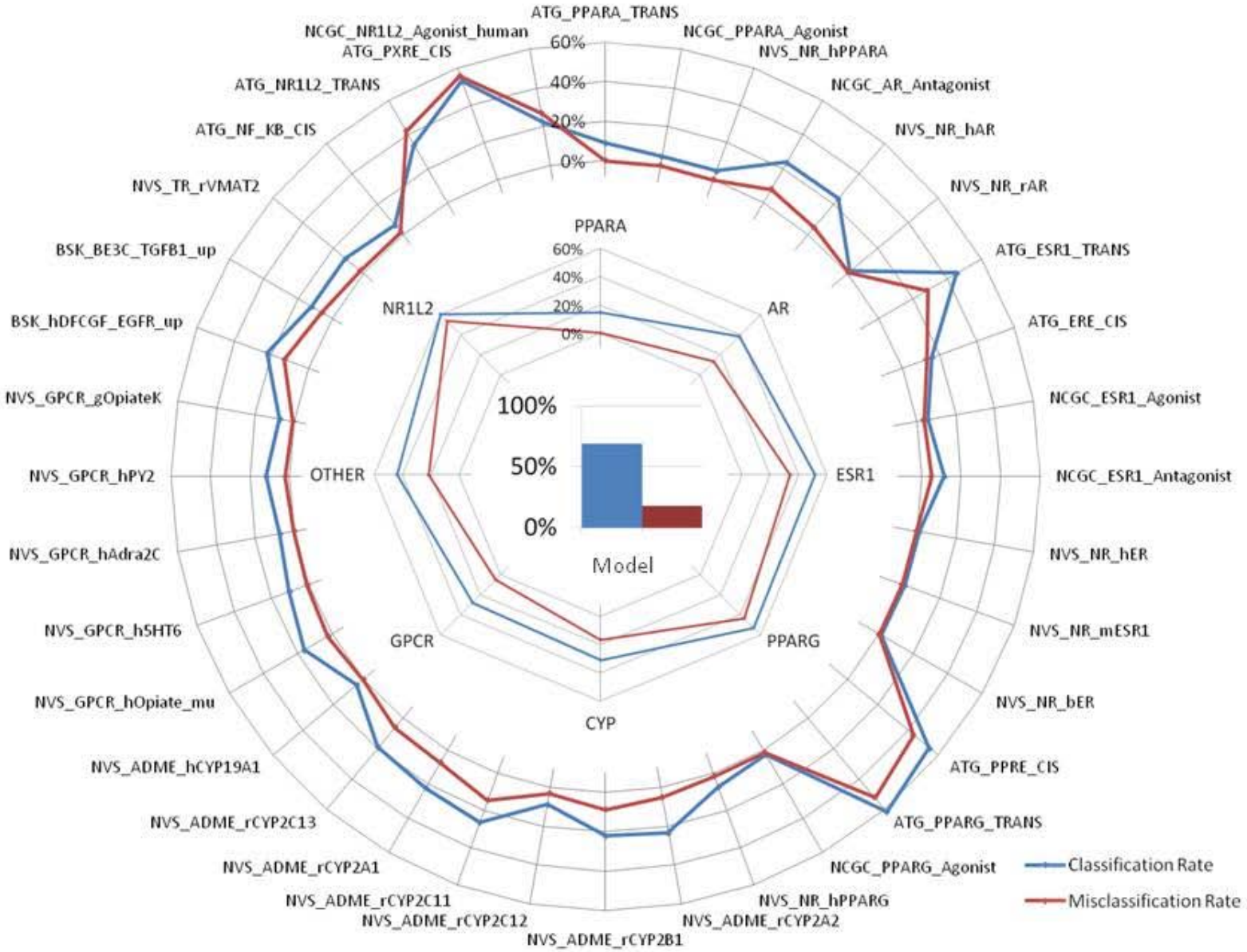
Predictive Model of Reproductive Toxicity



Predictive Model of Reproductive Toxicity

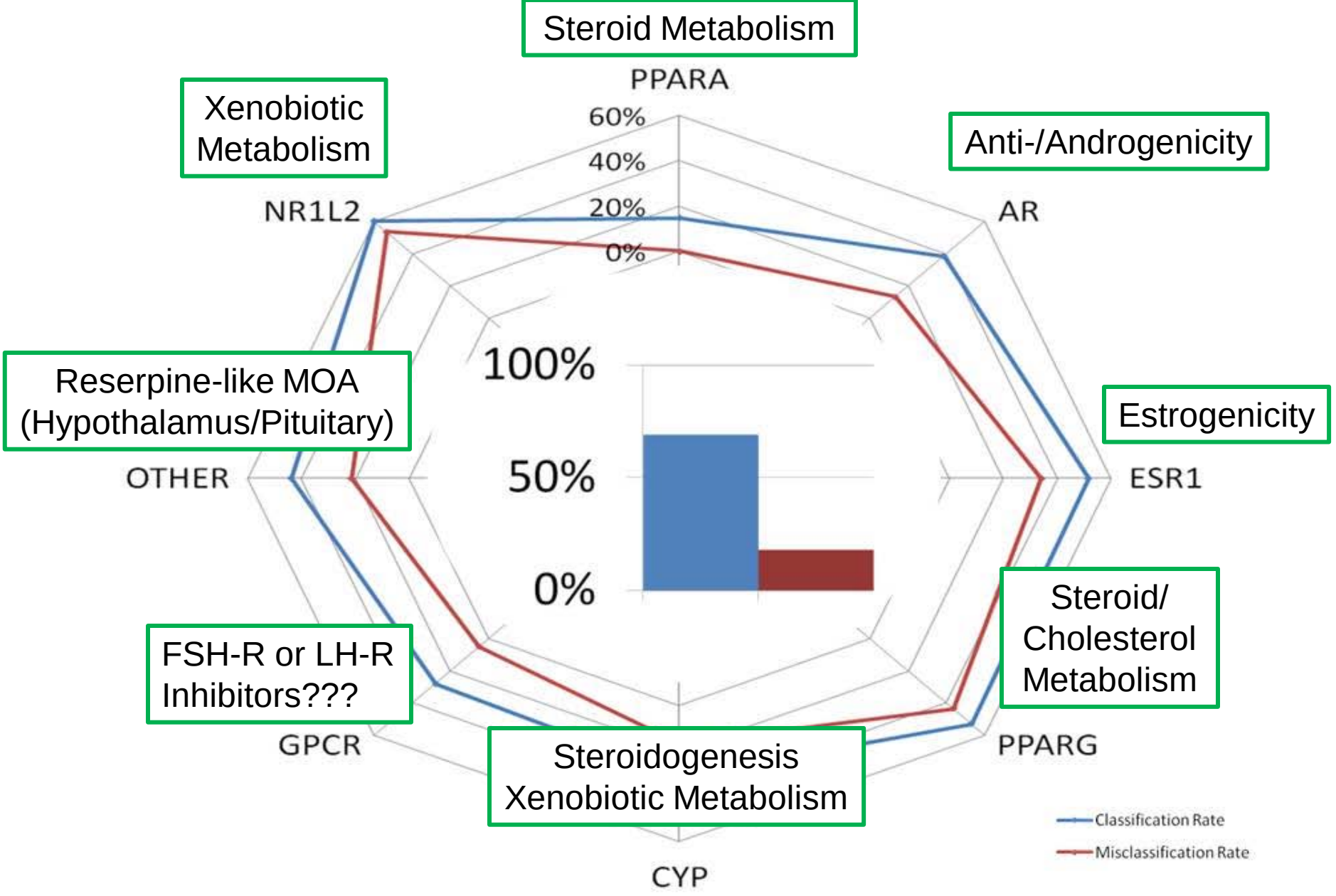
	In vitro Activity	Little to No *In vitro* Activity (<2% Active)	Total *In vivo* Chemical Counts
Acceptable Reproductive Study	**206** (A)	50 (B)	256
Unacceptable Reproductive Study	31 (C)	8 (D)	39
No Reproductive Study Available	10 (E)	4 (F)	14
Total *In vitro* Chemical Counts	247	62	309

86 of 206 Chemicals Positive for Reproductive Toxicity



Classification Rate = TP / Total # of Positives

Misclassification Rate = FP / Total # of Negatives



Classification Rate = TP / Total # of Positives

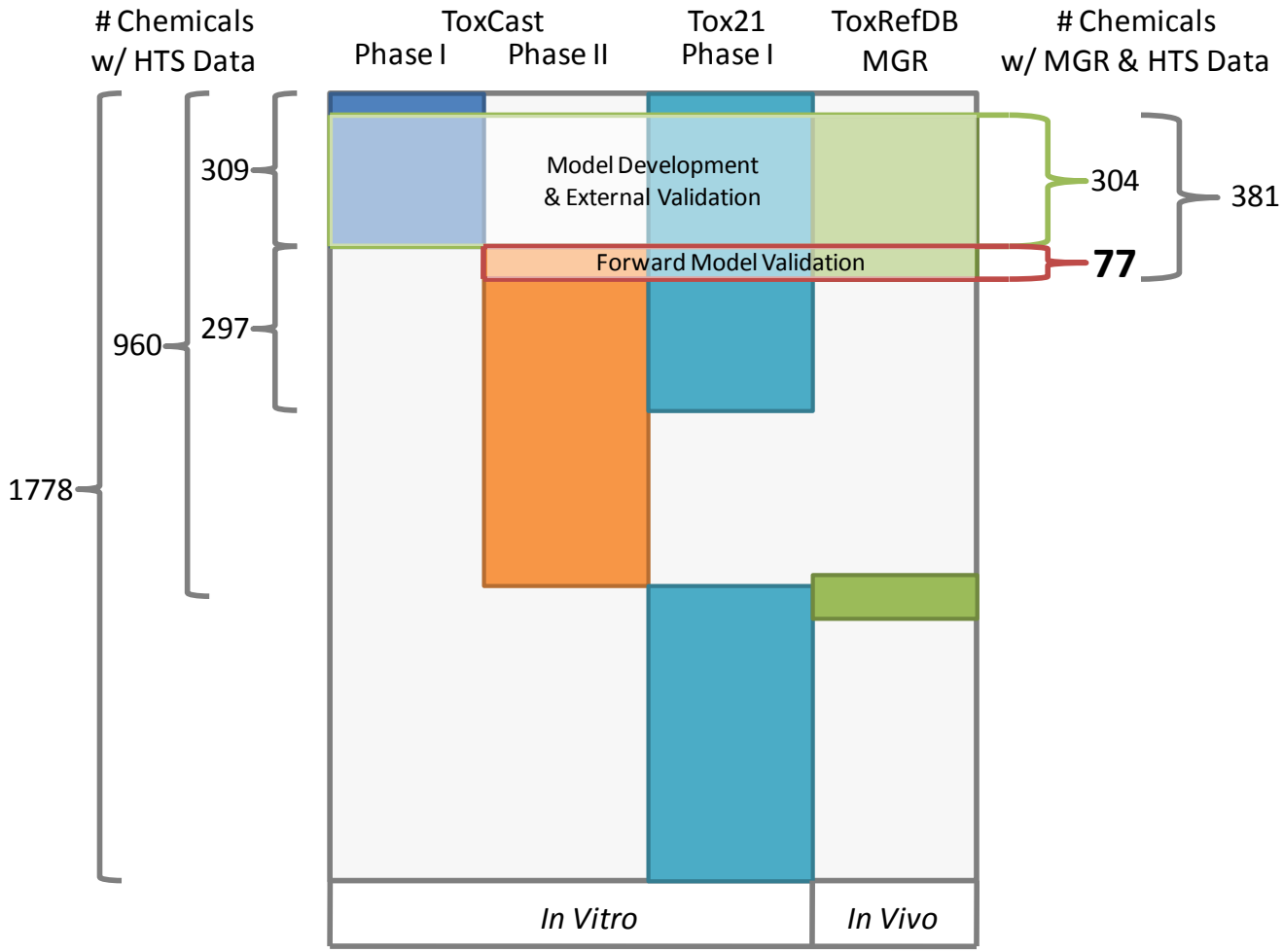
Misclassification Rate = FP / Total # of Negatives

Model Summary Statistics

Cross-Validation Statistics		Full Model Statistics				Parameter Coefficients	
Learner	**LDA**	TP	**55**	F1	**73%**	**PPARα**	1.37
CV	**5-fold**	FP	**28**	RR	**6.3**	**AR**	0.98
No. F	**8**	FN	**13**	OR	**17**	**ERα**	0.45
Assays	**36**	TN	**110**	PPV	**66%**	**PPARγ**	0.23
BA Train	**77%**	SENS	**81%**	NPV	**90%**	**CYP**	0.28
SD Train	**2%**	SPEC	**80%**	Pred	**78%**	**GPCR**	0.5
BA Test	**74%**	BA	**80%**	P-Value	**4.2E-17**	**OTHER**	0.45
SD Test	**5%**	A	**80%**	Cutoff	**0.6**	**PXR**	-0.21

Forward Validation Study

Forward Validation Study



Forward Validation Study

	Model Development Chemical Set	Forward Validation Chemical Set
TP	55	**37**
FP	28	**8**
FN	13	**6**
TN	110	**11**
NA	50	**15**
SENSIVITY	81%	**86%**
SPECIFICITY	80%	**58%**
BALANCED ACCURACY	80%	**72%**
ACCURACY	80%	**77%**

77

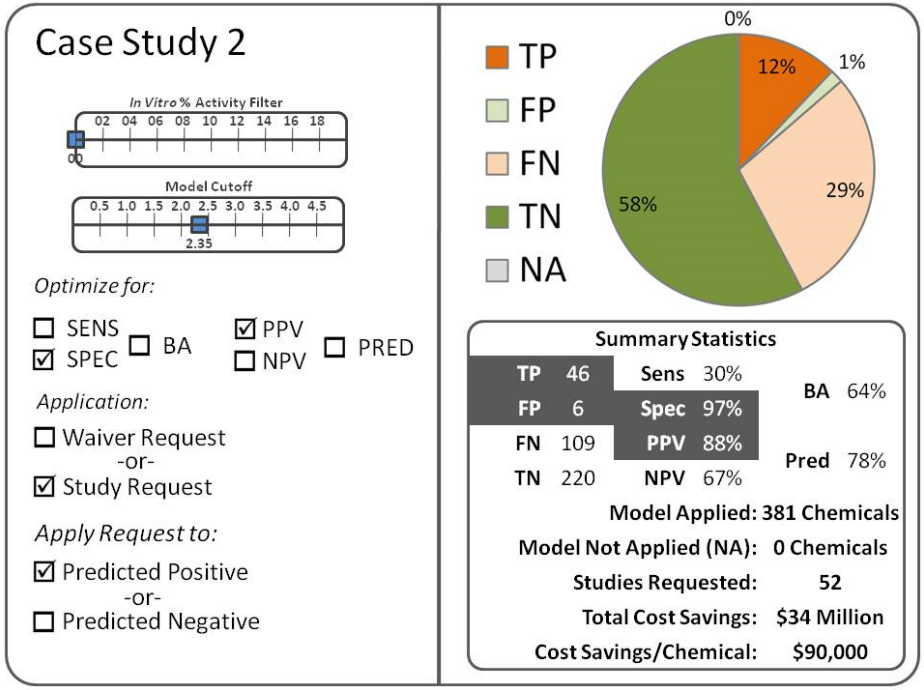
Forward Validation Study

- Maintained predictivity in light of real-world confounders
  - Increased chemical diversity
  - Assay attrition & replacement
  - High positive prevalence
- High sensitivity and overall accuracy
  - **FP**: Sodium dodecyl sulfate is detergent most likely interfering with assays
  - **FN**: Acrylamide and benzene containing compounds acting through germ-cell mutagenicity mode-of-action (MOA)
  - **TP** vs **TN**: Structurally-related chemicals with different reproductive outcomes (Benzyl Butyl Phthalate vs Octyl Phthalate)
- Impact
  - Capable of predicting reproductive toxicity across a diverse chemical set
  - MOA : Bronopol - CYP Inhibition – Disruption of Steroidogenesis
  - Immediate impact on chemical testing decision making

Future Directions

- Assay development to fill biological gaps in model
  - Steroidogenesis
  - Germ cell mutagenicity
  - Incorporation of metabolism
- Systems modeling of the neuroendocrine system
  - Moving from a classification model to a systems model
  - Account for timing, dose/concentration, life-stage
- Developing an Integrated Testing Strategy
  - Combine model with alternative test methods
  - Incorporate systems models for mechanistic guidance
  - Combine model with other HTS-derived models
  - **Prioritize chemicals for further testing**

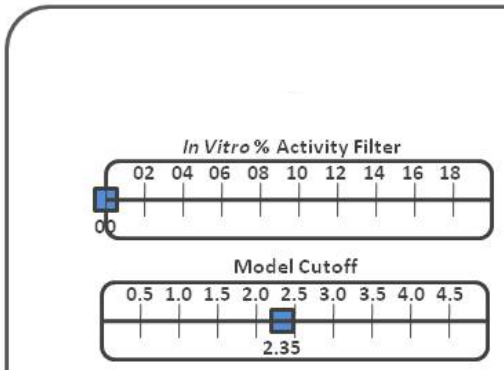
Case Study in Testing Prioritization



Case Study in Testing Prioritization

- Applied to 381 chemicals (155 chemicals positive)
  - 78% overall model balanced accuracy using default parameters
  - Adjustable parameters
- Case Study Context
  - Authority to request MGR study
  - Must prioritize a portion of chemical set
- Performed with no additional information
- Cost savings estimates = Increased efficiency of using the model to select which chemicals to test vs. selecting chemicals randomly

Case Study in Testing Prioritization



Optimize for:

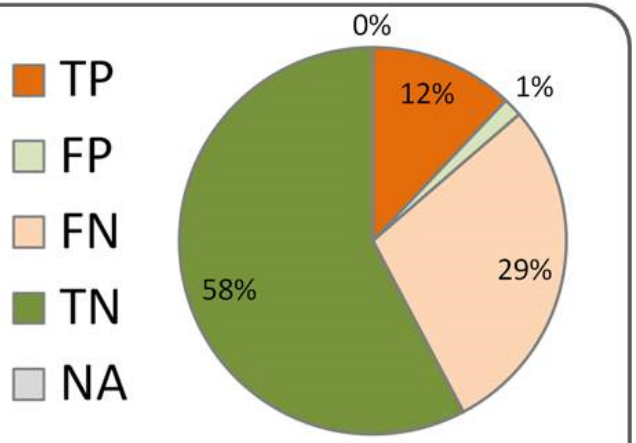
☐ SENS ☐ BA ☑ PPV ☐ PRED
☑ SPEC ☐ NPV

Application:

☐ Waiver Request
-or-
☑ Study Request

Apply Request to:

☑ Predicted Positive
-or-
☐ Predicted Negative



Summary Statistics

TP	46	Sens	30%	BA	64%
FP	6	Spec	97%		
FN	109	PPV	88%	Pred	78%
TN	220	NPV	67%		

Model Applied: 381 Chemicals
Model Not Applied (NA): 0 Chemicals
Studies Requested: 52
Total Cost Savings: $34 Million
Cost Savings/Chemical: $90,000

Conclusions

- Captured 30 years of traditional reproductive toxicity data using a standardized vocabulary enabling consistent endpoint definitions
- Analyzing and interpreting HTS data requires bioinformatic workflows and understanding of assay confounders and other considerations
- Used HTS to develop forward validated predictive model of reproductive toxicity with biological plausability
- Demonstrated ability of model to impact chemical testing decision making
- Dashboard concept enables user interaction with model and direct input into decision points