United States Environmental Protection Agency

Applying a PBTK model for IVIVE

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In Vitro to In Vivo Extrapolation (IVIVE)

Enables use of high throughput toxicity assays as an alternative to animal testing

<u>*In vivo* dose (mg/kg/day)</u>

- Point of departure (POD: LOEL, LOAEL, etc.)
- Lowest dose where a specific effect (e.g. nonneoplastic pathology in the liver, etc.) was observed

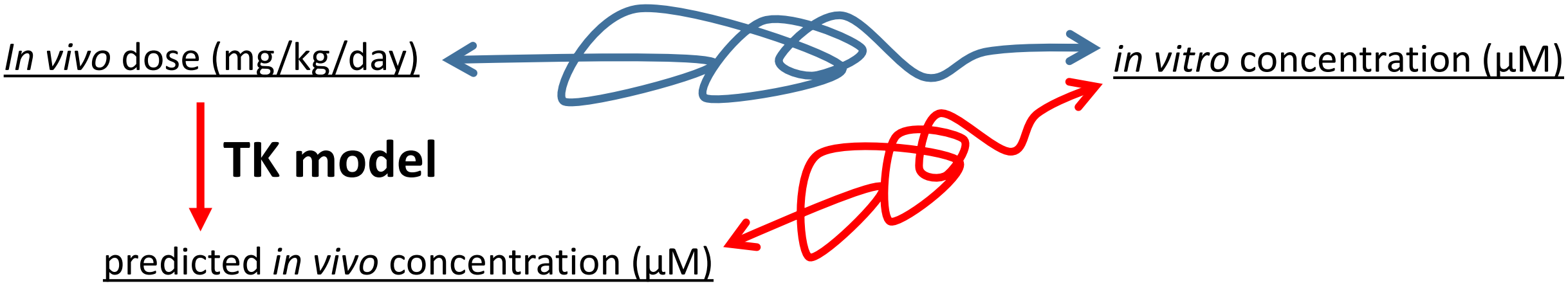
Some kind of model (statistical, machine learning etc.)

<u>*In vitro* concentration (µM)</u>

- An individual assay
- Ensemble of assays

What about toxicokinetics?

In Vitro to In Vivo Extrapolation (IVIVE)

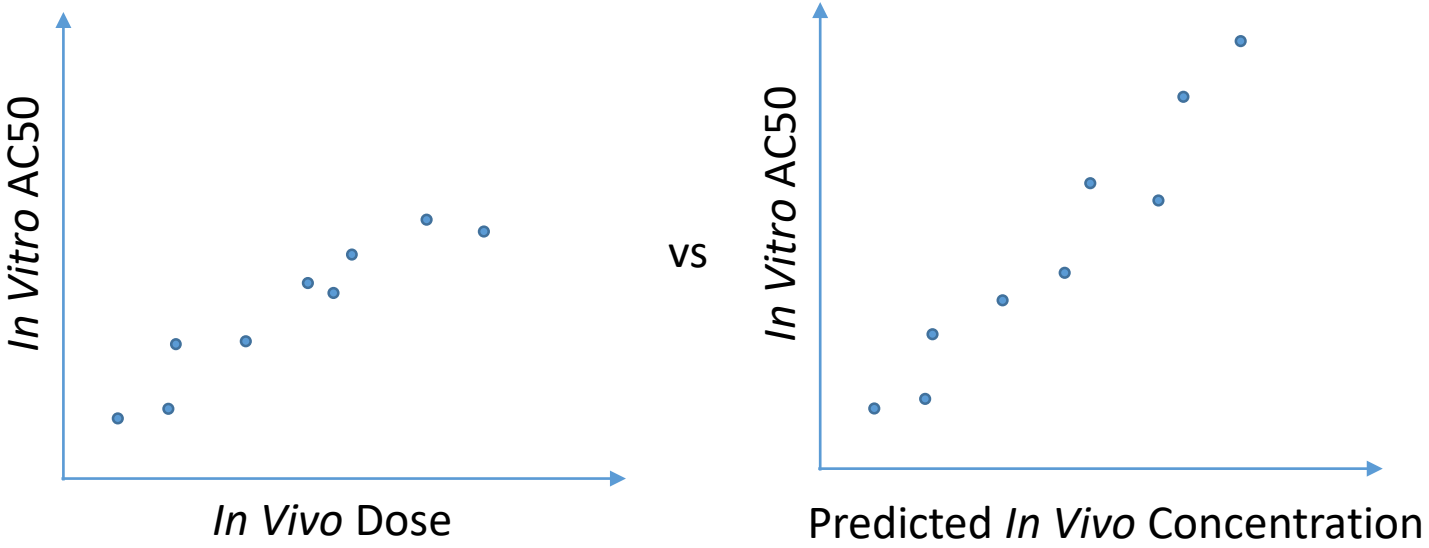


Does incorporating toxicokinetics improve the correlation between *in vitro* and *in vivo* toxicity data?

What are the effects of the assumptions in the application of TK?

In Vitro to In Vivo Extrapolation (IVIVE)

Ideally, we would know of an *in vitro* assay that is related to a specific *in vivo* effect (pathology); use this to evaluate the effect of incorporating TK



In the absence of this information, we:

1. **Evaluate all *in vitro* assay endpoints against all *in vivo* effects (ToxRef)**
2. **Evaluate all *in vitro* assay endpoints against points of departure (ToxVal POD; determined across all *in vivo* effects)**

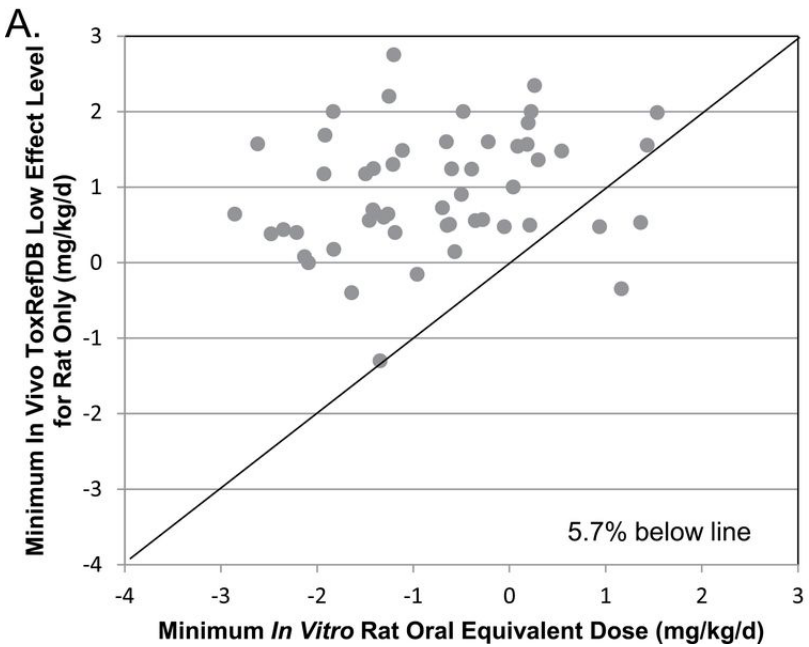
Prior Work

Comparison of the *in vitro* assay with the lowest oral equivalent dose with the *in vivo* response with the lowest LEL for each chemical.

Measured rat-specific intrinsic clearance (Cl_{int}) and fraction of unbound chemical in plasma (f_{up}) for **56 chemicals** with rat ToxRefDB data.

Used **steady-state** PK model for *in-vitro* to *in-vivo* extrapolation.

$$\text{Rat}\,C_{ss} = \frac{\text{ko}}{(\text{GFR} \times F_{ub}) + \left(\dfrac{Q_l \times F_{ub} \times \text{Cl}_{int}}{Q_l + F_{ub} \times \text{Cl}_{int}}\right)}$$



- **Using TK for IVIVE may help define exposure heuristics**
- **Incorporating TK did not otherwise improve predictive performance**
  - **Assumptions in the application of TK may have influenced this result**

Wetmore, B. A.; Wambaugh, J. F.; *et al.* "Relative Impact of Incorporating Pharmacokinetics on Predicting *In Vivo* Hazard and Mode of Action from High-Throughput *In Vitro* Toxicity Assays." *Toxicol. Sci.* (2013) 132 (2): 327-346.

This Work

- **PBTK model**
- **Evaluate assumptions:**
  - Clearance
  - PBTK concentration selection (mean vs. max, etc.)
  - Accounting for partitioning in cell based assays
- **104 chemicals w/ rat specific *in vitro* measured values for f_{up} and Cl_{int} (Cyprotex)**

To prepare this analysis:

1) **Measured values for f_{up} and Cl_{int}**
2) **Select doses and examine scope of the data (i.e. what assays and *in vivo* effects can we look at?)**
3) **What assumptions to evaluate?**

ToxCast *in-vitro* AC50

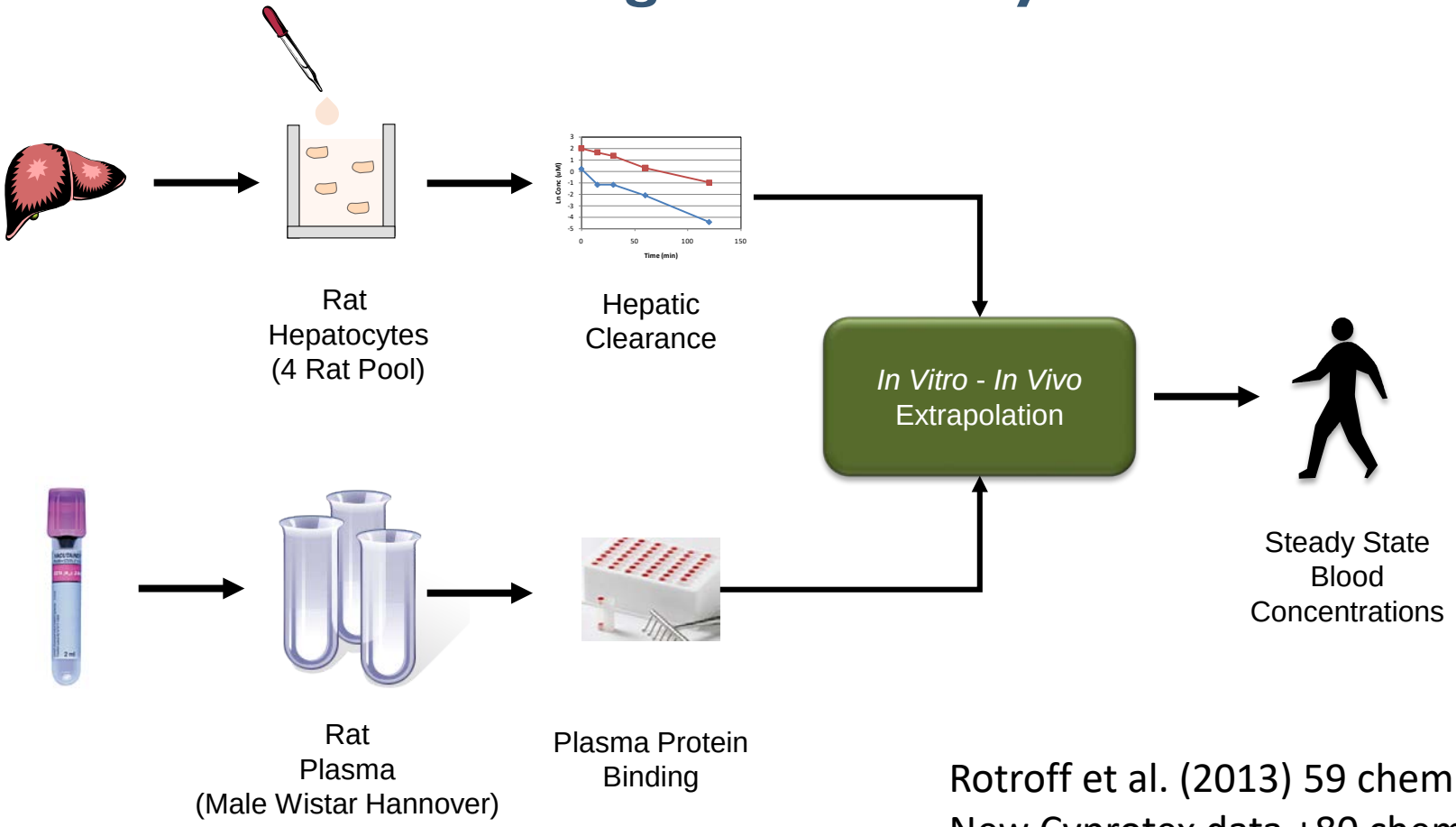
For each assay (and ToxRef effect), compare AC50 vs x_{PBTK}, x_{rand}, x_{dose}

$$AC50 \sim \beta_{i,1} x_{PBTK} + \gamma$$
$$AC50 \sim \beta_{i,2} x_{rand} + \gamma$$
$$AC50 \sim \beta_{i,3} x_{dose} + \gamma$$

Multiple Regression

$$AC50 \sim \beta_{j,1} x_{PBTK} + \beta_{j,2} x_{rand} + \beta_{j,3} x_{dose} + \gamma$$

Characterizing Rat *In Vivo* Toxicokinetics Using *In Vitro* Assays



Rotroff et al. (2013) 59 chemicals
New Cyprotex data +80 chemicals

Slide from Barbara Wetmore

Scope of the Data – Selecting Dose

ToxRef Effect Level Dose (ELD)

- **ToxRef*:** Detailed database of *in vivo* effect and dose
- **Effect level dose** defined as the **minimum dose** at which a **particular effect** (category-type-target, e.g. systemic-nonneoplastic pathology-liver) was observed for a given study and chemical, ignoring gender and strain; **a specific effect**

For a given chemical

- x_{dose} – doses for specific effect and study
- x_{PBTK} – concentration from transforming x_{dose} via PBTK
- x_{rand} – concentration from transforming x_{dose} via the randomly parameterized PBTK model

ToxVal POD Dose

- **ToxVal:** General database of *in vivo* POD
- Lowest observed effect level (**LOEL**) or lowest observed adverse effect level (**LOAEL**) for a given chemical and study; all usable rat studies; **across all effects**

For a given chemical

- x_{dose} – minimum POD across all studies
- x_{PBTK} – minimum concentration from transforming all POD via the PBTK model
- x_{rand} – minimum concentration from transforming all POD via the randomly parameterized PBTK model

Compare against every *in vitro* toxicity assay

*Martin, *et al. Environ Health Perspect.* 2009; 117 (3): 392-399

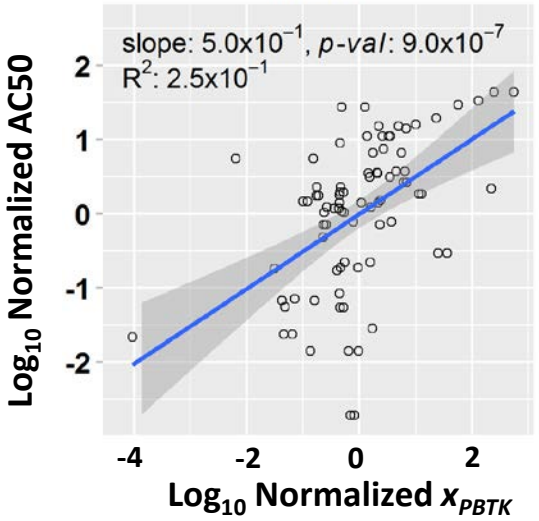
Scope of the Data – An Example

ToxRef Effect: systemic-nonneoplastic pathology-liver
- Possibly multiple points for a given chemical

Assay endpoint: ATG_PXRE_CIS_up
- Single point for a given chemical

Number of points in the regression: 85
Number of chemicals in the regression: 49

ToxRef PBTK Result



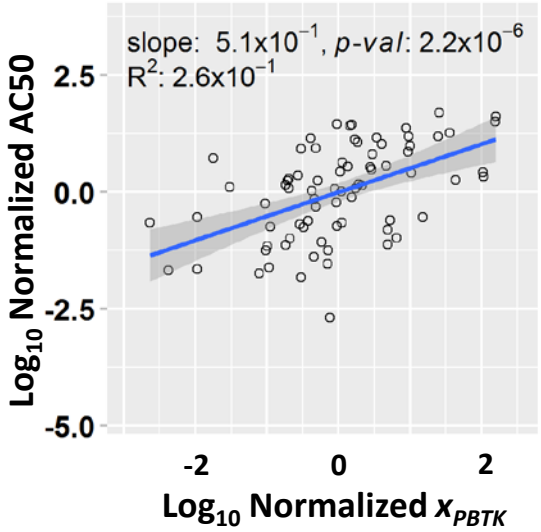
1 of ~40,000 assay and *in vivo* effect pairs

ToxVal POD: LOEL or LOAEL
- Single point for a given chemical (minimum taken across studies)

Assay endpoint: ATG_PXRE_CIS_up
- Single point for a given chemical

Number of points in the regression: 76
Number of chemicals in the regression: 76

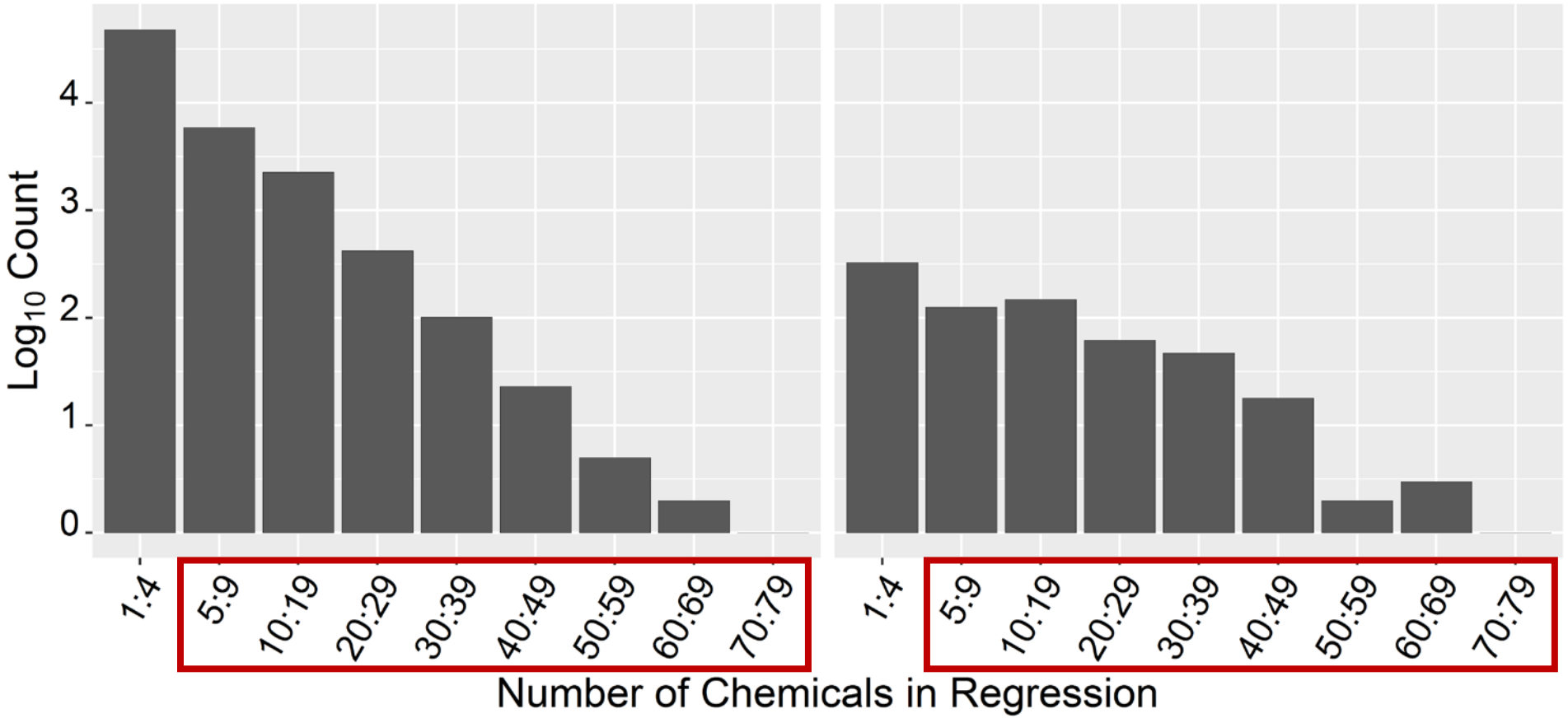
ToxVal PBTK Result



1 of ~1,000 assay endpoints

Scope of the Data

ToxRef Effect Level Dose **ToxVal POD** (1 POD per chem. across all effects)



ToxRef Analysis:

- Total: 56,973 *in vitro* assay-*in vivo* effect pairs
- ≥ 5 chemicals: 8,731 *in vitro* assay-*in vivo* effect pairs
- ≥ 20 chemicals: 552 *in vitro* assay-*in vivo* effect pairs

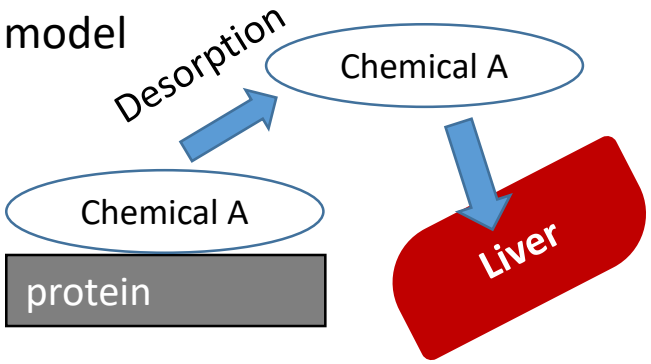
ToxVal Analysis:

- Total: 734 *in vitro* assays
- ≥ 5 chemicals: 405 *in vitro* assays
- ≥ 20 chemicals: 133 *in vitro* assays

Evaluated Assumptions in Applying TK for IVIVE

Hepatic Clearance – Restrictive vs. Nonrestrictive

An assumption in the PBTK model



Restrictive Clearance

- **Slow** desorption rate
- **Clearance** $\propto f_{up}$

Non-Restrictive Clearance

- **Fast** desorption rate
- **Clearance independent of** f_{up}

Multiplication or Division by f_{up}

Multiplication: $C_{PBTK} * f_{up}$

- Assume AC50 is closer to a free concentration

Division: $C_{PBTK} * f_{up}^{-1}$

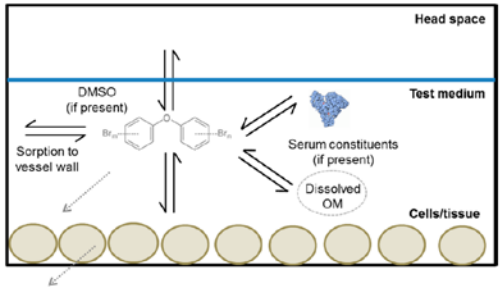
- Assume AC50 is nominal concentration and convert to free concentration

Concentration Selection from the PBTK model

- C_{PBTK}
- Plasma vs tissue concentration (matched to cell type of cell based assay)
- Mean vs max concentration

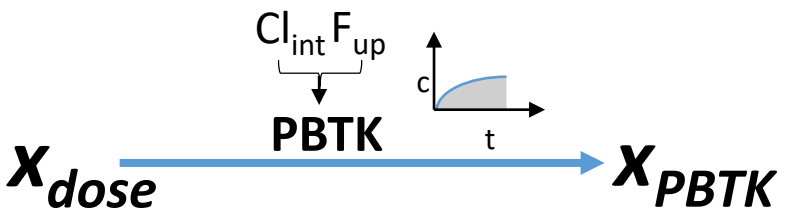
Partition model for cell based assays

- Nominal concentration (AC50) doesn't account for partitioning of chemical into serum and cells
- Determine free concentration from Armitage model
- Account for free concentration by defining a factor $\eta = \frac{C_{assay,free}}{C_{assay,nominal}}$ so that $x_{PBTK} = C_{PBTK}/\eta$



Armitage, J. M.; Wania, F.; Arnot, J. A. *Env. Sci. & Tech.* 2014, 49, 9770-9779.

Methods Summary



x_{rand} : result from randomly parameterized application

All assays:

- 8731 *in vitro* assay- *in vivo* effect pairs (analysis of ToxRef ELD)
- 405 *in vitro* assays (analysis of ToxVal POD)
- 24 combinations of assumptions to evaluate
- ~219,000 regressions (4 regressions per comparison)

Simple Regressions

$$AC50 \sim \beta_{i,1} x_{PBTK} + \gamma$$
$$AC50 \sim \beta_{i,2} x_{rand} + \gamma$$
$$AC50 \sim \beta_{i,3} x_{dose} + \gamma$$

Multiple Regression

$$AC50 \sim \beta_{j,1} x_{PBTK} + \beta_{j,2} x_{rand} + \beta_{j,3} x_{dose} + \gamma$$

Cell based assays: nominal vs free concentration (Armitage)

- 1531 *in vitro* assay- *in vivo* effect pairs(ToxRef ELD) with known FBS%
- 78 *in vitro* assays (ToxVal POD) with known FBS%
- an additional 24 combinations of assumptions to evaluate
- ~38,000 additional regressions

Clearance	f_{up} multiplier	Concentration	Concentration value
restrictive	none	plasma	mean
			max
		tissue	mean
			max
	f_{up}	plasma	mean
			max
		tissue	mean
			max
	f_{up}^{-1}	plasma	mean
			max
		tissue	mean
			max
non-restrictive	none	plasma	mean
			max
		tissue	mean
			max
	f_{up}	plasma	mean
			max
		tissue	mean
			max
	f_{up}^{-1}	plasma	mean
			max
		tissue	mean
			max

Example Results

Example Regressions – ToxRef ELD

***in vivo* Effect: systemic-nonneoplastic pathology-liver**

Assumptions

Clearance	f_{up} multiplier	Concentration	Concentration Value
nonrestrictive	none	plasma	mean

Simple Regressions

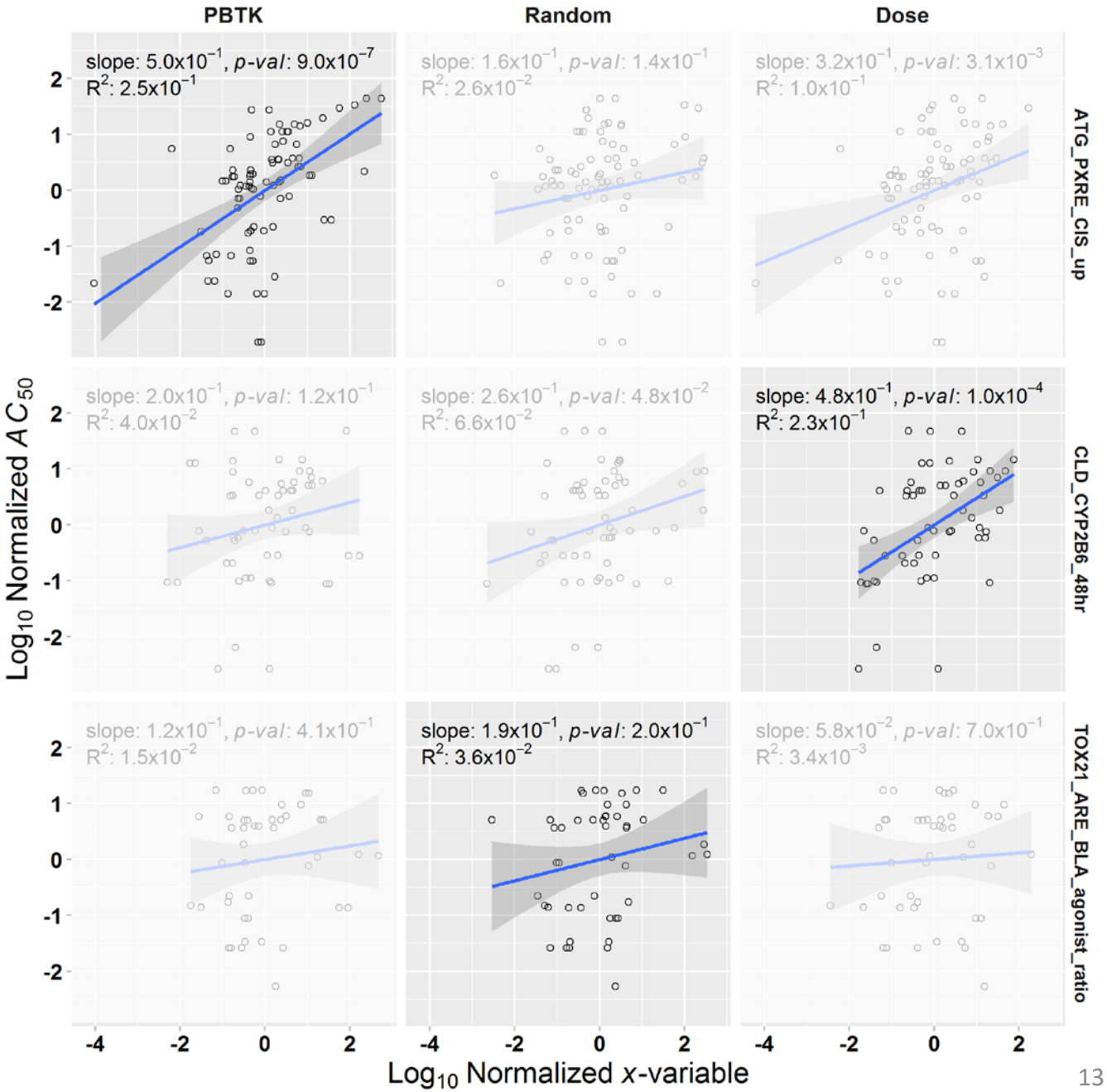
$$AC50 \sim \beta_{i,1} x_{PBTK} + \gamma$$
$$AC50 \sim \beta_{i,2} x_{rand} + \gamma$$
$$AC50 \sim \beta_{i,3} x_{dose} + \gamma$$

Multiple Regression:

$$AC50 \sim \beta_{j,1} x_{PBTK} + \beta_{j,2} x_{rand} + \beta_{j,3} x_{dose} + \gamma$$

	Slopes			p-values		
Assay	PBTK	Random	Dose	PBTK	Random	Dose
ATG_PXRE_CIS_up	**0.50**	-0.05	0.04	1.1E-04	6.8E-01	8.1E-01
CLD_CYP2B6_48_hr	0.06	-0.01	**0.47**	6.5E-01	9.7E-01	2.2E-03
TOX21_ARE_BLA agonist_ratio	0.09	**0.22**	-0.10	5.9E-01	2.4E-01	5.8E-01

Filter based on result of multiple regression



Example Regressions – ToxVal POD

Clearance	f_{up} multiplier	Concentration	Concentration Value
nonrestrictive	none	plasma	mean

Simple Regressions

$$AC50 \sim \beta_{i,1} x_{PBTK} + \gamma$$

$$AC50 \sim \beta_{i,2} x_{rand} + \gamma$$

$$AC50 \sim \beta_{i,3} x_{dose} + \gamma$$

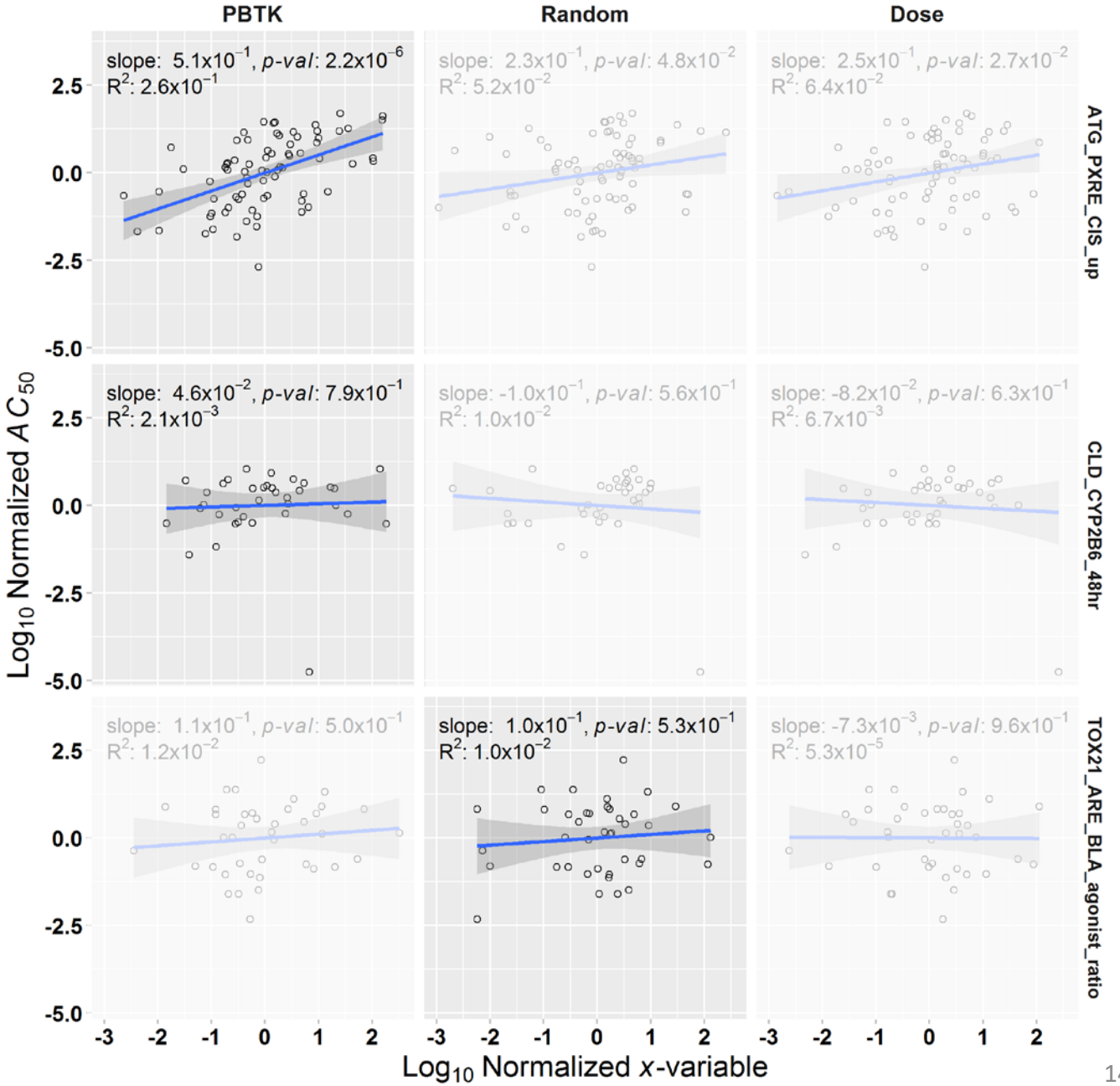
Summarize by looking **at histograms of slope, p-value, and R^2** for every **pair of ToxRef Effect and assay endpoint** and every assay endpoint with corresponding **ToxVal POD**

Multiple Regression:

$$AC50 \sim \beta_{j,1} x_{PBTK} + \beta_{j,2} x_{rand} + \beta_{j,3} x_{dose} + \gamma$$

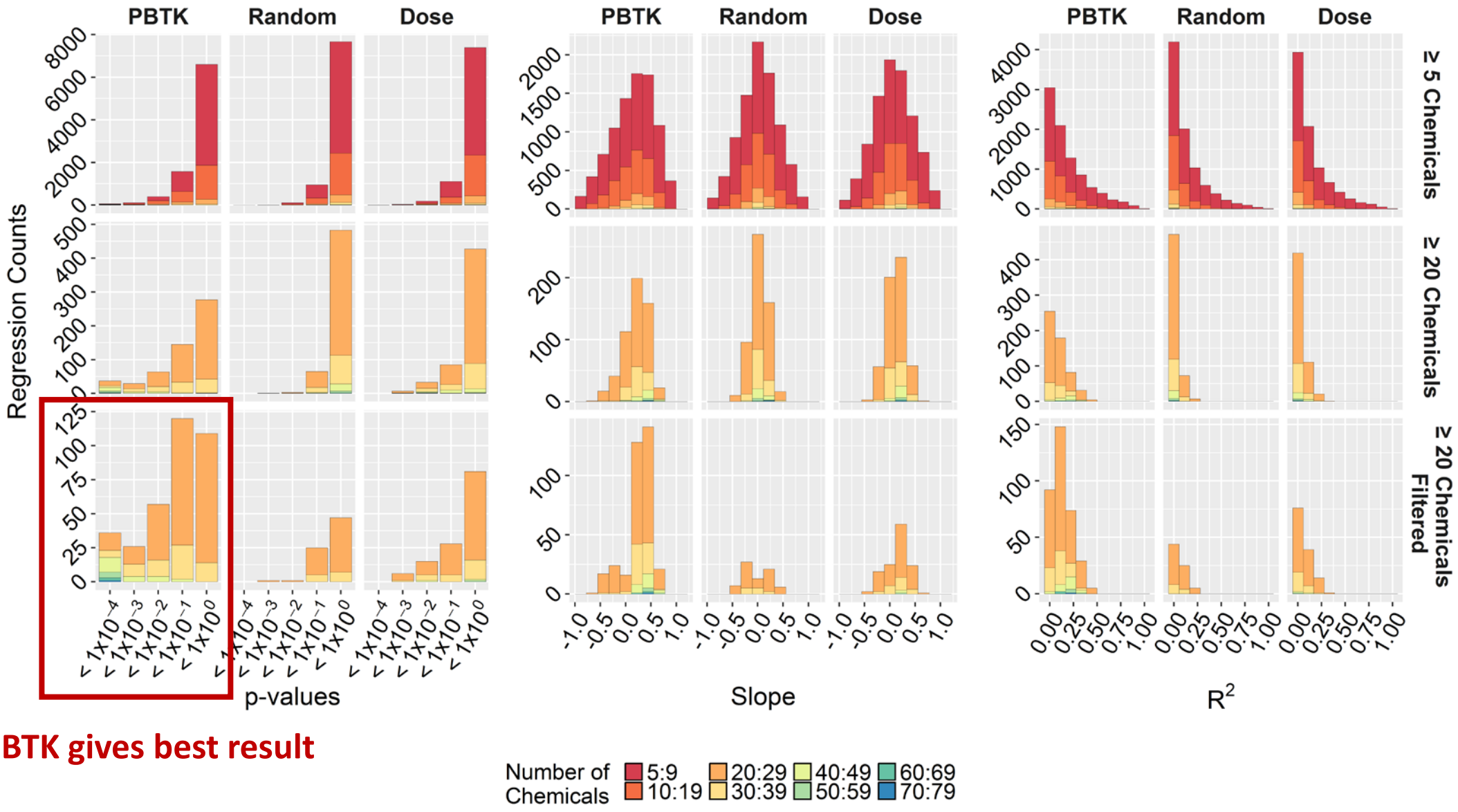
	Slopes			p-values		
Assay	PBTK	Random	Dose	PBTK	Random	Dose
ATG_PXRE_CIS_up	0.61	0.17	-0.24	1.3E-05	2.1E-01	1.3E-01
CLD_CYP2B6_48_hr	0.11	-0.05	-0.10	6.2E-01	8.1E-01	6.8E-01
TOX21_ARE_BLA agonist_ratio	0.19	0.20	-0.25	3.5E-01	3.6E-01	3.2E-01

Filter based on result of multiple regression



ToxRef ELD Example Result

Clearance	f_{up} multiplier	Concentration	Concentration Value
nonrestrictive	none	plasma	mean

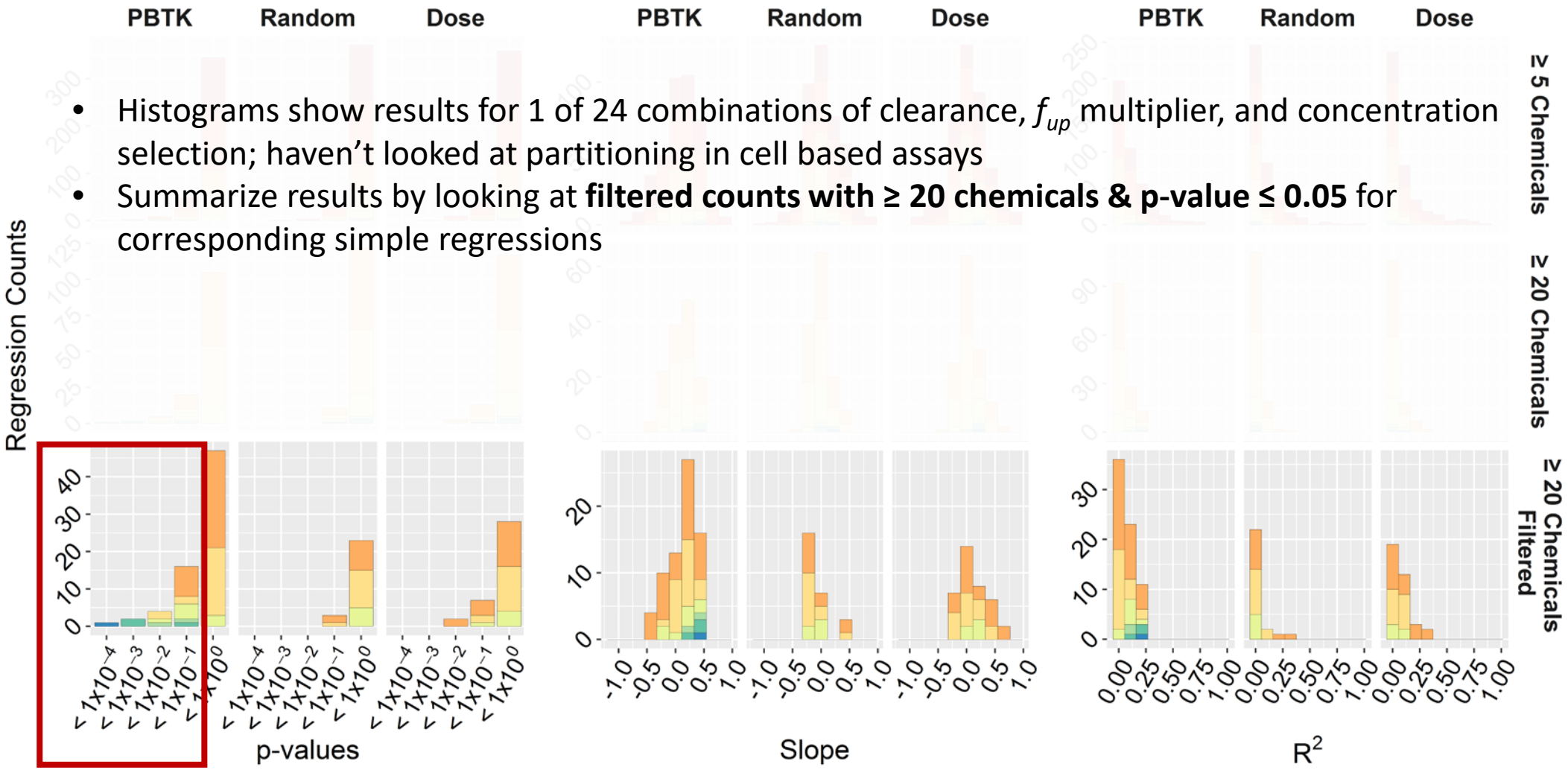


PBTK gives best result

ToxVal POD Example Result

Clearance	f_{up} multiplier	Concentration	Concentration Value
nonrestrictive	none	plasma	mean

- Histograms show results for 1 of 24 combinations of clearance, f_{up} multiplier, and concentration selection; haven't looked at partitioning in cell based assays
- Summarize results by looking at **filtered counts with ≥ 20 chemicals & p-value ≤ 0.05** for corresponding simple regressions



PBTK gives best result

Filtered counts with p-value ≤ 0.05 and # of chemicals ≥ 20

All Assays

Clearance	f_{up} multiplier	Concentration	Concentration value	ToxRef ELD PBTK	ToxRef ELD Random	ToxRef ELD Dose	ToxVal POD PBTK	ToxVal POD Random	ToxVal POD Dose
restrictive	none	plasma	mean	108	23	50	2	6	7
			max	101	20	39	4	5	4
		tissue	mean	94	19	59	3	3	7
			max	75	17	54	5	2	6
	f_{up}	plasma	mean	189	22	30	9	4	5
			max	183	20	28	13	2	5
		tissue	mean	145	24	48	5	5	8
			max	133	25	39	7	3	5
	f_{up}^{-1}	plasma	mean	40	17	77	1	5	9
			max	38	26	72	2	5	8
		tissue	mean	50	25	77	4	6	8
			max	35	25	76	2	5	8
non-restrictive	none	plasma	mean	214	19	34	15	2	6
			max	210	19	29	15	2	6
		tissue	mean	198	16	36	11	4	8
			max	206	14	29	11	2	8
	f_{up}	plasma	mean	213	12	34	19	2	6
			max	224	15	28	20	2	6
		tissue	mean	187	21	38	15	2	8
			max	192	20	34	14	2	8
	f_{up}^{-1}	plasma	mean	141	29	53	7	5	7
			max	124	25	56	5	4	7
		tissue	mean	124	24	51	6	6	7
			max	106	25	52	6	4	7

Cell Based Assays

nonrestrictive clearance, no f_{up} multiplier, mean plasma concentration

Cell Assay Model	ToxRef PBTK	ToxRef Random	ToxRef Dose	ToxVal PBTK	ToxVal Random	ToxVal Dose
None	22	7	0	1	0	2
Armitage	30	2	1	3	1	0

- **Nonrestrictive** clearance appears to give better result
- Using a f_{up} multiplier has an effect
- **Plasma** slightly better than tissue concentration
- No significant difference between using mean or max concentration
- Using a cell assay partition model appears to improve results for cell based assays

ToxRef: total pairs ≥ 20 chemicals = 552
ToxVal: assays ≥ 20 chemicals = 133

Conclusions

- In general, **using the PBTK model improves the correlation between the evaluated *in vitro* and *in vivo* toxicity data**
- In some cases, untransformed dose remains a better predictor
- The assumptions in the application of the PBTK model for IVIVE matter
- **Nonrestrictive clearance** with **plasma concentration** gave the best result and should be used as a starting basis when applying PBTK for IVIVE, using the predicted *in vivo* unbound concentration (multiplying by f_{up}) may be beneficial
- Although some correlations were significant, they were not predictive on their own
- Toxicokinetics should be considered when building ensemble models to relate *in vitro* toxicity assay results to particular *in vivo* endpoints – *but only had data for 104 chemicals in this analysis*

Acknowledgements

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Cyprotex