Quantitative variability in repeat dose toxicity studies: Implications for scientific confidence in NAMs

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What is needed to understand the acceptability of NAMs for risk assessment?

- In US, Section 4(h) in the Lautenberg amendment to TSCA:
  - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - New approach methods (NAMs) need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models
- "Directive to Prioritize Efforts to Reduce Animal Testing" memorandum signed by Administrator Andrew Wheeler on September 10, 2019
  - "1. Validation to ensure that NAMs are equivalent to or better than the animal tests replaced."

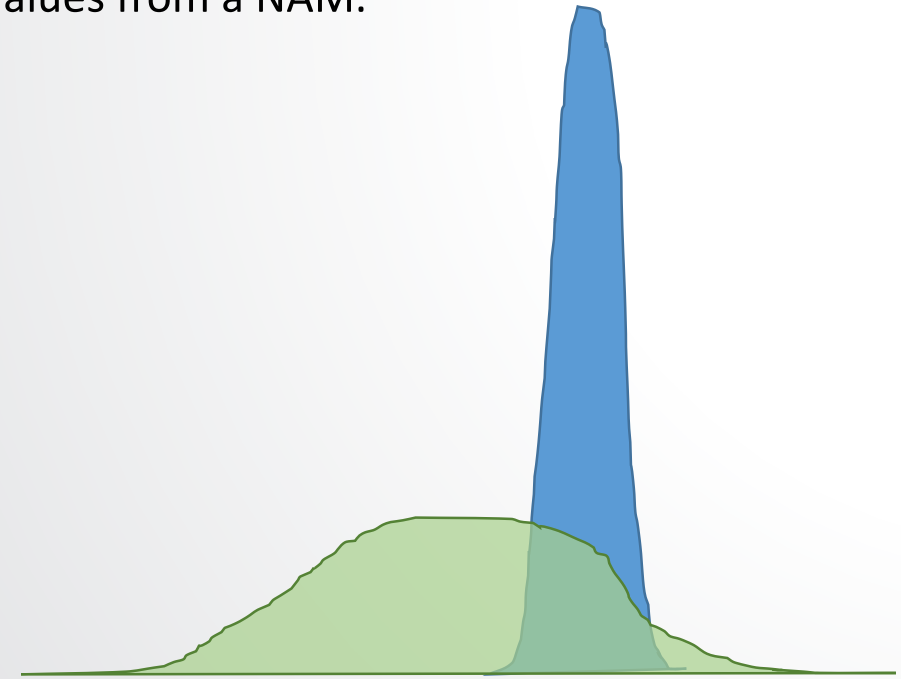
How do we define expectations of *in silico, in chemico,* and *in vitro* models for predicting repeat-dose toxicity?

In silico, in chemico, **and *in vitro* models cannot predict *in vivo* systemic effect values with greater accuracy than those animal models reproduce themselves.**

How do we express variability in traditional animal toxicity tests?

Quantitative: variance is a measure of how far values are spread from the average.

We need to know what the "spread" or variability of traditional effect levels (e.g., lowest effect levels, LELs, or lowest observable adverse effect levels, LOAELs) might be to know the range of acceptable or "good" values from a NAM.



Qualitative: We need to know if a specific effect is always observed or not.

		"Truth" (traditional toxicology)	
		Negative	Positive
Predicted (NAM)	Negative	True negative	False negative
	Positive	False positive	True positive

Research questions for understanding this variability

3 main questions	What is the range of possible systemic effect values (mg/kg/day) in replicate studies?	What is the maximal accuracy of a model that attempts to predict a systemic effect values for an unknown chemical?	What is the probability that an effect in adult animals will be observed in replicate studies?
Statistical approach to the question	• Residual root mean square error (RMSE) is an estimate of variance in the same units as the systemic effect values. • The RMSE can also be used to define a minimum prediction interval, or estimate range, for a model.	• The mean square error (MSE) is used to approximate the unexplained variance (not explained by study descriptors). • This unexplained variance limits the R-squared on a new model.	• Understand the reproducibility of treatment-related changes in specific endpoint targets (e.g., any effect on liver).

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ToxRefDB v2.0 is a source for a dataset to address these questions of quantitative variability.

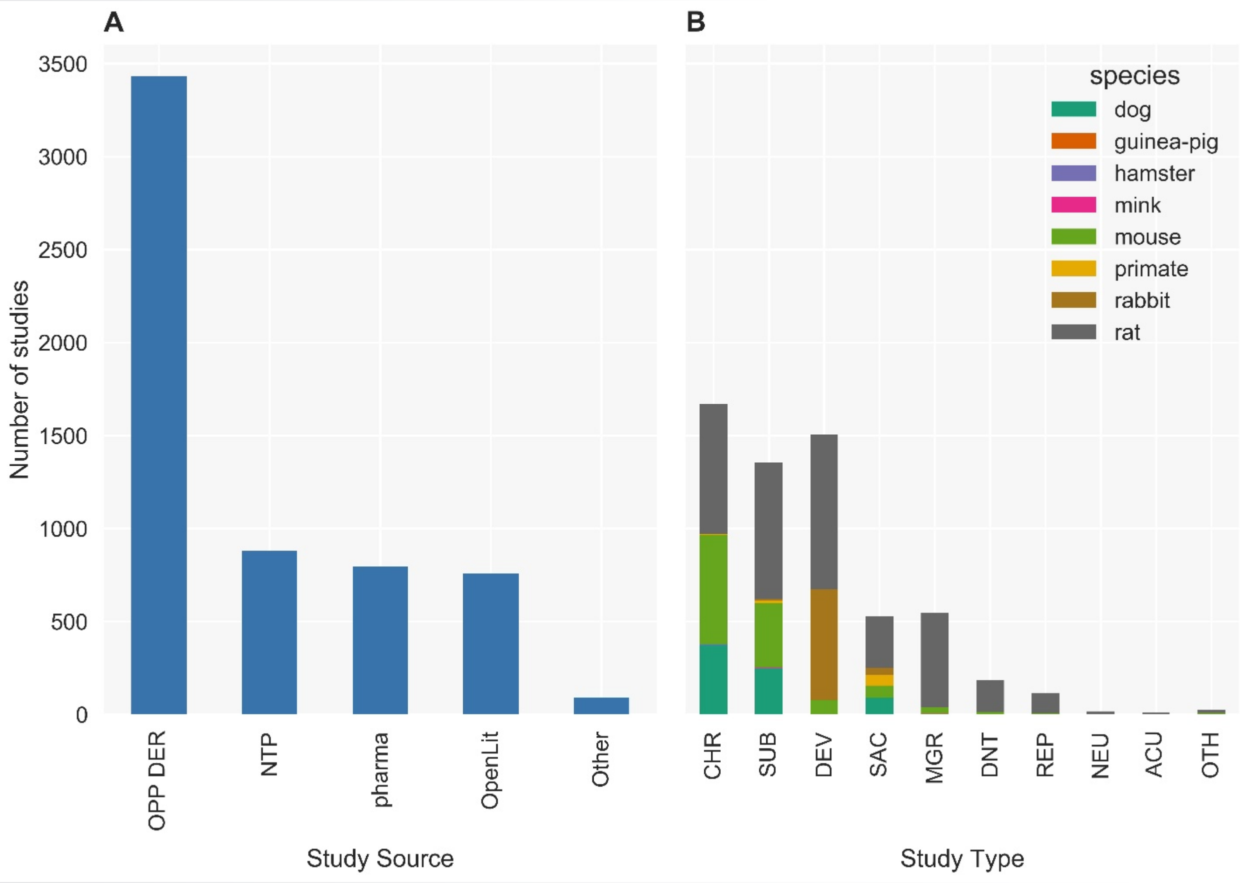


Figure 1. Number of studies by study type and species in ToxRefDB v2.0. *The study designs include chronic (CHR), sub-chronic (SUB), developmental (DEV), subacute (SAC), multigeneration reproductive (MGR), developmental neurotoxicity (DNT), reproductive (REP), neurotoxicity (NEU), acute (ACU), and other (OTH) for numerous species, but mostly for rat, mouse, rabbit, and dog.*

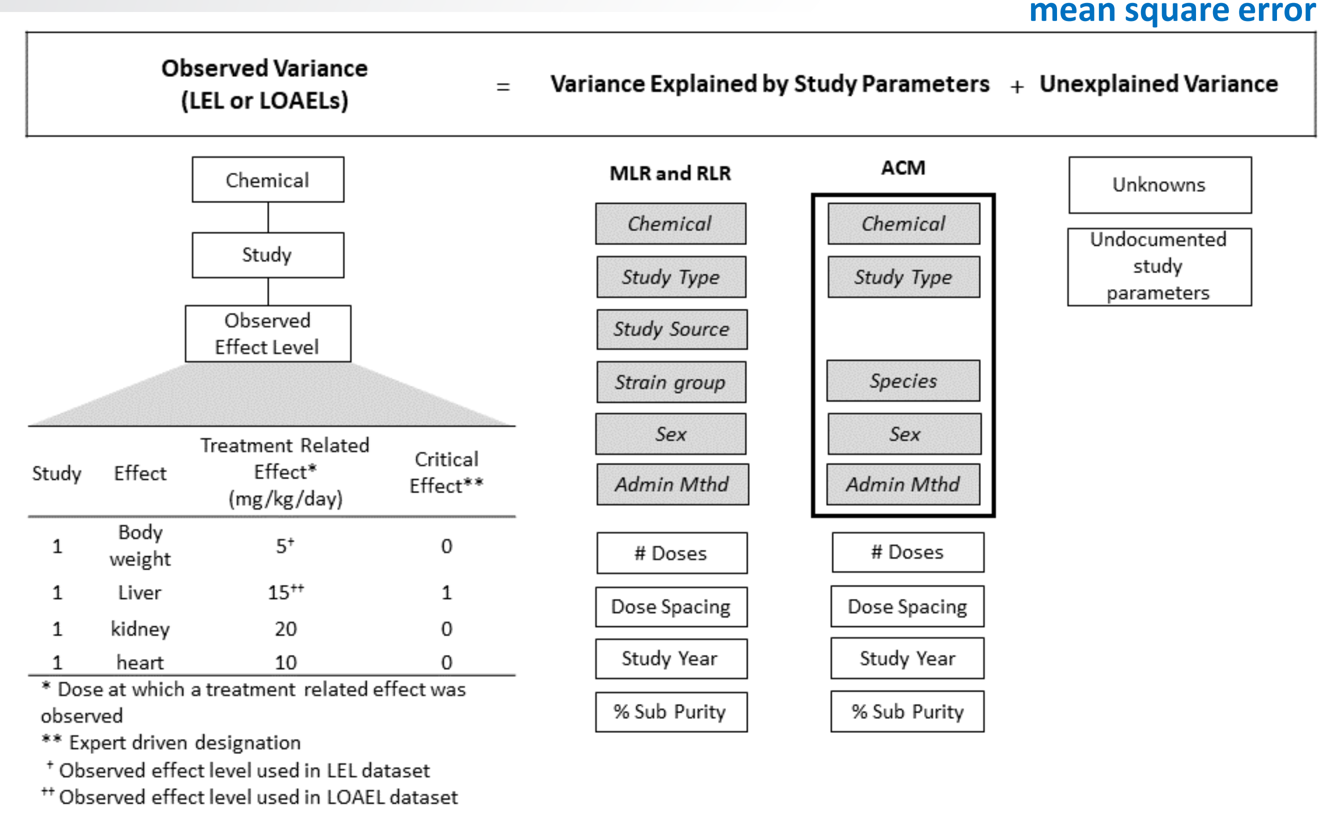
ToxRefDB v2.0 contains relevant study data to evaluate variability in traditional data for >1000 chemicals and >5000 studies.

Figure from Watford S, Pham LL, Wignall J, Shin R, Martin MT, Paul Friedman K. 2019. "ToxRefDB version 2.0: Improved utility for predictive and retrospective toxicology analyses." Reproductive Toxicology; 89: 145-158. https://doi.org/10.1016/j.reprotox.2019.07.012

Based on the study descriptors in ToxRefDB v2.0, we developed statistical models of the variance in quantitative systemic effect level values.

Total variance

Approximated by mean square error



Observed Variance (LEL or LOAELs) = Variance Explained by Study Parameters + Unexplained Variance

Chemical

Study

Observed Effect Level

Study	Effect	Treatment Related Effect* (mg/kg/day)	Critical Effect**
1	Body weight	5^{+}	0
1	Liver	15^{++}	1
1	kidney	20	0
1	heart	10	0

* Dose at which a treatment related effect was observed

** Expert driven designation

[+] Observed effect level used in LEL dataset

[++] Observed effect level used in LOAEL dataset

MLR and RLR

Chemical, *Study Type*, *Study Source*, *Strain group*, *Sex*, *Admin Mthd*, # Doses, Dose Spacing, Study Year, % Sub Purity

ACM

Chemical, *Study Type*, *Species*, *Sex*, *Admin Mthd*, # Doses, Dose Spacing, Study Year, % Sub Purity

Unknowns

Undocumented study parameters

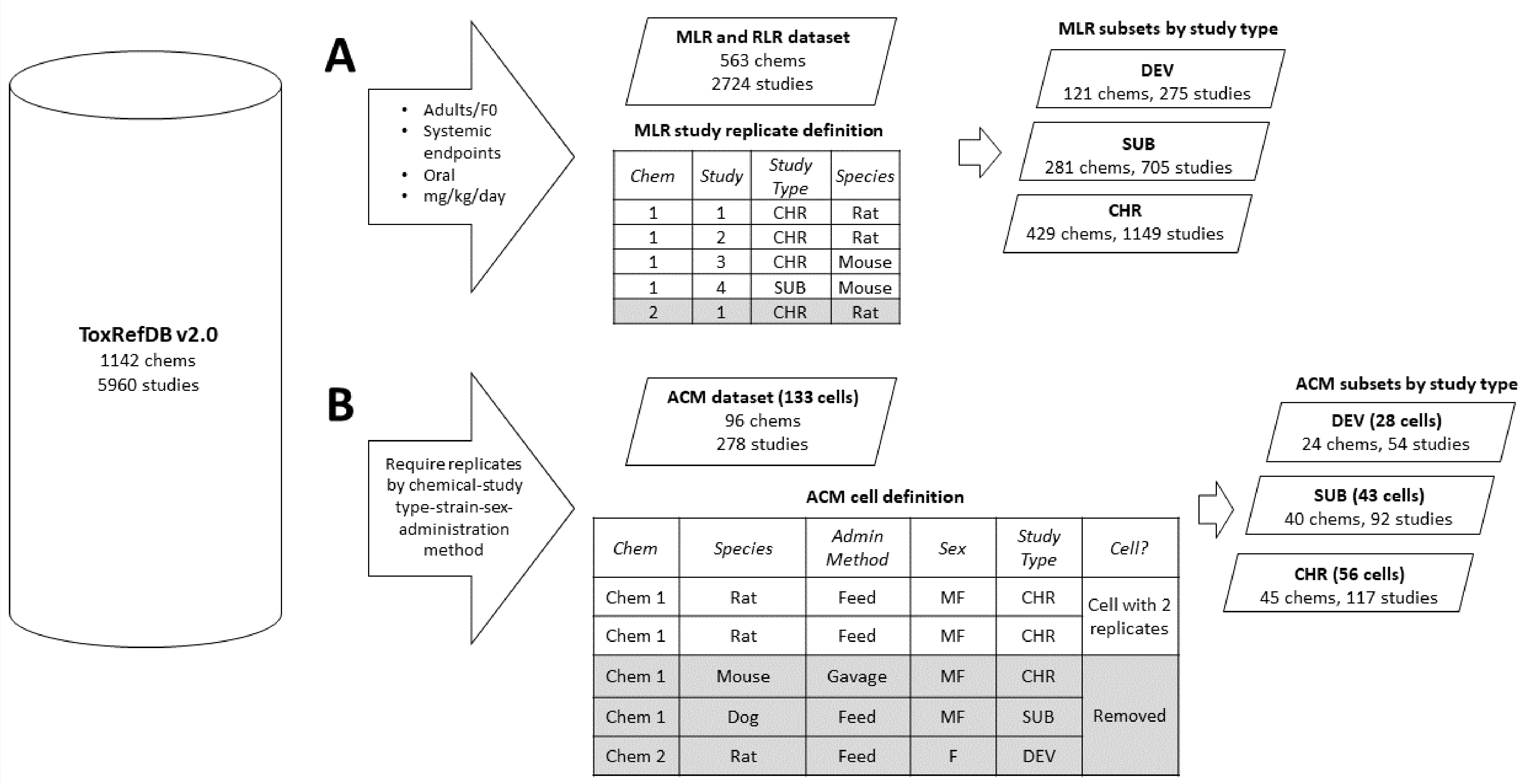
Using two approaches:

	Multilinear regression (MLR, RLR)	Augmented cell means (ACM)
Aggregation level	Chemical	Chemical-Study Type-Species-Sex-Admin Method combination
Replicate definition stringency	Not stringent	Stringent
N	Maximized; ↓ impact of outliers/database error rate	Small; may bias variance estimate
Study descriptors	Contribute independently to variance	Accounts for possible interactions among descriptors

Figure 2. Statistical model of the variance. *LEL = lowest effect level; LOAEL = lowest observable adverse effect level. The LEL is the lowest treatment-related effect observed for a given chemical in a study, and the LOAEL is defined by expert review as coinciding with the critical effect dose level from a given study. Multiple studies for a given chemical yield multiple LELs and LOAELs for computation of variance. MLR = multilinear regression; RLR = robust linear regression; ACM = augmented cell means; Adm. Method = administration method; % Sub Purity = % substance purity used in the study. The gray shaded study descriptor boxes are categorical variables, and the white study descriptor boxes are continuous variables. The box around five categorical study descriptors for the ACM indicates these were concatenated to a factor to define study replicates.*

Pham LL, Watford S, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, Paul Friedman K. *Accepted.* "Variability in in vivo studies: Defining the upper limit of performance for predictions of systemic effect levels." Computational Toxicology.

Our workflow for evaluating variance in repeat dose toxicity information



ToxRefDB v2.0
1142 chems
5960 studies

A

- Adults/F0
- Systemic endpoints
- Oral
- mg/kg/day

MLR and RLR dataset
563 chems
2724 studies

MLR study replicate definition

Chem	*Study*	*Study Type*	*Species*
1	1	CHR	Rat
1	2	CHR	Rat
1	3	CHR	Mouse
1	4	SUB	Mouse
2	1	CHR	Rat

MLR subsets by study type

DEV
121 chems, 275 studies

SUB
281 chems, 705 studies

CHR
429 chems, 1149 studies

B

Require replicates by chemical-study type-strain-sex-administration method

ACM dataset (133 cells)
96 chems
278 studies

ACM cell definition

Chem	*Species*	*Admin Method*	*Sex*	*Study Type*	*Cell?*
Chem 1	Rat	Feed	MF	CHR	Cell with 2 replicates
Chem 1	Rat	Feed	MF	CHR	
Chem 1	Mouse	Gavage	MF	CHR	Removed
Chem 1	Dog	Feed	MF	SUB	
Chem 2	Rat	Feed	F	DEV	

ACM subsets by study type

DEV (28 cells)
24 chems, 54 studies

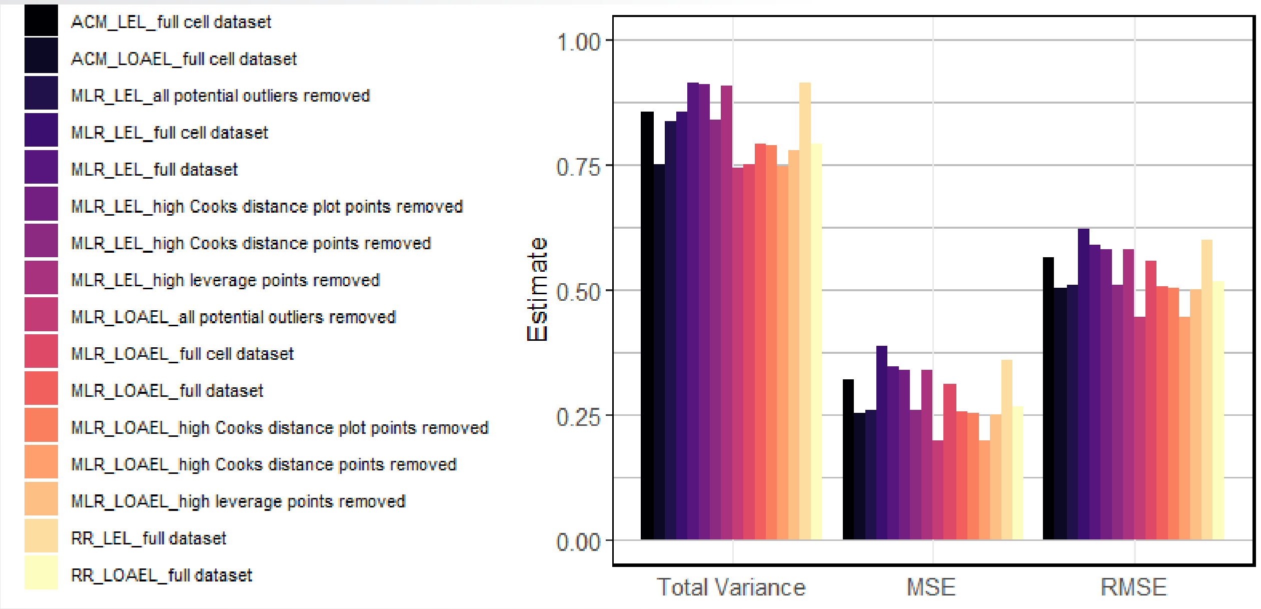
SUB (43 cells)
40 chems, 92 studies

CHR (56 cells)
45 chems, 117 studies

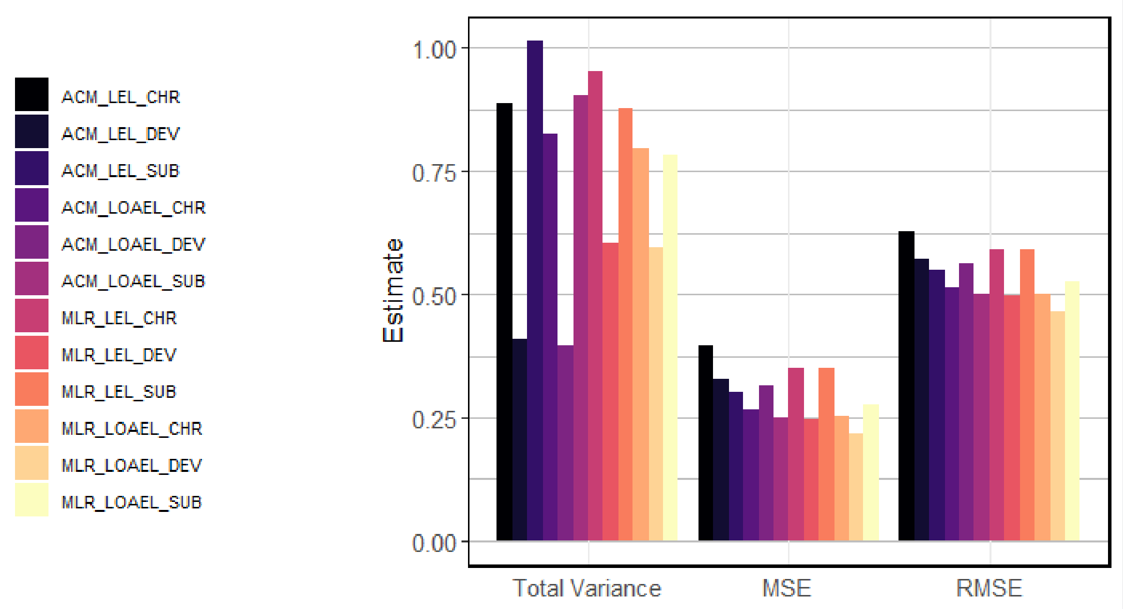
Figure 1. Variance estimation workflow.
CHR = chronic; DEV = developmental (adults only); SUB = subchronic; cells are defined by the factor of all categorical variables; MF = males and females; F = females; MLR = multilinear regression; RLR = robust linear regression; ACM = augmented cell means.

28 models to approximate total variance, unexplained variance (MSE), and then the spread of the residuals from the statistical models (RMSE)

Statistical models for LELs and LOAELs for the full dataset



Statistical models for LELs and LOAELs for datasets subset by study type

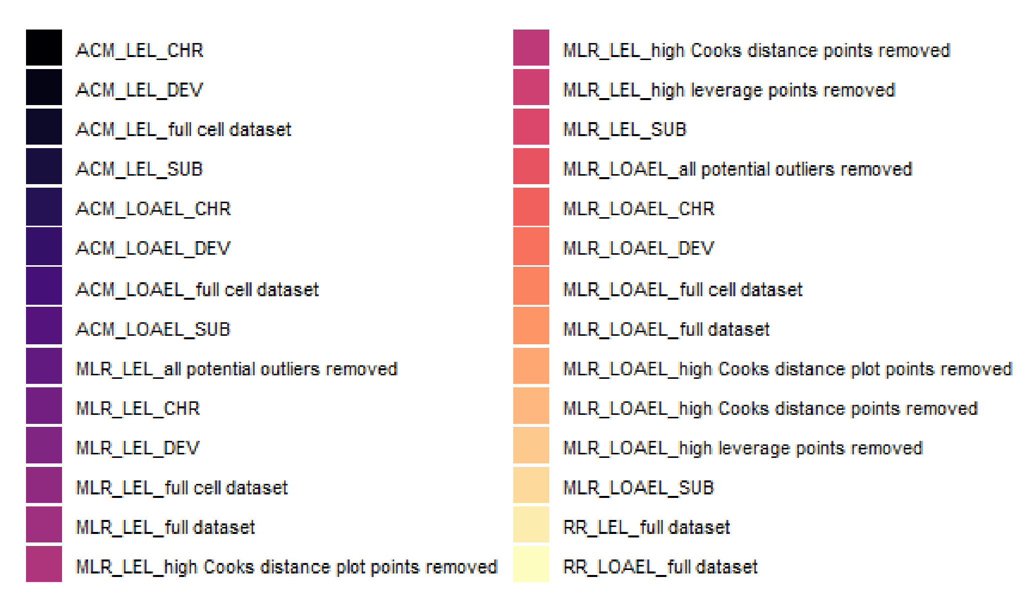


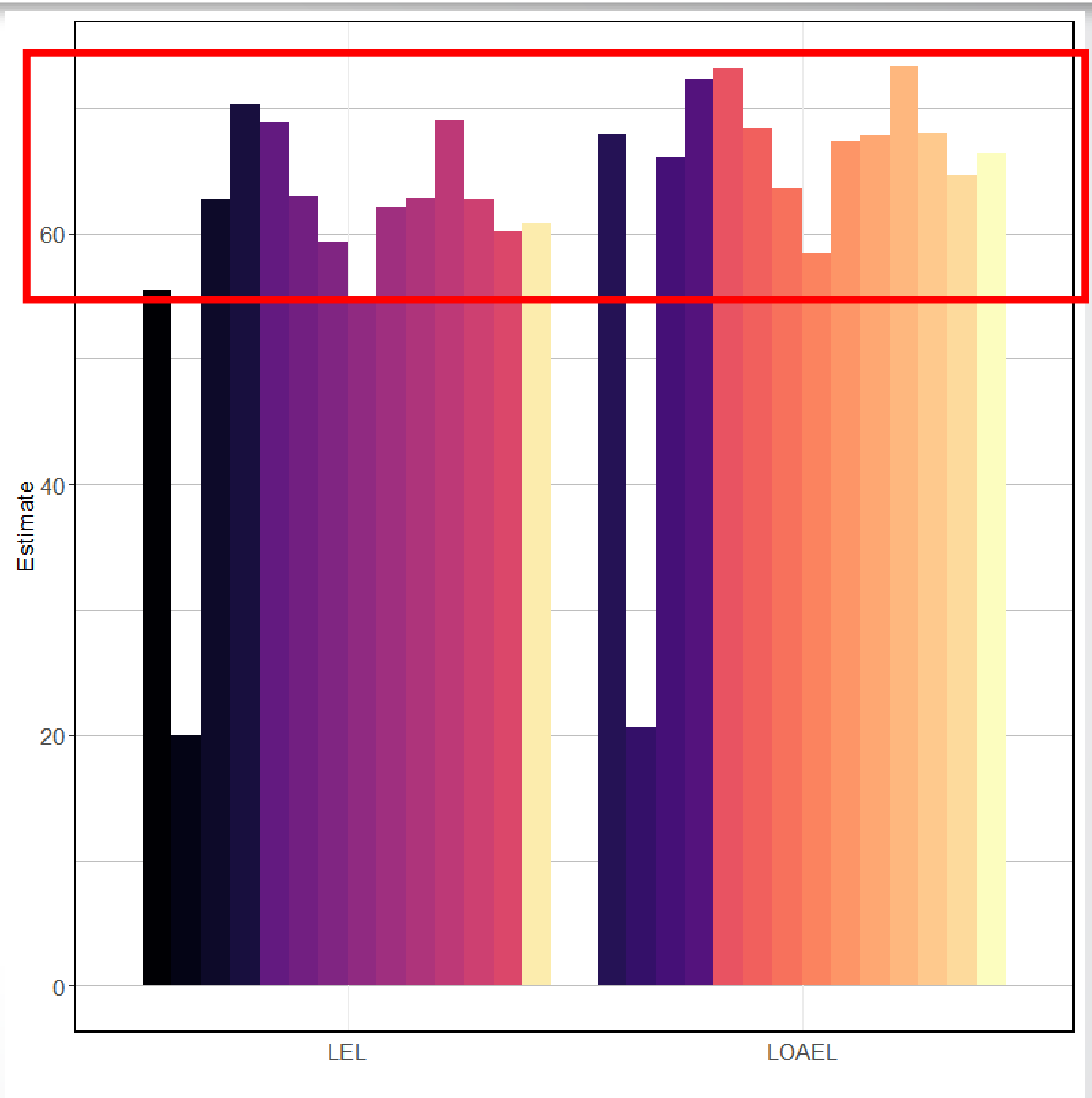
- Total variance in systemic toxicity effect values likely approaches 0.75-1 (units of ($\log_{10}$-mg/kg/day$)^2$)
- MSE (unexplained variance) is 0.2 – 0.4 (units of ($\log_{10}$-mg/kg/day$)^2$)
- RMSE is 0.45-0.60 $\log_{10}$-mg/kg/day
- RMSE is used to define a 95% minimum prediction interval (i.e., based on the standard deviation or spread of the residuals)

Based on tables in Pham LL, Watford S, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, Paul Friedman K. *Accepted.* "Variability in in vivo studies: Defining the upper limit of performance for predictions of systemic effect levels." Computational Toxicology.

Percent explained variance is also stable across statistical models.

- The % explained variance (amount explained by study descriptors) likely approaches 55-73%.
- This means that the R^2 on some new, predictive model would approach 0.55 to 0.73 as an upper bound on accuracy.





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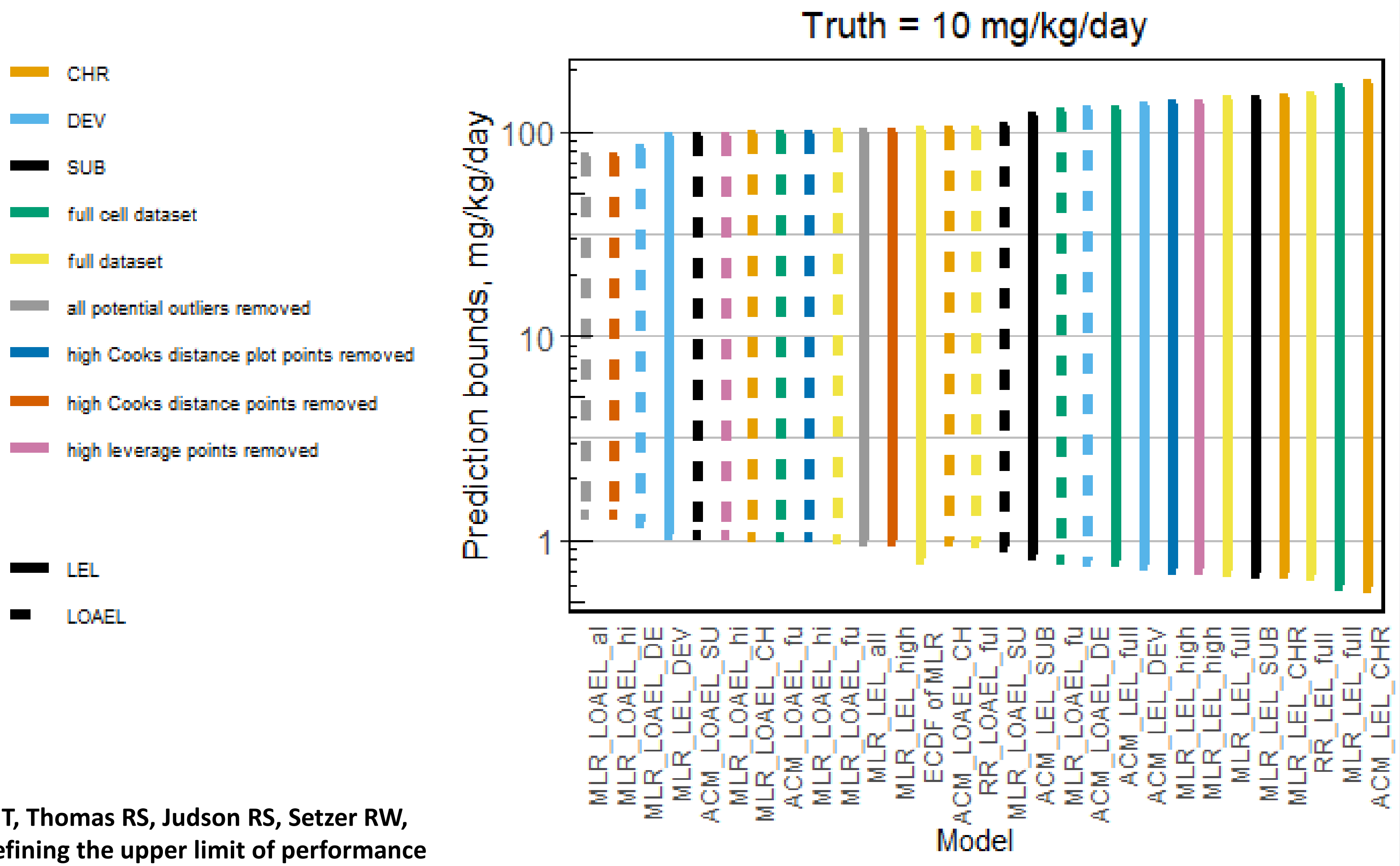
Range of 95% minimum prediction intervals across the modeling approaches, effect levels, and study types is 58-284-fold

If attempting to use a NAM-based predictive model for prediction of a reference systemic effect level value of 10 mg/kg/day, it is likely that given the variability in reference data of this kind, that a model prediction of somewhere between 1 and 100 mg/kg/day would be the greatest amount of accuracy achievable.



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How does this compare to previous work in this area?

- Previous QSAR models of subchronic oral rat NOAEL values: R^2 approaches 0.46-0.71, i.e. 46-71% of residual variance could be explained for the reference set (Veselinovic et al. 2016; Toropov et al. 2015; Toropova et al. 2017).
- A multi-linear regression QSAR model of chronic oral rat LOAEL values for approximately 400 chemicals, demonstrated a RMSE of 0.73 $\log_{10}$(mg/kg-day), which was similar to the size of the variability in the training data, ±0.64 $\log_{10}$(mg/kg-day), suggested that the error in the model approached the error in the reference data from different laboratories (Mazzatorta et al. 2008; Helma et al. 2018).

Few examples of quantitative variability in this domain to cite, but suggest that similar thresholds of 50-70% explained variance and RMSE of 0.5-0.7 may exist in other larger reference data sets for systemic toxicity in subchronic and chronic animal studies.

Primary conclusions of our work

- Variability in *in vivo* toxicity studies limits predictive accuracy of NAMs.
- Total variance in systemic effect levels and the fraction explained were quantified.
- Maximal R-squared for a NAM-based predictive model of systemic effect levels may be 55 to 73%; i.e., as much as 1/3 of the variance in these data may not be explainable using study descriptors.
- The estimate of variance (RMSE) in curated LELs and/or LOAELs approaches a 0.5 log10-mg/kg/day.
- **Understanding that a prediction of an animal systemic effect level within ± 1 log10-mg/kg/day fold demonstrates a *very good* NAM is important for acceptance of NAMs for chemical safety assessment.**
- Finally, construction of NAM-based effect level estimates that offer an equivalent level of public health protection as effect levels produced by methods using animals may provide a bridge to major reduction in the use of animals as well as identification of cases in which animals may provide scientific value.

Data variability informs model uncertainty

Model Uncertainty

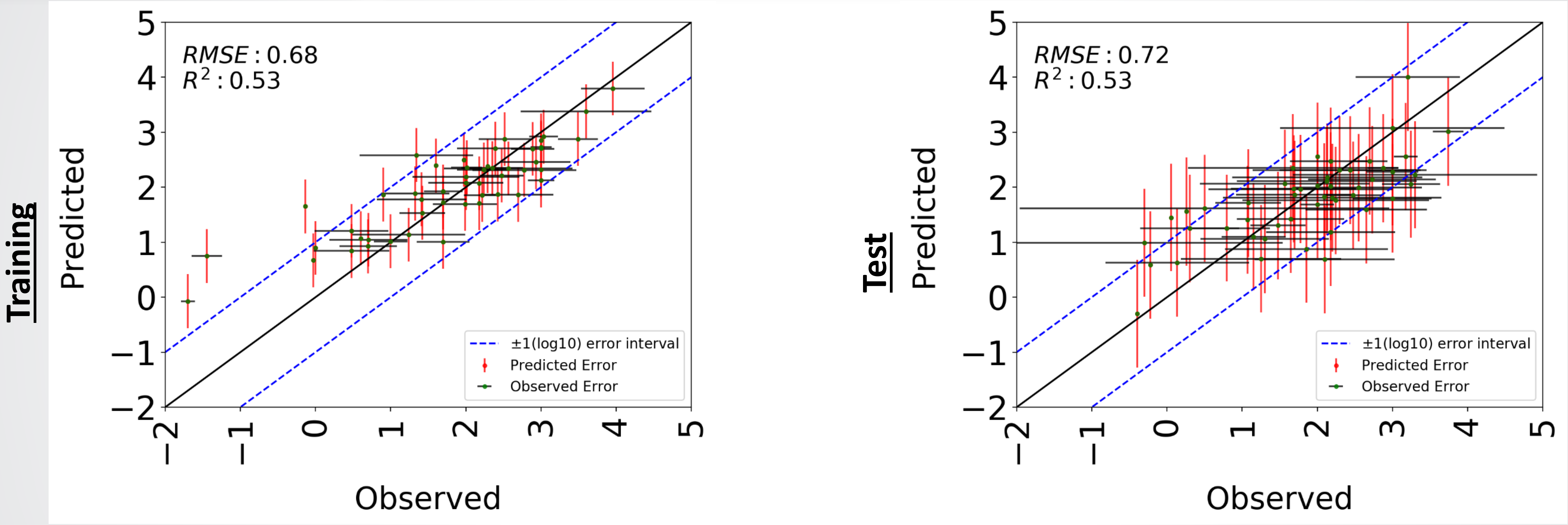
- A model gives a result (a POD), but this is an estimate of the "true" POD. The true POD is mostly unknown.
- Uncertainty in the evaluation data will lead to uncertainty in the model and our estimate of its quality

Point-estimate with confidence interval models

- A POD distribution was constructed for each chemical (μ = Median experimental POD value from all studies, σ = 0.5 $\log_{10}$-units)
- *100* bootstrap models were built with random sampling of POD values for each chemical from the pre-generated POD distribution.
- Predicted POD_{QSAR} = mean of 100 bootstrap predictions
- Confidence interval of POD_{QSAR} = ±1 standard deviation of 100 bootstrap predictions

Based on Pradeep P, Paul Friedman K, Judson RS. Submitted. "Structure-based QSAR models to predict repeat dose toxicity points of departure." Computational Toxicology.

Unpublished results for systemic toxicity POD_{QSAR}



Observed versus predicted plot for 50 (random) chemicals with the observed and predicted confidence intervals

- The predicted 95% confidence interval (error bar) for each chemical is calculated as two standard deviations of the predictions from the models.
- The observed 95% confidence interval (error bar) is calculated as two standard deviations of the experimental data for each chemical.

Based on Pradeep P, Paul Friedman K, Judson RS. Submitted. "Structure-based QSAR models to predict repeat dose toxicity points of departure." Computational Toxicology.

Thank you for listening

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