Accelerating the Pace of Chemical Risk Assessment Case Studies and Implications for New Approach Methods-Based PODs

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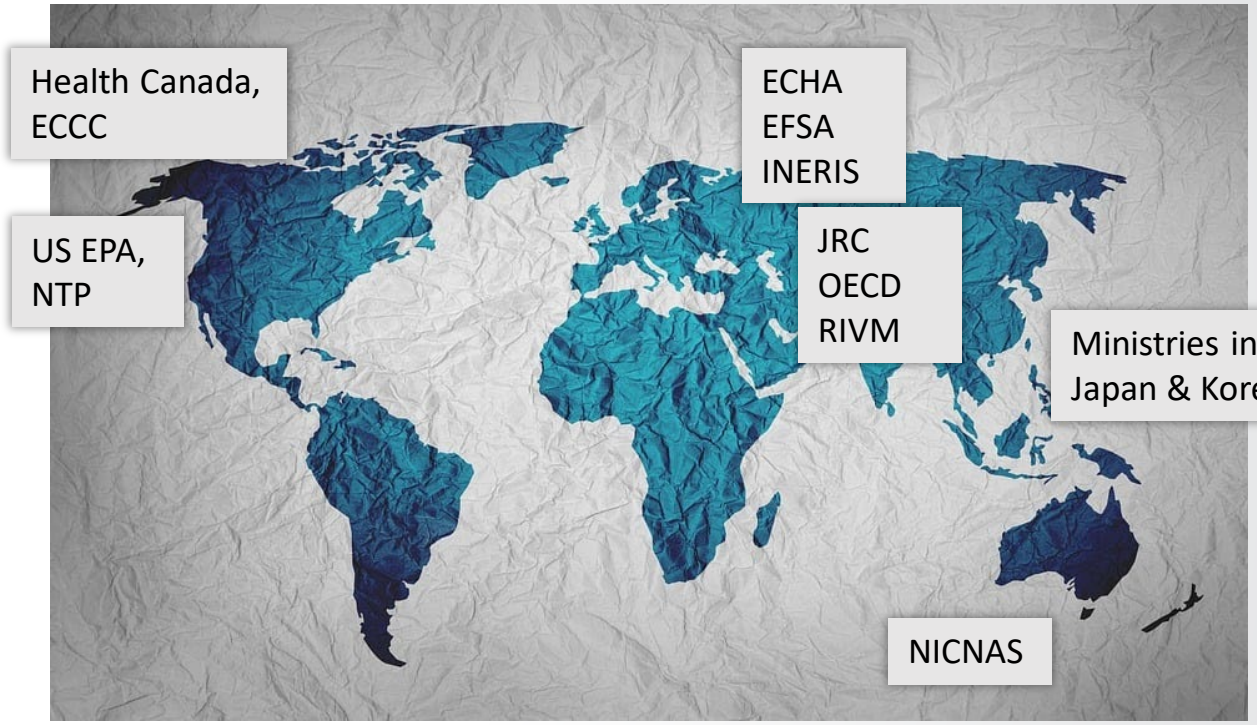
Goals of the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

- To bring together international regulators to discuss progress and barriers in applying new approach methods (NAMs) to prioritization, screening, and quantitative risk assessment applications
- To formulate and execute collaborative case studies to advance this primary objective



Key questions that unify APCRA case study collection

How to make progress on NAM-based:

Prioritization
First tier assessment
Full assessments
Replacement of animal studies
Classification and labeling

- Current barriers?
  - *Benchmarking NAMs against animal studies*
  - *Potential technology limitations*
  - *Lack of confidence in NAM application*
  - *Differing regulatory needs for decision-making*
- Near-term efforts?
  - *Exploring ways of describing hazard and exposure with NAMs*
  - *Safety instead of adversity*
  - *Analysis of NAM uncertainties*
- Greater acceptance and scientific confidence by the regulators and public?
  - *Training and communication*
  - *Case studies*

Much of our work in some way has been aimed at evaluating POD_{NAM} and its utility

- Part 1: POD learnings and limitations from the APCRA **retrospective** case study
- Part 2: POD learnings from APCRA **prospective** case study (in preparation)
- Part 3: POD learnings from organ-level reproducibility study (in preparation)

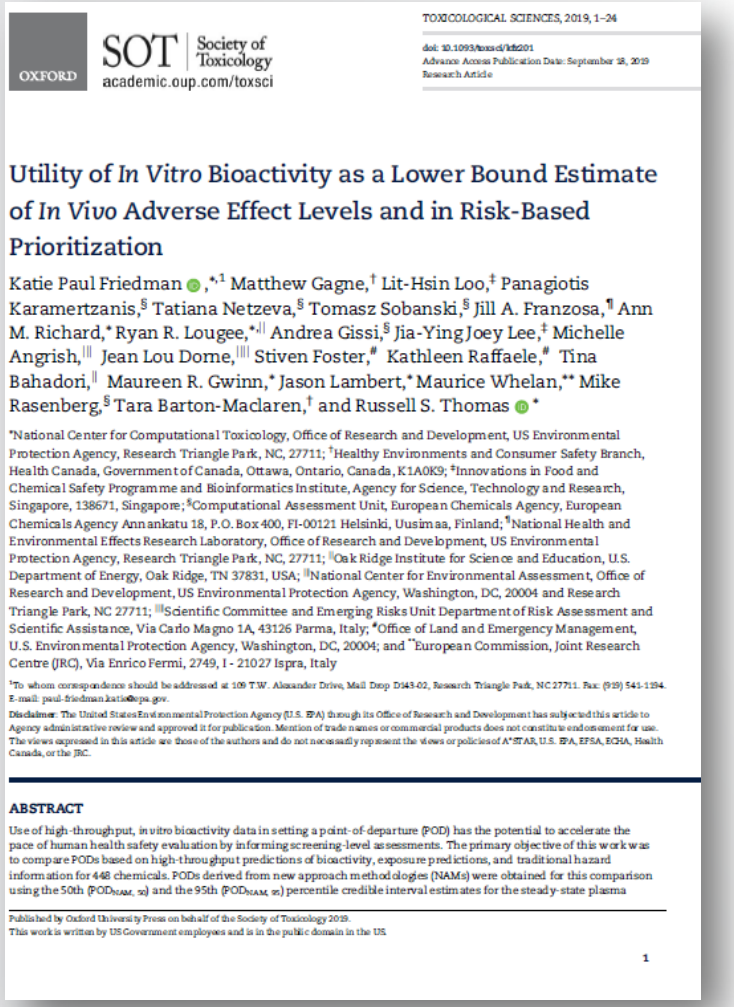
Based on current case studies, what quantitative uncertainty factor would be needed to ensure conservatism of the POD_{NAM} for all chemicals?

Thinking ahead to the conclusion: What could be done to customize the uncertainty factor to the toxicokinetic profile of the chemical?

Part 1

APCRA retrospective case study (focus on POD_{NAM})

APCRA retrospective case study developed confidence in a straight-forward workflow for a protective POD_{NAM}



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Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

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ABSTRACT

Use of high-throughput, *in vitro* bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening-level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals. PODs derived from new approach methodologies (NAMs) were obtained for this comparison using the 50th ($POD_{NAM,50}$) and the 95th ($POD_{NAM,95}$) percentile credible interval estimates for the steady-state plasma

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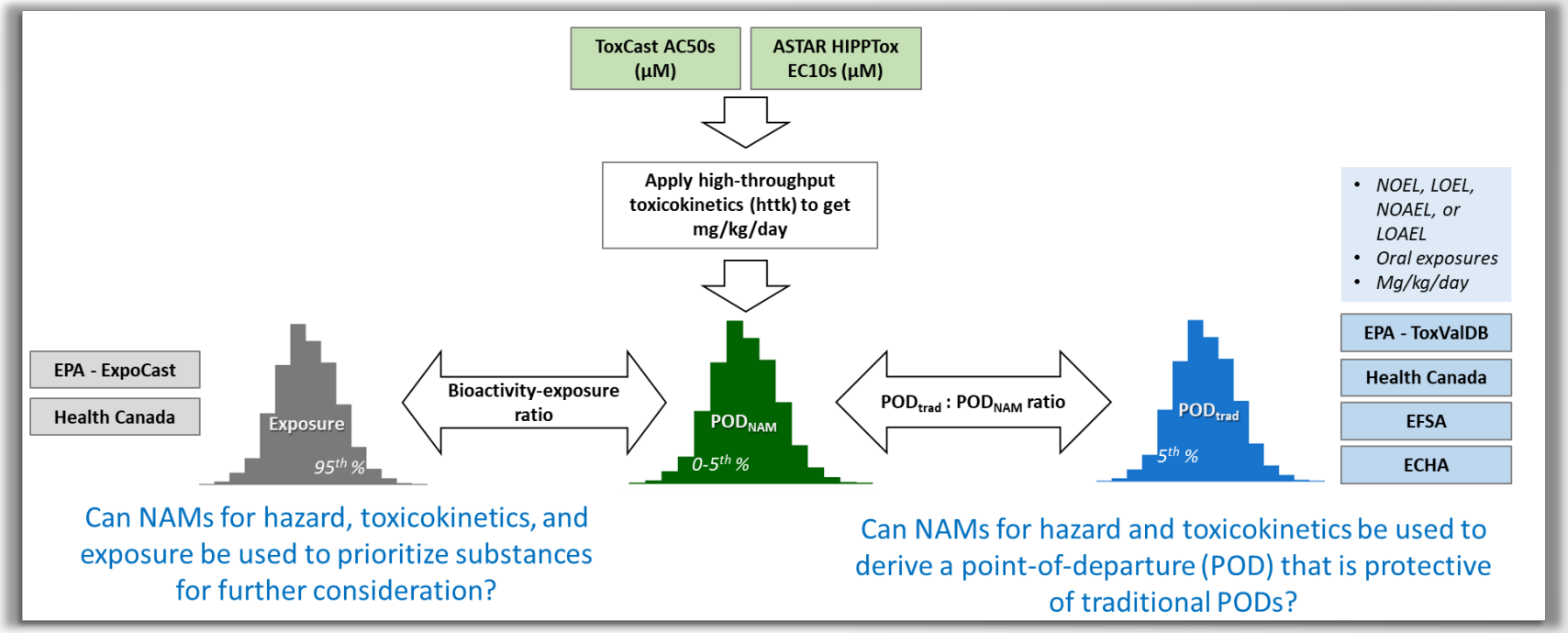
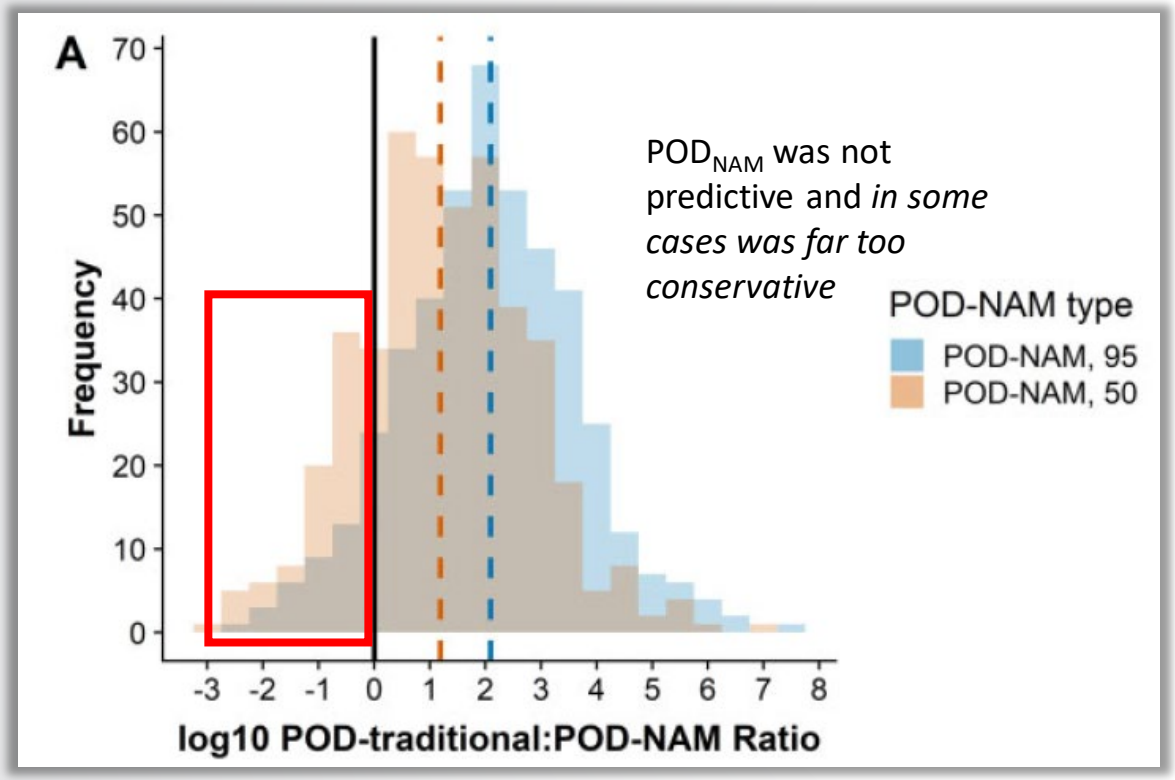


Figure from Paul Friedman *et al.* 2020

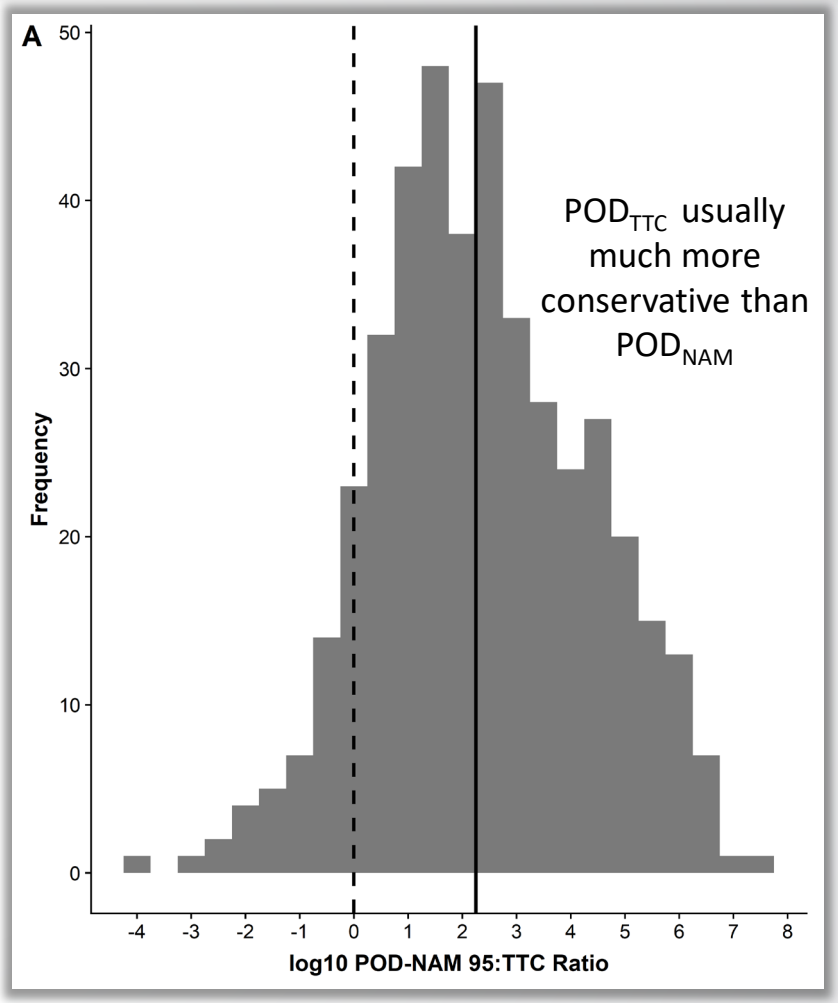
10.1093/toxsci/kfz201

Convinced the field we could produce POD_{NAM} < $POD_{traditional}$ and further popularized a simple workflow for IVIVE and bioactivity:exposure ratio



POD_{NAM} was not predictive and *in some cases was far too conservative*

….but with as much as ~2-3 orders of magnitude uncertainty for capturing $POD_{traditional}$ for a minority (<10%) of the 448 chemicals included



POD_{TTC} usually much more conservative than POD_{NAM}

POD_{NAM} not conservative enough for chemistries we could catch with TTC, e.g. organophosphates and carbamates

Figures from Paul Friedman *et al.* 2020

Part 2

APCRA prospective case study (focus on POD_{NAM})

APCRA prospective case study aims to bridge new approach methods (NAMs) to the need for any additional *in vivo* data in an international context (*in prep*)

In silico and *in vitro* NAMs for toxicodynamics and toxicokinetics

~200 substances

Goal: Point of departure (POD) estimates and insights into hazard

5-day rodent studies using transcriptomics in liver/kidney

~20 substances

Goal: Greater certainty in POD

Development of a NAM-enhanced 90-day study?

# of substances tbd

Goal: Confirmation of POD from 5-day studies and/or hazard profile, if needed

- Building confidence in the connections between NAMs and traditional toxicology studies
- Inform needs for data-poor substances in an international context
- POD_{NAM} calculation and development of "hazard flags" to suggest particular biological indications

ECHA EUROPEAN CHEMICALS AGENCY

EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

European Commission ENVIRONMENT

SINGAPORE Agency for Science, Technology and Research

UNIVERSITY OF BIRMINGHAM

Health Canada Santé Canada

rivm National Institute for Public Health and the Environment

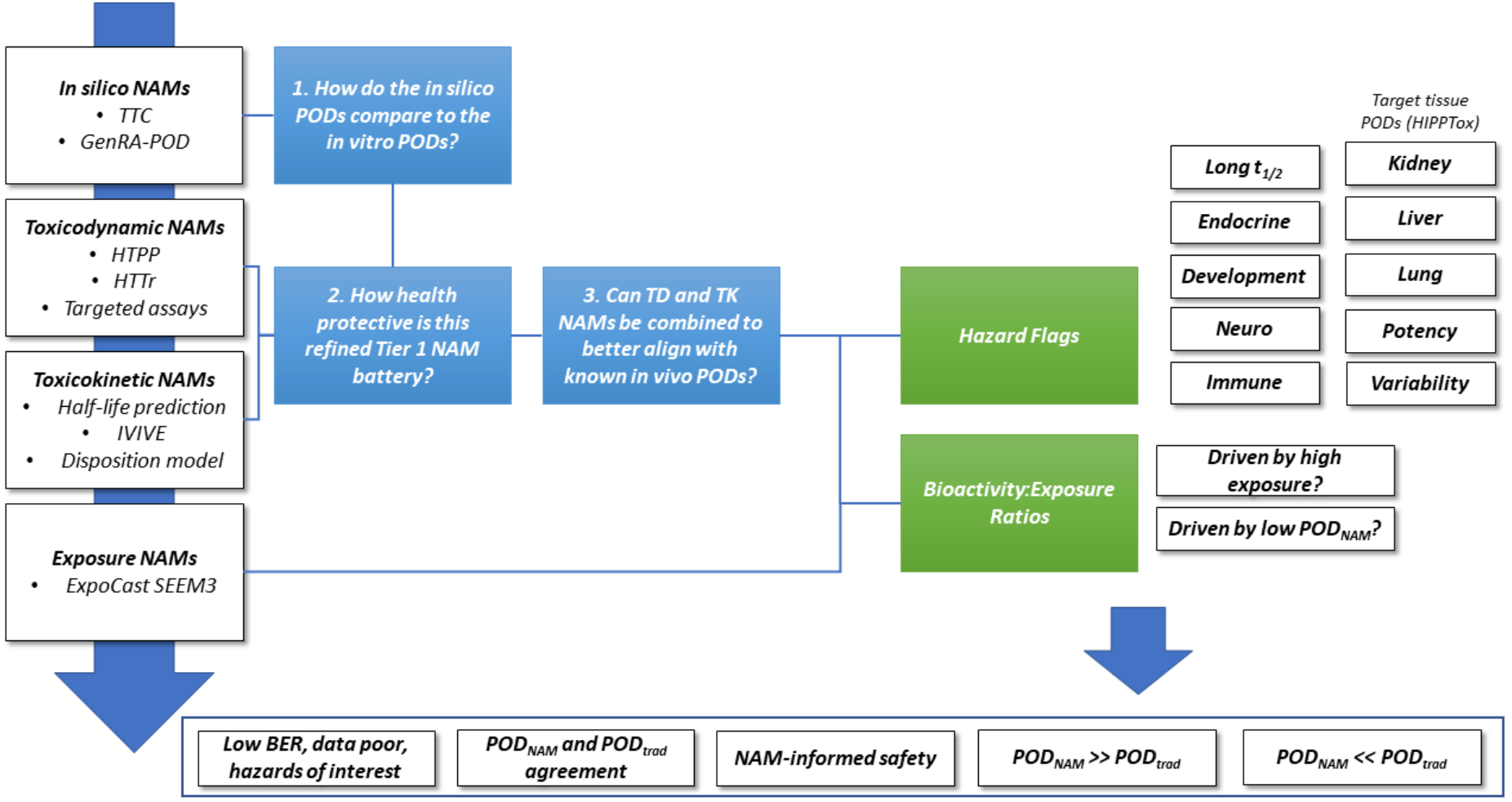
EPA United States Environmental Protection Agency

NTP National Toxicology Program U.S. Department of Health and Human Services

APCRA ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

In silico and *in vitro* NAMs are combined prospectively to identify chemicals with putative hazard and BER based prioritization

Figure 1. Mapping the approach.



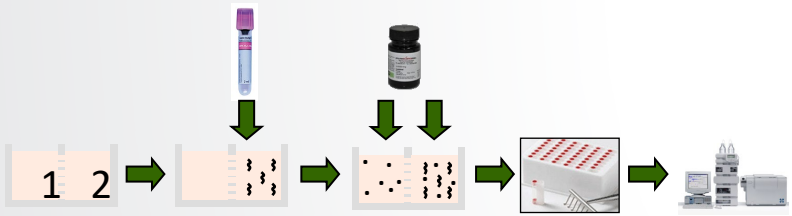
- Refine assay battery and include assays with broad biological coverage
- Refine IVIVE approach
- Experiment to understand which data may be most informative of $POD_{traditional}$
- Include indicators of putative hazard and related interests (hazard flags)
- Include updated exposure predictions for BER

5 potentially overlapping groups that the NAM data can inform for selection of chemicals for additional screening

IVIVE approach based on R library 'httk'

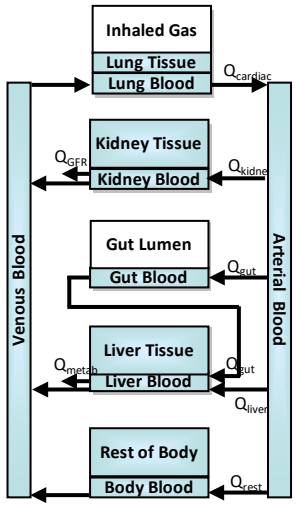
in vitro data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



= ***httk***

- Preference to PBTK model over 3 compartment steady state model
- Preference to in vitro HTTK data over in silico HTTK predictions
- Predictive modeling of available estimates of a lower bound *in vivo* POD using AEDs from 3 compartment steady state or PBTK modeling failed to show unique improvement

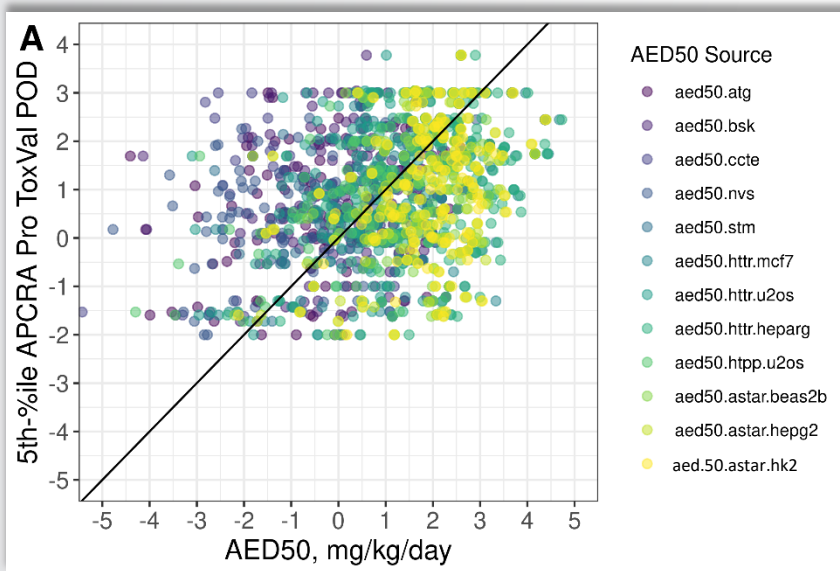
IVIVE = in vitro to in vivo extrapolation (of dose)

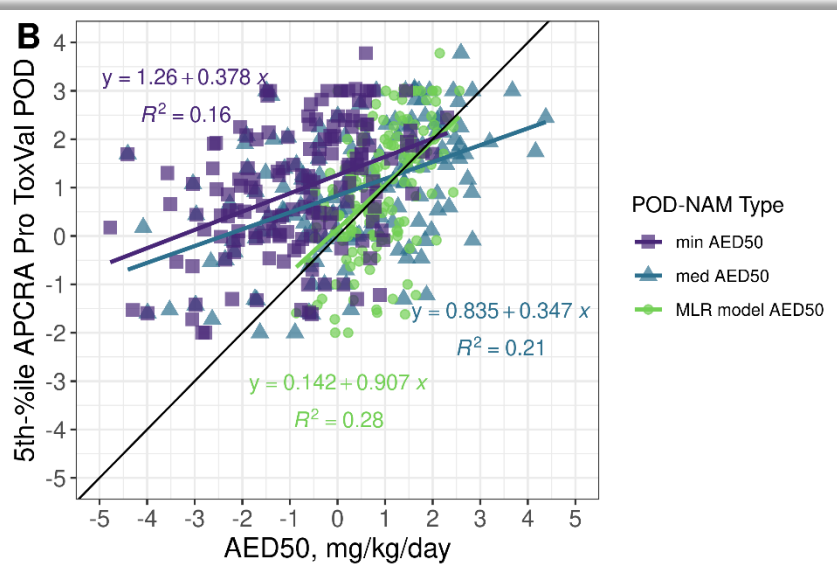
Is POD_{AED50} predictive of $POD_{traditional}$? With some amount of error

(A) Minimum AED50s by assay technology fail to suggest that a single technology can accurately predict estimates of $POD_{traditional}$

(B) The median from the set of minimum AED50s by assay technology performs fairly well in predicting estimates of $POD_{traditional}$

(C) Predicting estimates of $POD_{traditional}$ with TD and TK NAMs resulted in RMSE that approach 1 to 1.2 log_{10}-mg/kg/day





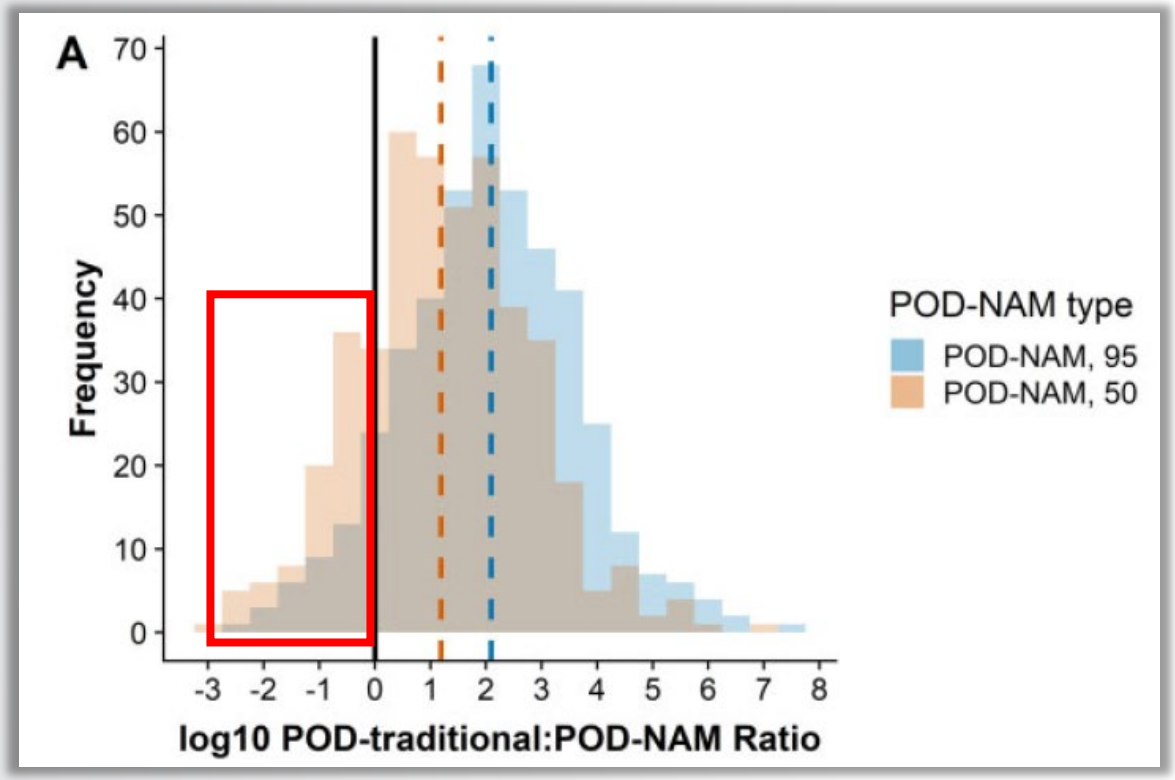
C

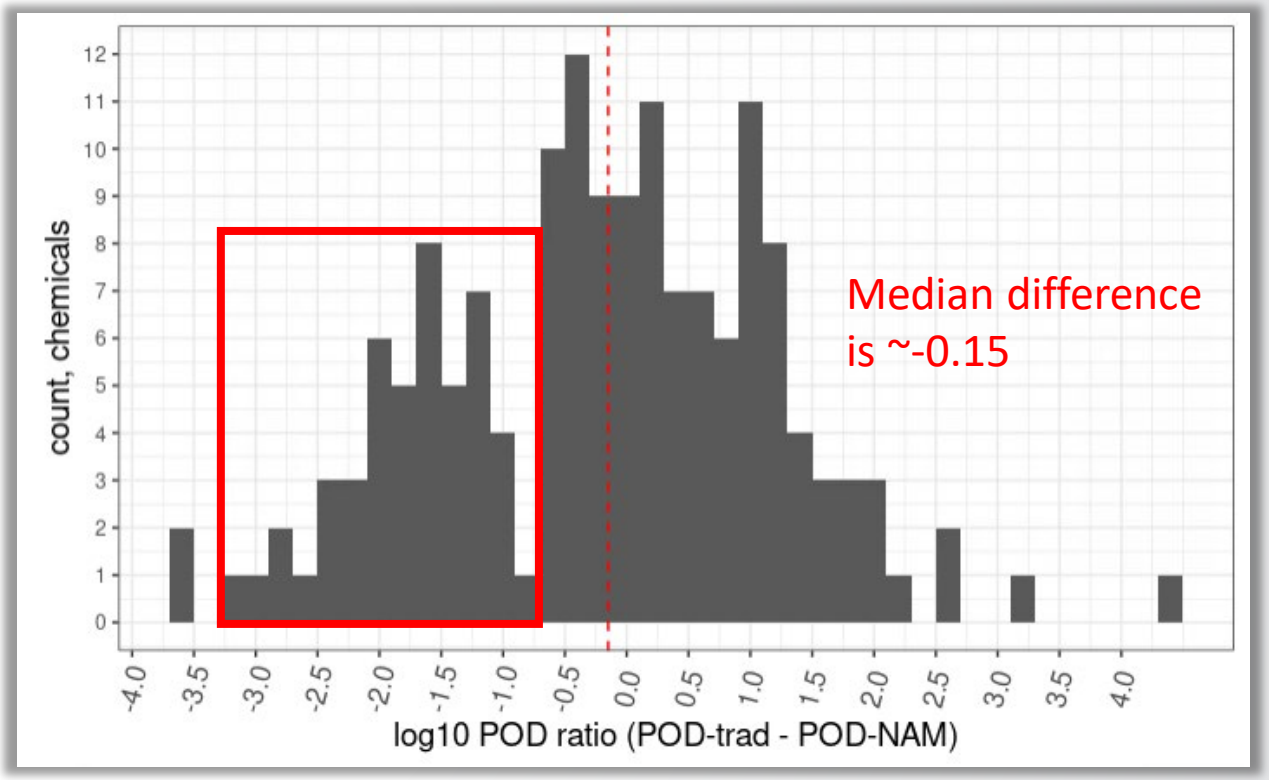
	Min AED50 Model	Med AED50 Model	MLR AED50 Model
RMSE	1.182	1.147	1.098
R-squared	0.164	0.213	0.279

A multi-linear regression model performs slightly better than the median (as did a random forest model). Models failed to reduce the RMSE below 1.1 log_{10}-mg/kg/day.

If no other data were available, a possible adjustment factor to ensure conservatism for using POD_{AED50} could be ~ 2-2.5 log_{10}-mg/kg/day (-1.96*RMSE)

How does the overall level of conservatism of POD compare to the retrospective case study?



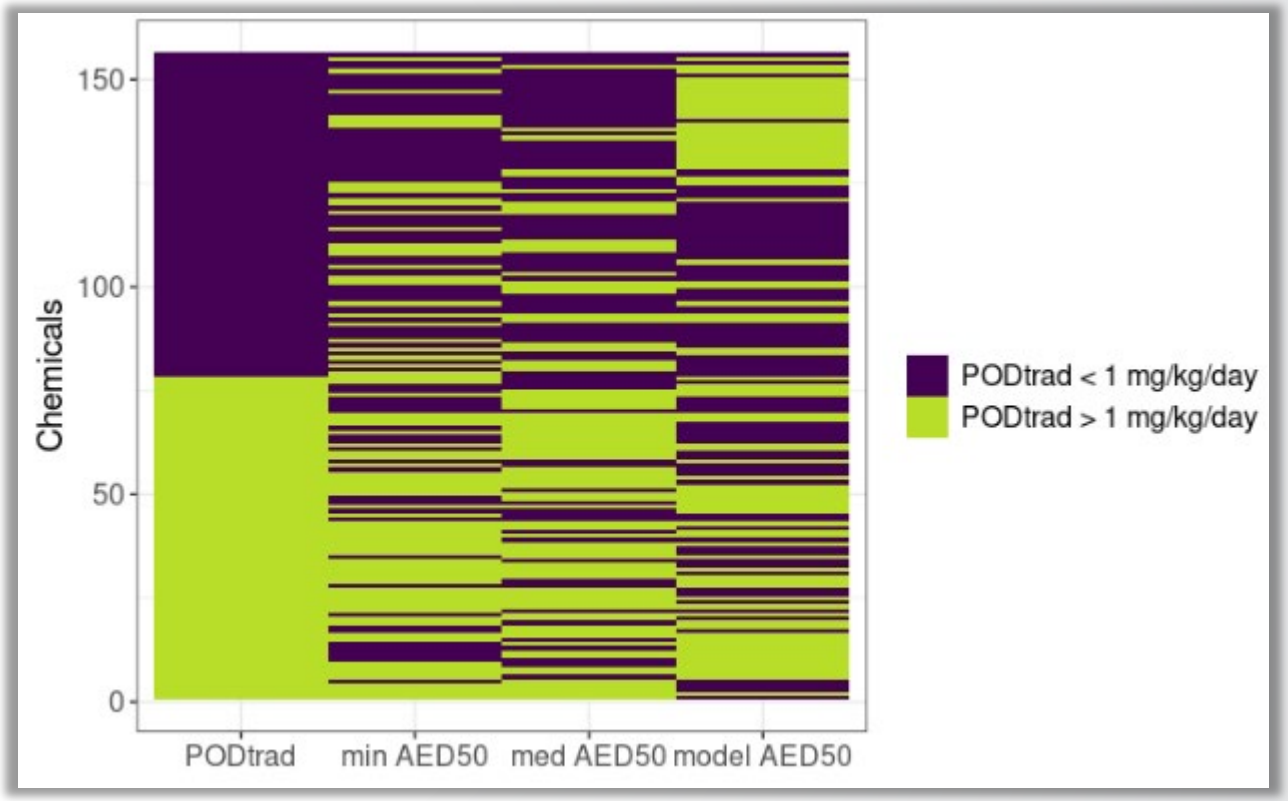


The median difference in the APCRA prospective case study is smaller, but the tails of these distributions still suggest that for a subset of chemicals we may not be conservative enough (perhaps by 3 orders of magnitude).

How well does POD_{AED50} recapitulate the order of $POD_{traditional}$?

Condition	% of chemicals with $POD_{traditional} < 10$
$POD_{medAED50} < 10$	61%
$POD_{medAED50} < 100$	85%
$POD_{medAED50} < 1000$	99%

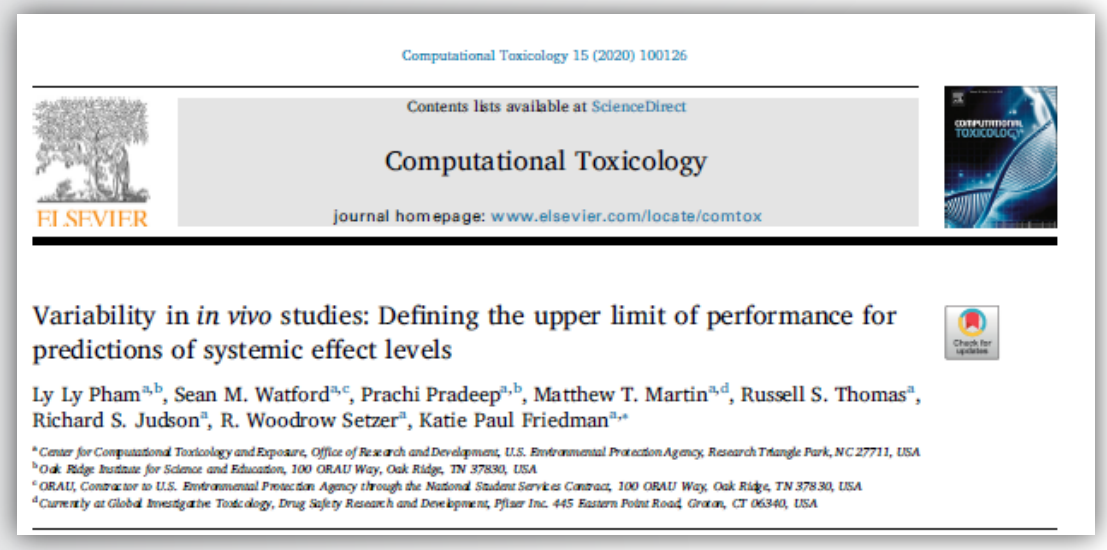
An uncertainty factor of 1000 (3 orders of magnitude on a log10-mg/kg/day scale) would ensure low POD was captured for practically all chemicals in the case study



Part 3

Paul Friedman *et al.* (in prep) Qualitative and Quantitative Variability of Repeat Dose Animal Toxicity Studies

We defined a benchmark for quantitative reproducibility of systemic findings in repeat dose animal studies



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Variability in *in vivo* studies: Defining the upper limit of performance for predictions of systemic effect levels

Ly Ly Pham[a,b], Sean M. Watford[a,c], Prachi Pradeep[a,b], Matthew T. Martin[a,d], Russell S. Thomas[a], Richard S. Judson[a], R. Woodrow Setzer[a], Katie Paul Friedman[a,*]

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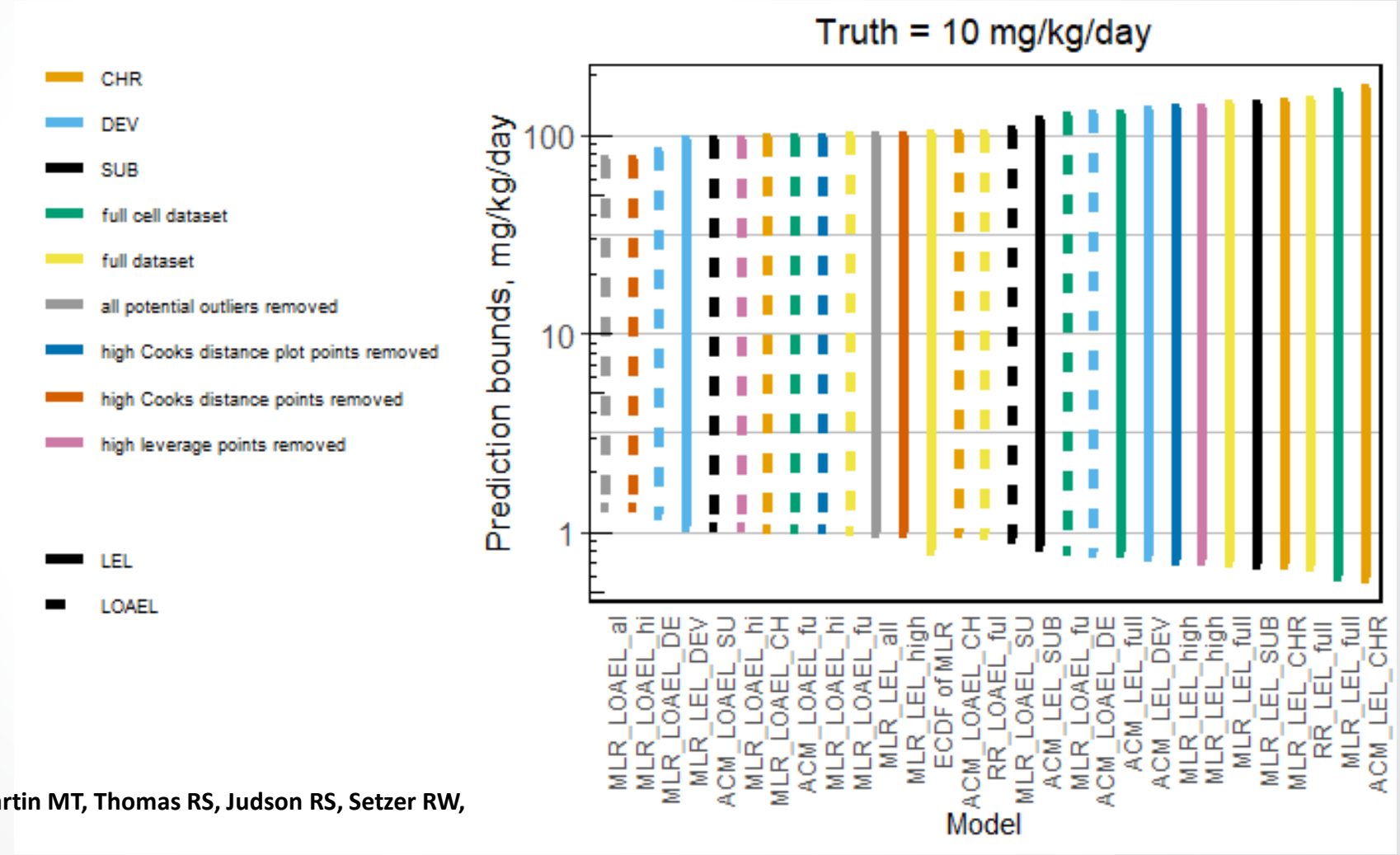
Pham LL, Watford S, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, Paul Friedman K. 2020. 10.1016/j.comtox.2020.100126

Primary Research Question	Statistical approaches
What is the range of possible effect values (mg/kg/day) in replicate studies for a given chemical?	• Residual root mean square error (RMSE) is an estimate of variance in the same units as the systemic effect values. • The RMSE can be used to define a minimum prediction interval, or estimate range, for a model.
What is the maximal accuracy of a new model that attempts to predict effect values for a chemical?	• The mean square error (MSE) is used to approximate the unexplained variance (not explained by study descriptors) for comparison to total variance. • This % unexplained variance limits the maximal R-squared on a new model.

A key learning was that 95% minimum prediction intervals across the modeling approaches, effect levels, and study types were 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 (100-fold wide).



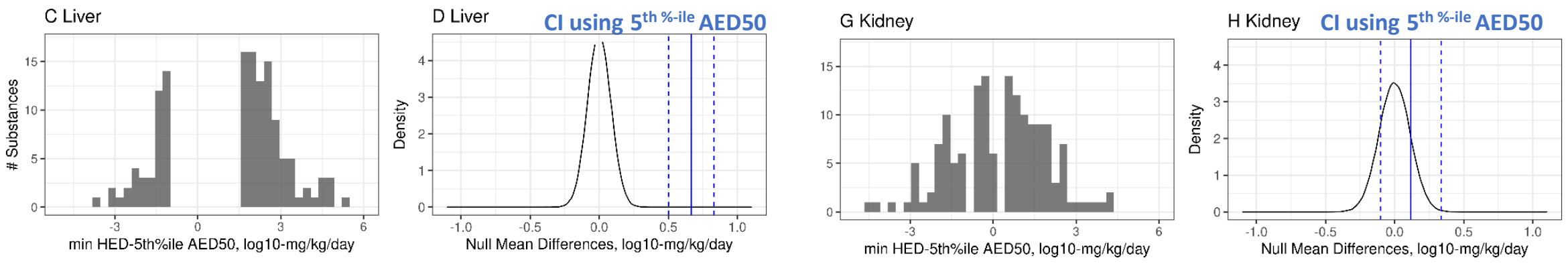
Based on tables from Pham LL, Watford S, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, Paul Friedman K. 2020. 10.1016/j.comtox.2020.100126

In new work in preparation, we examine organ-level effects and their quantitative and qualitative reproducibility

- A component of this work is: How well do currently available liver and kidney-related NAMs in ToxCast predict liver and kidney lowest effect level values *in vivo*?

The distribution of LEL-AED_{50} differences demonstrated very long tails, signaling the differences in LELs or HEDs and AEDs can be extreme

- Distributions of raw differences suggest the mean difference approaches 0
- But these distributions demonstrated much longer tails, with minimum LEL to AED_{50} comparisons at times suggesting differences in excess of 3 orders of magnitude in either direction at the tails
- The *mean* differences (HED or LEL – summary AED50 metrics) are all within 1 log10-mg/kg/day



The distribution of LEL-AED$_{50}$ differences demonstrated very long tails, signaling that for smaller numbers of chemicals, the differences in LELs and AEDs can be extreme

Organ	# Chemicals	In vivo POD (log_{10}-mg/kg/day)	AED type (log_{10}-mg/kg/day)	Mean difference, in vivo POD - AED (log10-mg/kg/day)	p-value	Lower CI bound	Upper CI bound
Liver	365	min LEL	mean AED	0.3203	<0.0001	0.1736	0.4670
Liver	365	min LEL	5th %-ile AED	1.3755	<0.0001	1.172	1.579
Kidney	194	min LEL	mean AED	0.5060	<0.0001	0.290	0.7223
Kidney	194	min LEL	5th %-ile AED	0.8586	<0.0001	0.608	1.110
Liver	365	min HED	mean AED	-0.3900	<0.0001	-0.5394	-0.2405
Liver	365	min HED	5th %-ile AED	0.6652	<0.0001	0.5013	0.8291
Kidney	194	min HED	mean AED	-0.2357	0.0245	-0.4418	-0.0295
Kidney	194	min HED	5th %-ile AED	0.1169	0.2953	-0.1027	0.3366

Table 3, Paul Friedman et al. (in prep).

It is possible that existing NAMs that indicate organ-level effects, on average, may predict liver- or kidney-related HEDs within estimates of variability in replicate *in vivo* studies, *but caution should be employed in viewing this result due to the tails on the distribution of raw differences*

Conclusions

- Work in the APCRA and in EPA-ORD-CCTE has advanced our understanding of the utility of POD_{NAM}, among other objectives
- It is likely that an uncertainty factor of 100-1000 is necessary to maintain conservatism of POD_{NAM} for $POD_{traditional}$ for all chemicals…*unless*…
  - Triage chemicals by the degree of certainty in their toxicokinetic profile such that chemicals with a higher degree of certainty in IVIVE (e.g., pharmaceuticals) could have a lower uncertainty factor applied to the POD_{NAM}
  - Refine IVIVE approach with more information (bioavailability, *in vitro* disposition, more curation of concentration vs. time data for training)
  - Combine POD_{vitro} with POD_{QSAR} for a consensus POD_{NAM}

Thank you for listening

Thanks especially to John Wambaugh, Richard Judson, Woody Setzer, Ly Ly Pham, Prachi Pradeep, MJ Foster, Sean Watford, and Rusty Thomas

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Prospective case study members

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A*STAR	JRC	Uni Birmingham	
Lit-Hsin Loo	Thomas Cole Maurice Whelan	Mark Viant	