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United States Environmental Protection Agency

The Influence of *In Vitro* Disposition and Toxicokinetics on the Association of *In Vitro* Bioactivity and *In Vivo* Toxicity Data

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New Data and Tools for Understanding Chemical Distribution *In Vitro*
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Office of Research and Development
Center for Computational Toxicology and Exposure

EPA at 50 Progress for a Stronger Future

Conflict of Interest Statement

The authors declare no conflict of interest

US EPA Office of Research and Development

- The Office of Research and Development (ORD) is the scientific research arm of EPA
  - 562 peer-reviewed journal articles in 2018

- Research is conducted by ORD's four national centers, and three offices organized to address:
  - Public health and env. assessment; comp. tox. and exposure; env. measurement and modeling; and env. solutions and emergency response.

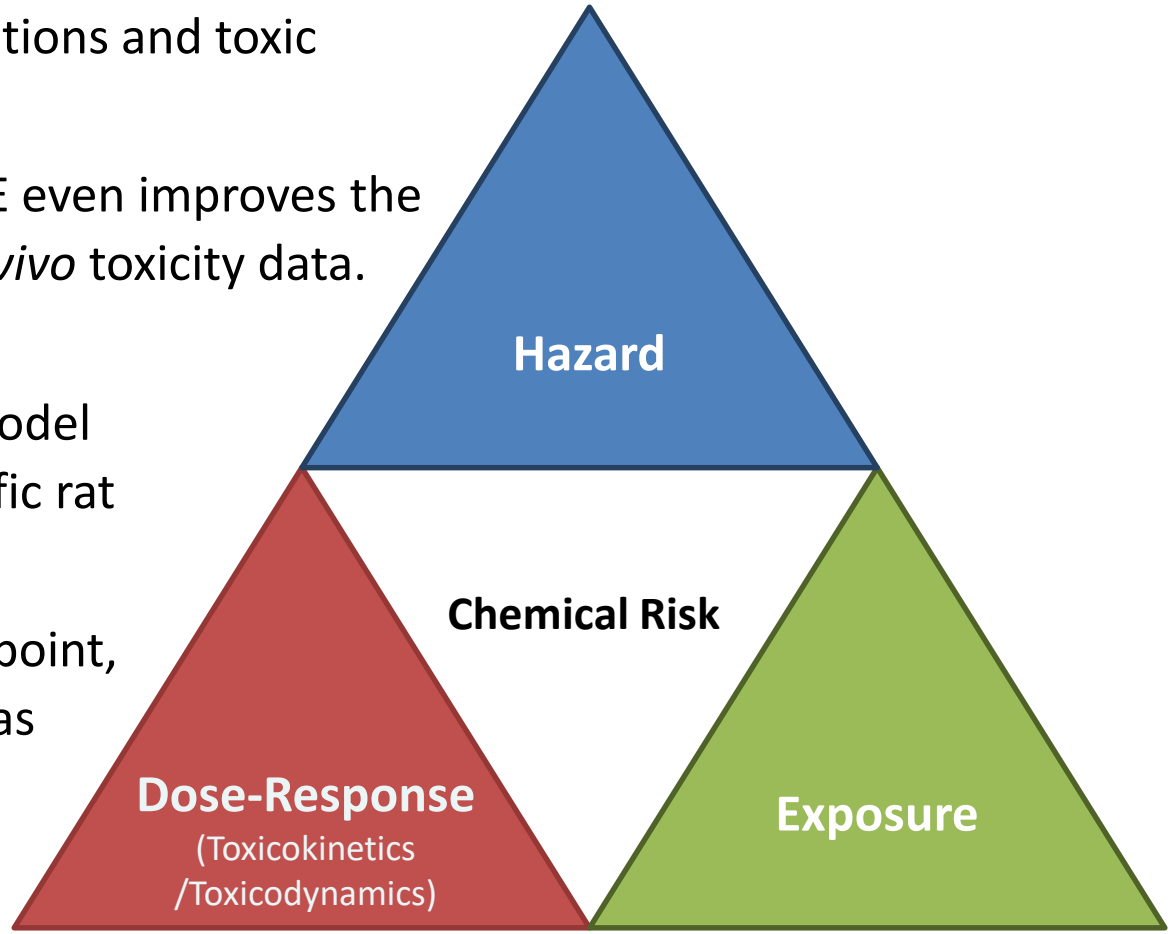
- 13 facilities across the United States

- Research conducted by a combination of Federal scientists (including uniformed members of the **Public Health Service**); contract researchers; and postdoctoral, graduate student, and post-baccalaureate trainees

ORD Facility in
Research Triangle Park, NC

Introduction

- To use high-throughput screening (HTS) assays as an alternative to traditional animal studies we must link *in vitro* bioactivity concentrations and toxic doses via IVIVE.
- Previously, it has not been clear whether the use of IVIVE even improves the observed association between *in vitro* bioactivity and *in vivo* toxicity data.
- We have used an *in vitro* disposition model and a high-throughput, physiologically based toxicokinetic (PBTK) model to relate *in vitro* bioactivity (ToxCast) and endpoint specific rat *in vivo* toxicity data.
- For every possible comparison of *in vitro* and *in vivo* endpoint, the concordance between the *in vivo* and *in vitro* data was evaluated by a regression analysis.



The NRC (1883) outlined three components for determining chemical risk.

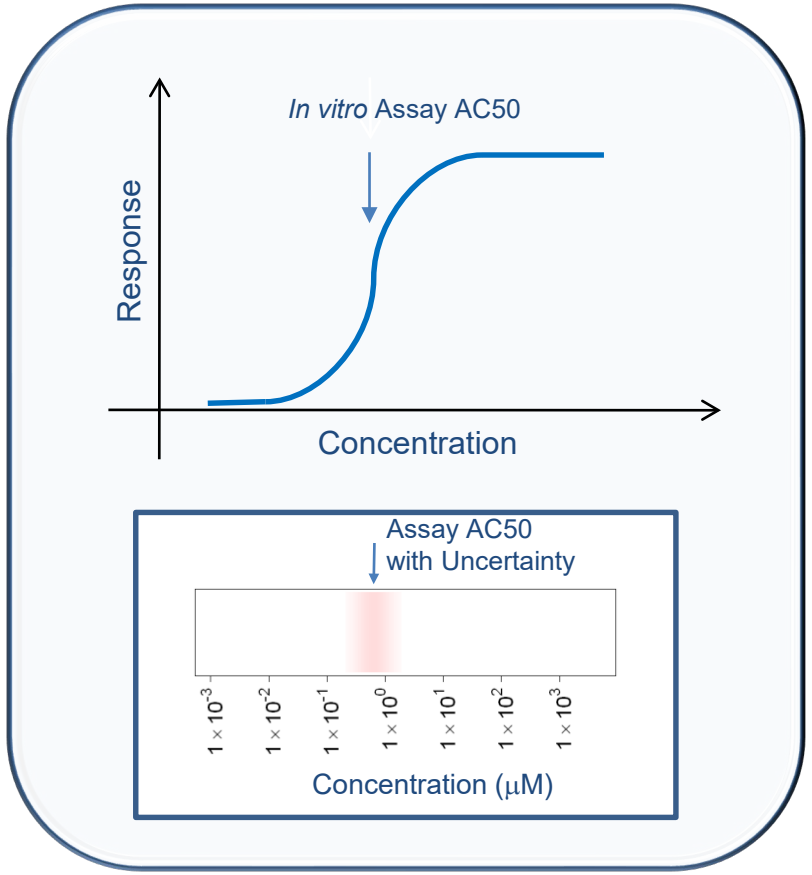
High-Throughput Bioact Projects

- We attempt to estimate points of departure *in vitro* using high throughput screening (HTS) for bioactivity as a surrogate for hazard data

- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)

- **ToxCast** (Toxicity Forecast): For a subset (>3000) of Tox21 chemicals EPA has measured >1100 additional assays-endpoints (Kavlock *et al.*, 2012)

- Most assays conducted in dose-response format (identify 50% activity concentration – AC_{50} – and efficacy if data described by a Hill function, Filer *et al.*, 2016)



All data are public: http://comptox.epa.gov/dashboard/

In Vitro - In Vivo Extrapolation (IVIVE)

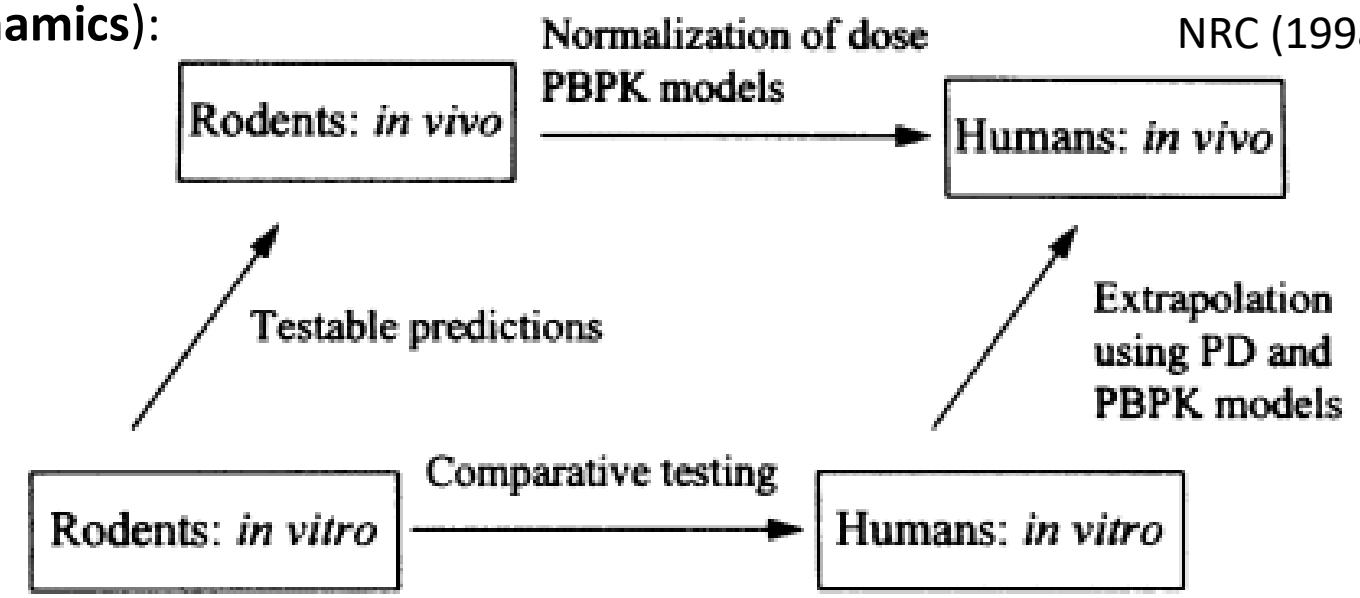
What do we do with an *in vitro* concentration? -- IVIVE is the use of *in vitro* experimental data to predict phenomena *in vivo*

- IVIVE-PK/TK (**Pharmacokinetics/Toxicokinetics**):
  - Fate of molecules/chemicals in body
  - Considers absorption, distribution, metabolism, excretion (ADME)
  - Uses empirical PK and physiologically-based (PBPK) modeling

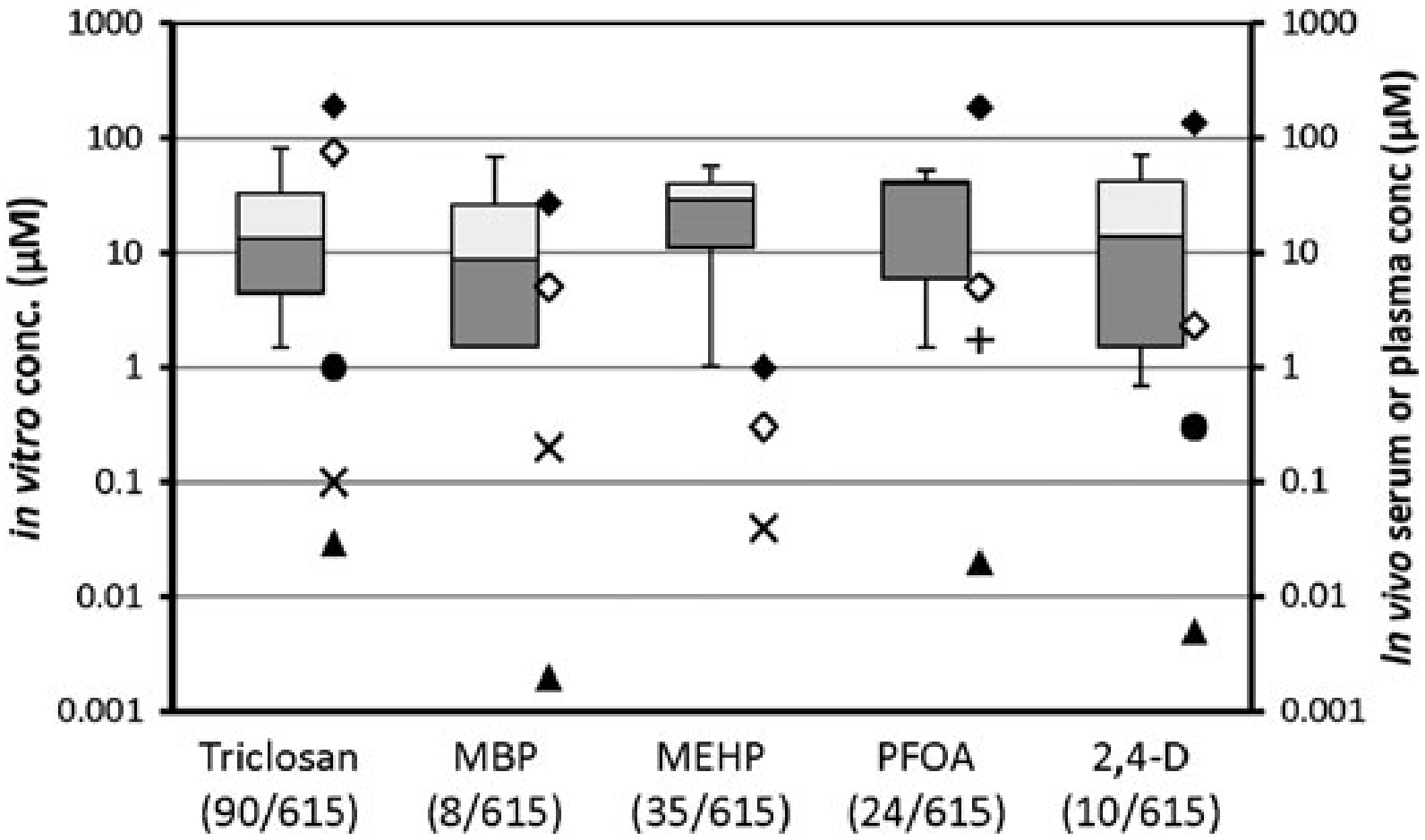
- IVIVE-PD/TD (**Pharmacodynamics/Toxicodynamics**):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/ irreversible effeccts

- Both contribute to *in vivo* effect prediction

NRC (1998)



Comparing on the Basis of Concentration



Range of bioactive concentrations across ToxCast assays

Estimated or measured average concentrations associated with the LOAEL in animal studies

NOAEL in animal studies

Humans with chronic exposure reference values (solid circles)

X Volunteers using products containing the chemical

\+ Bio-monitored occupational populations

General populations

The five chemicals (as of 2011) with plasma biomonitoring AND ToxCast data… what do we do about the other 1000's?

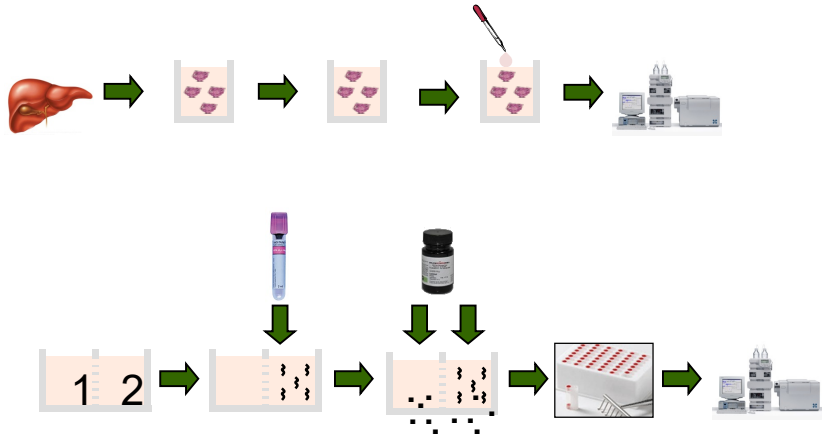
Aylward and Hays (2011)

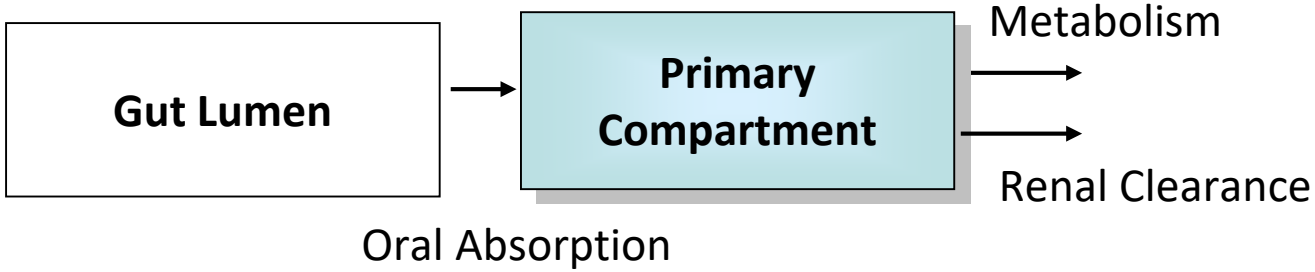
High Throughput Toxicokinetics (HTTK)

Most chemicals lack public toxicokinetic-related data (Wetmore et al., 2012):

In vitro toxicokinetic data + generic toxicokinetic model

= high(er) throughput toxicokinetics

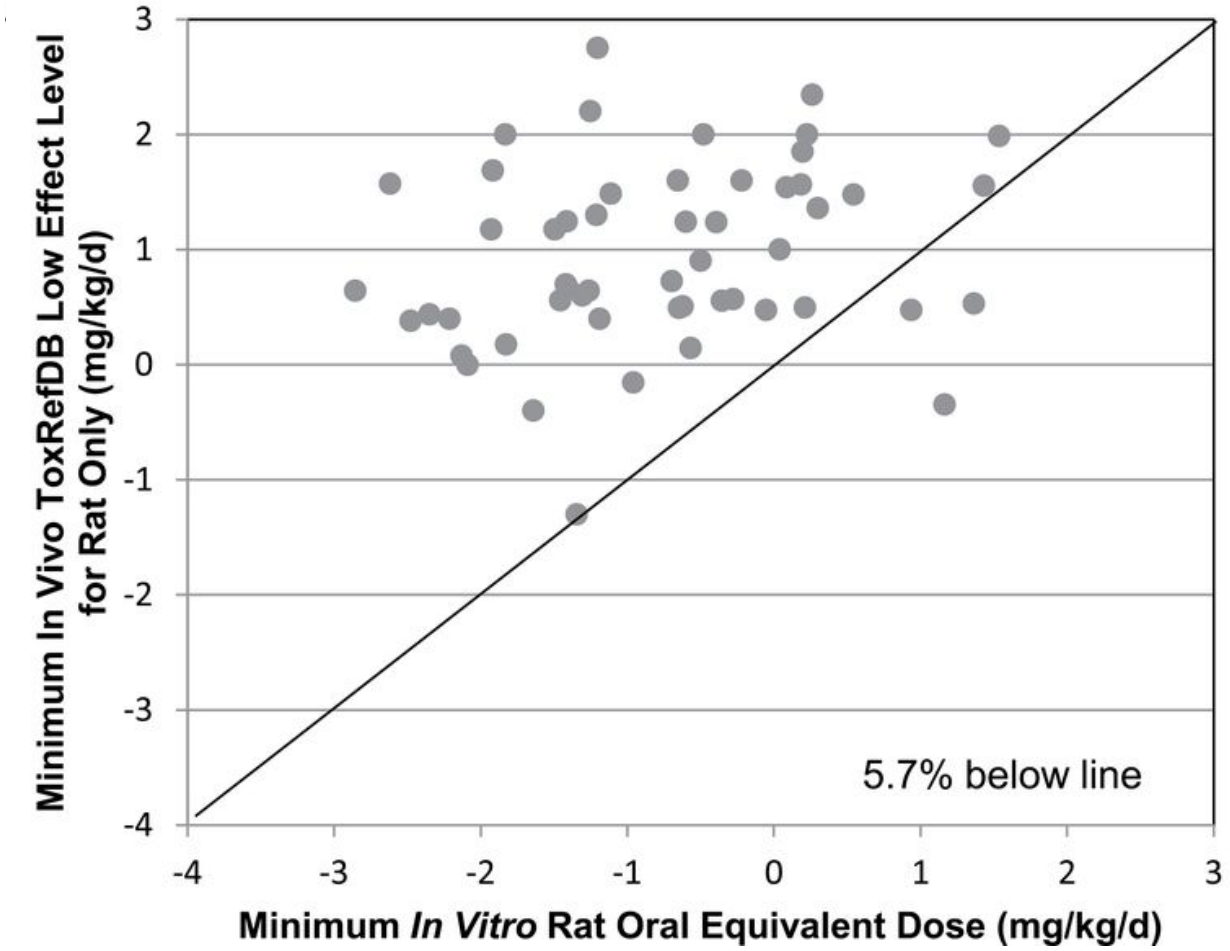


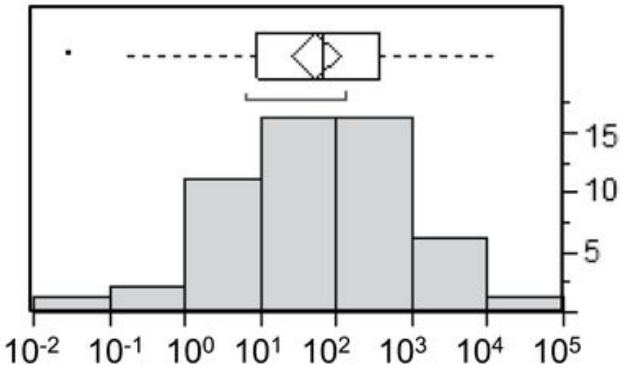


httk

Comparing IVIVE Predictions with Toxic Doses

Rat-specific HTTK data were collected in vitro for ~50 chemicals, allowing IVIVE with ToxCast Data



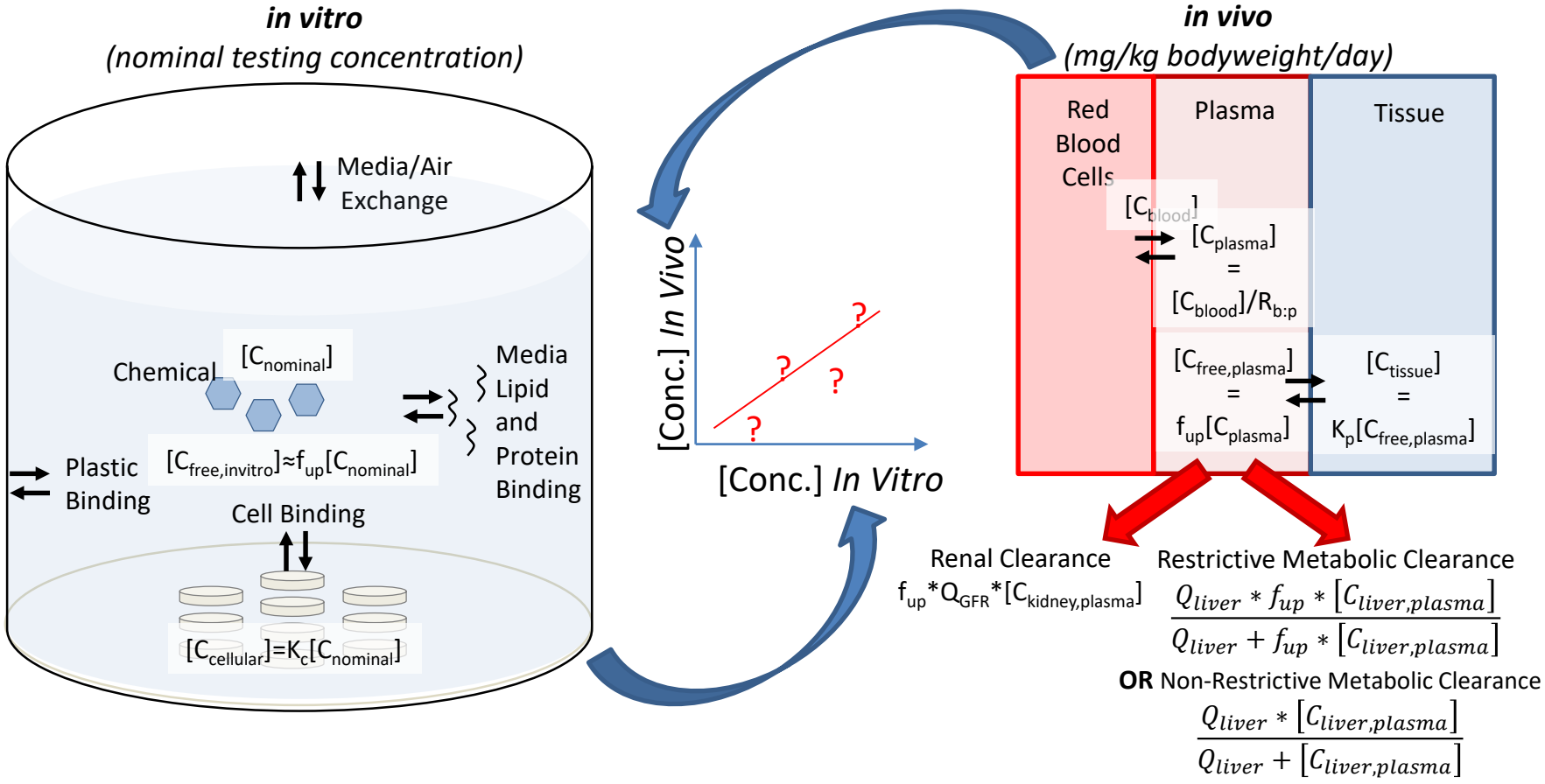


Log_{10} Ratio ToxRefDB Min LEL:ToxCast Min Oral Equivalent Dose

Distribution Summary Statistics		
Median	1.82	(66.07)
Upper Quartile	2.55	(354.81)
Lower Quartile	0.95	(8.91)

Wetmore et al. (2013)

There Are Many Considerations When Doing IVIVE

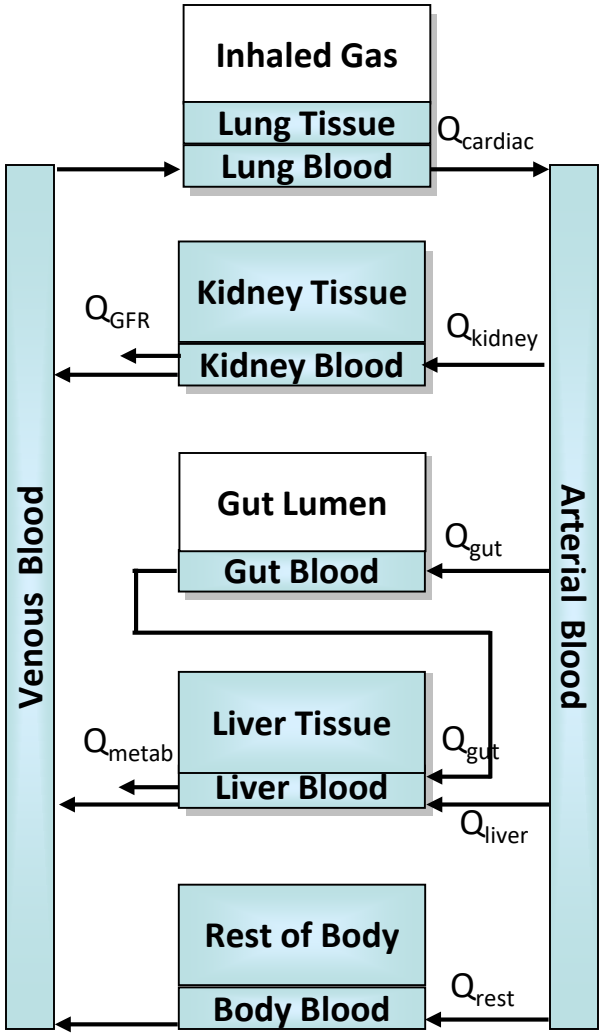


$$\frac{Q_{liver} * f_{up} * [C_{liver,plasma}]}{Q_{liver} + f_{up} * [C_{liver,plasma}]}$$

$$\frac{Q_{liver} * [C_{liver,plasma}]}{Q_{liver} + [C_{liver,plasma}]}$$

How do you select the appropriate *in vitro* and *in vivo* concentrations for extrapolation?

A General Physiologically-based Toxicokinetic (PBTK) Model

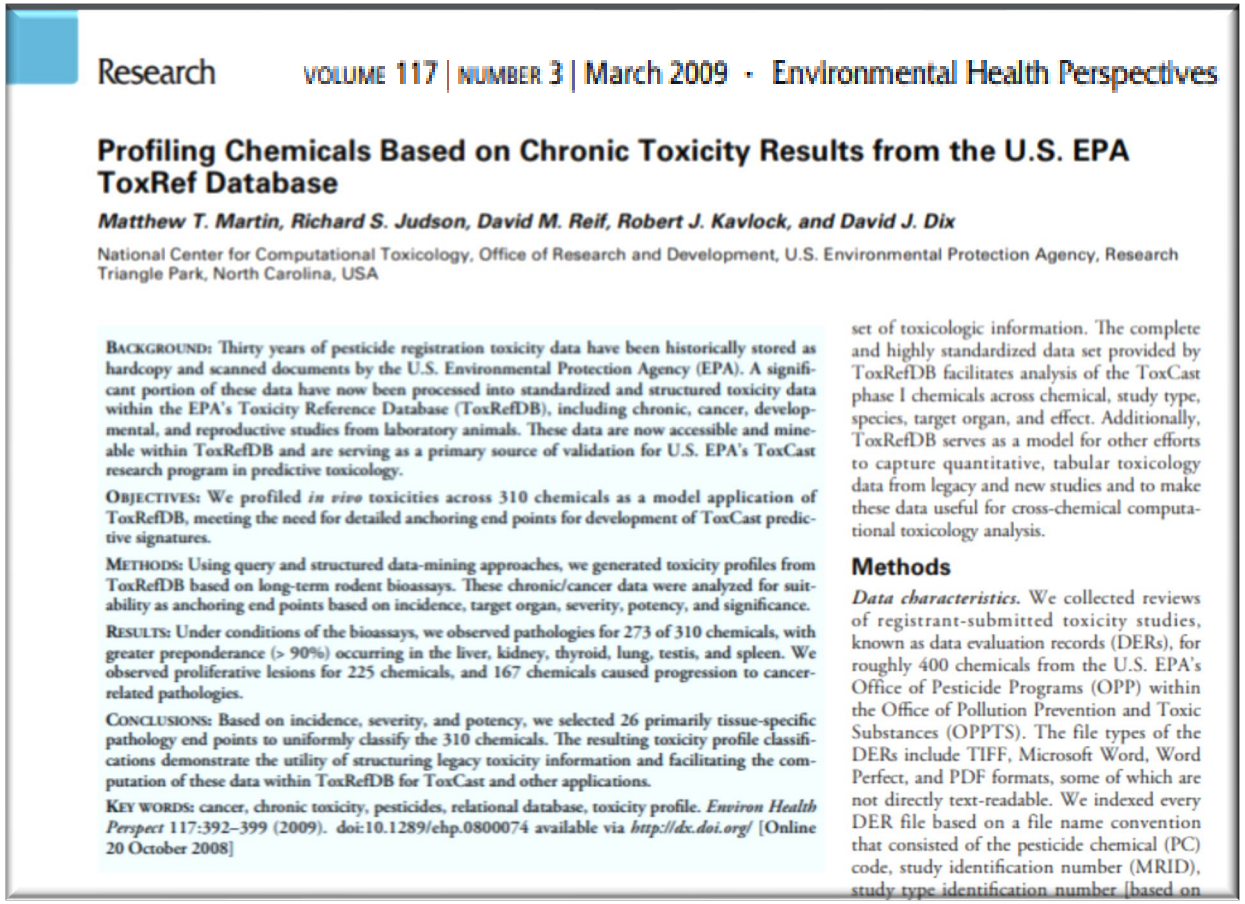


Pearce et al. (2017)

- R package "httk" includes a generic PBTK model
- Can be tailored to a chemical using in vitro data and predictions from chemical structure
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.
- The only ways chemicals "leave" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

ToxRefDB

- *In vivo* data for rat were accessed from the Toxicity Reference (ToxRef) database version 1
- Much of the data in ToxRefDB v1 was derived from studies or study summaries for study designs compliant with or similar to the EPA OCSPP 870 series guidelines
- ToxRefDB v1 is a "positives-only" database, and *in vivo* data were reported as the nominal dose at which an effect (not necessarily critical) was observed for a particular endpoint
- The analysis in this work included chronic (2 year), subchronic (90 day), and developmental (parental and fetal generations) study types



Research VOLUME 117 | NUMBER 3 | March 2009 · Environmental Health Perspectives

Profiling Chemicals Based on Chronic Toxicity Results from the U.S. EPA ToxRef Database

Matthew T. Martin, Richard S. Judson, David M. Reif, Robert J. Kavlock, and David J. Dix

National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

BACKGROUND: Thirty years of pesticide registration toxicity data have been historically stored as hardcopy and scanned documents by the U.S. Environmental Protection Agency (EPA). A significant portion of these data have now been processed into standardized and structured toxicity data within the EPA's Toxicity Reference Database (ToxRefDB), including chronic, cancer, developmental, and reproductive studies from laboratory animals. These data are now accessible and mineable within ToxRefDB and are serving as a primary source of validation for U.S. EPA's ToxCast research program in predictive toxicology.

OBJECTIVES: We profiled *in vivo* toxicities across 310 chemicals as a model application of ToxRefDB, meeting the need for detailed anchoring end points for development of ToxCast predictive signatures.

METHODS: Using query and structured data-mining approaches, we generated toxicity profiles from ToxRefDB based on long-term rodent bioassays. These chronic/cancer data were analyzed for suitability as anchoring end points based on incidence, target organ, severity, potency, and significance.

RESULTS: Under conditions of the bioassays, we observed pathologies for 273 of 310 chemicals, with greater preponderance (> 90%) occurring in the liver, kidney, thyroid, lung, testis, and spleen. We observed proliferative lesions for 225 chemicals, and 167 chemicals caused progression to cancer-related pathologies.

CONCLUSIONS: Based on incidence, severity, and potency, we selected 26 primarily tissue-specific pathology end points to uniformly classify the 310 chemicals. The resulting toxicity profile classifications demonstrate the utility of structuring legacy toxicity information and facilitating the computation of these data within ToxRefDB for ToxCast and other applications.

KEY WORDS: cancer, chronic toxicity, pesticides, relational database, toxicity profile. *Environ Health Perspect* 117:392–399 (2009). doi:10.1289/ehp.0800074 available via *http://dx.doi.org/* [Online 20 October 2008]

set of toxicologic information. The complete and highly standardized data set provided by ToxRefDB facilitates analysis of the ToxCast phase I chemicals across chemical, study type, species, target organ, and effect. Additionally, ToxRefDB serves as a model for other efforts to capture quantitative, tabular toxicology data from legacy and new studies and to make these data useful for cross-chemical computational toxicology analysis.

Methods

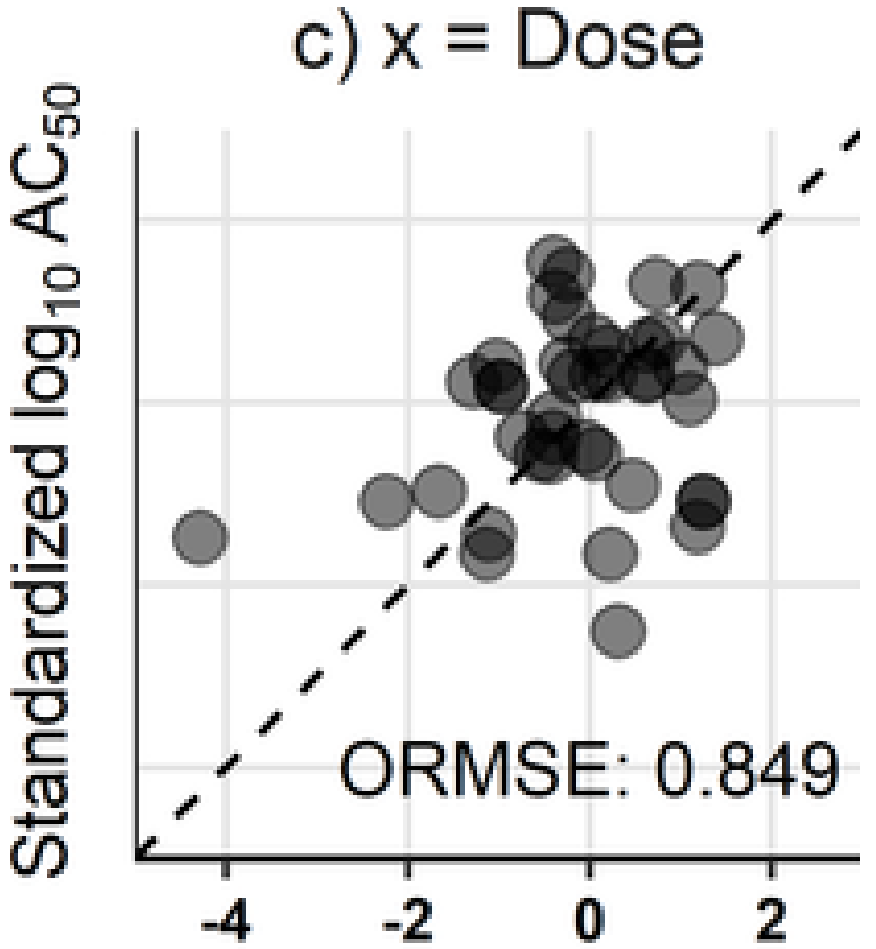
Data characteristics. We collected reviews of registrant-submitted toxicity studies, known as data evaluation records (DERs), for roughly 400 chemicals from the U.S. EPA's Office of Pesticide Programs (OPP) within the Office of Pollution Prevention and Toxic Substances (OPPTS). The file types of the DERs include TIFF, Microsoft Word, Word Perfect, and PDF formats, some of which are not directly text-readable. We indexed every DER file based on a file name convention that consisted of the pesticide chemical (PC) code, study identification number (MRID), study type identification number [based on

Martin et al. (2009)

Honda et al. (2019)

- New rat-specific HTTK data collected for ~80 chemicals in addition to ~50 from Wetmore et al. (2013)
- For each ToxRef endpoint (mg/kg/day) we did a **forward dosimetry** calculation (**predicted µM concentration**)
- For each ToxCast endpoint (µM) we did a **reverse dosimetry** IVIVE calculation (**predicted mg/kg/day dose**)
- We compared each ToxRef and ToxCast endpoint on both µM and mg/kg/day scales
- Calculated Orthogonal Root Mean Squared Error (ORMSE) – lower is better

Example for a single ToxCast assay and ToxRef endpoint, each point is a chemical:



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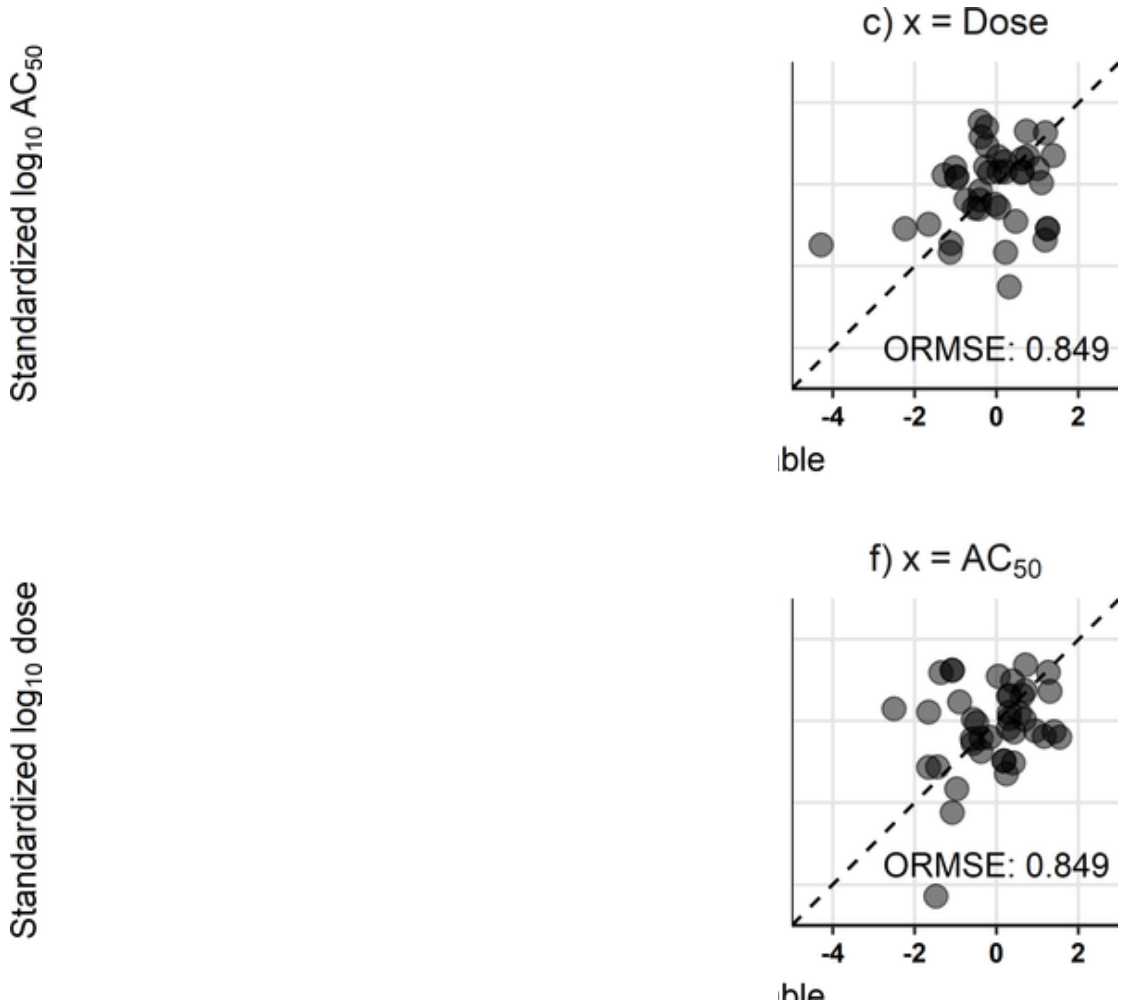

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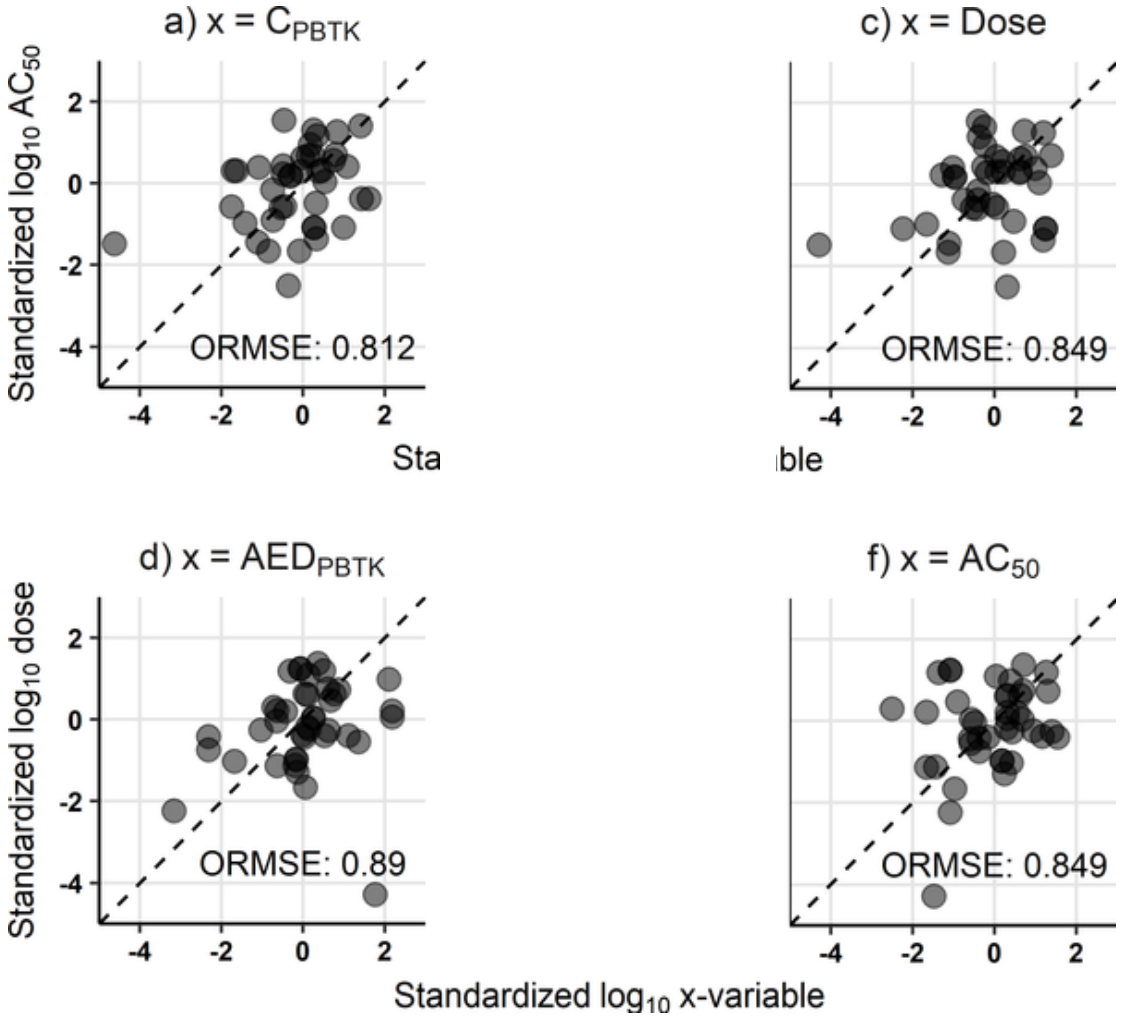
We compared the ORMSE for dose vs. AC_{50} with using PBTK to perform IVIVE:



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“Significance”

- >1000 ToxCast assay endpoints
- 106 specific ToxRef endpoints (68 pathological responses and 3 study types)
- 80 chemicals with observed effects in ToxRef and bioactivity in ToxCast

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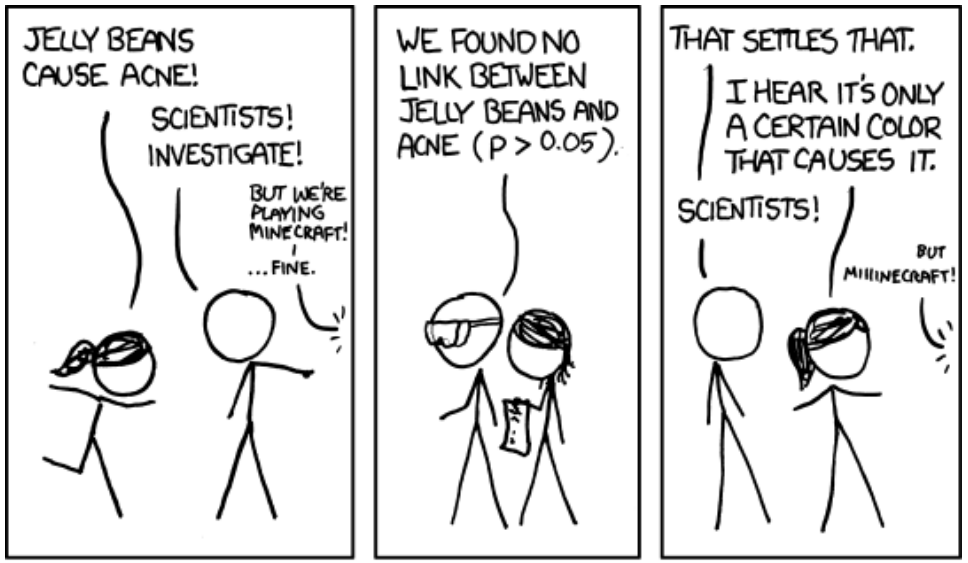

https://xkcd.com/882/

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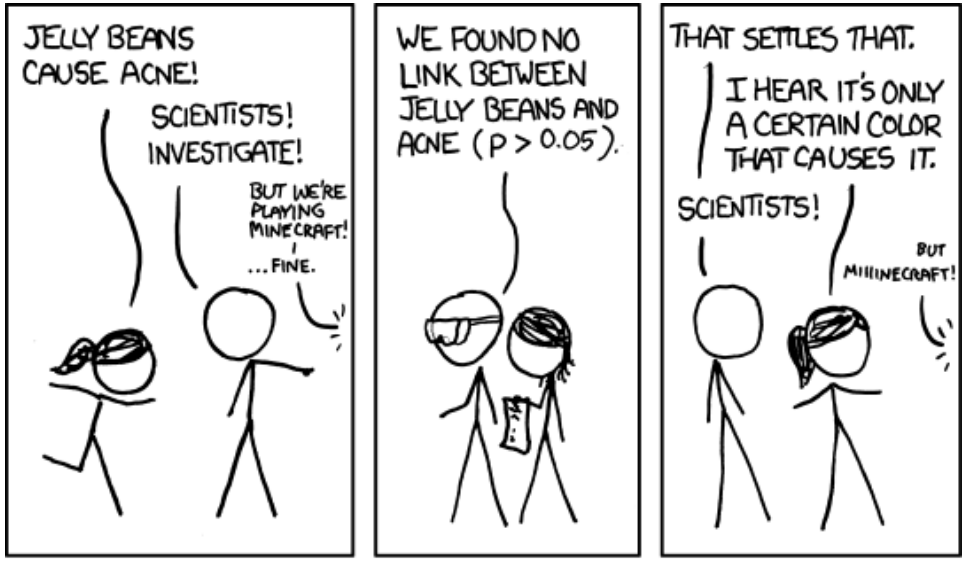

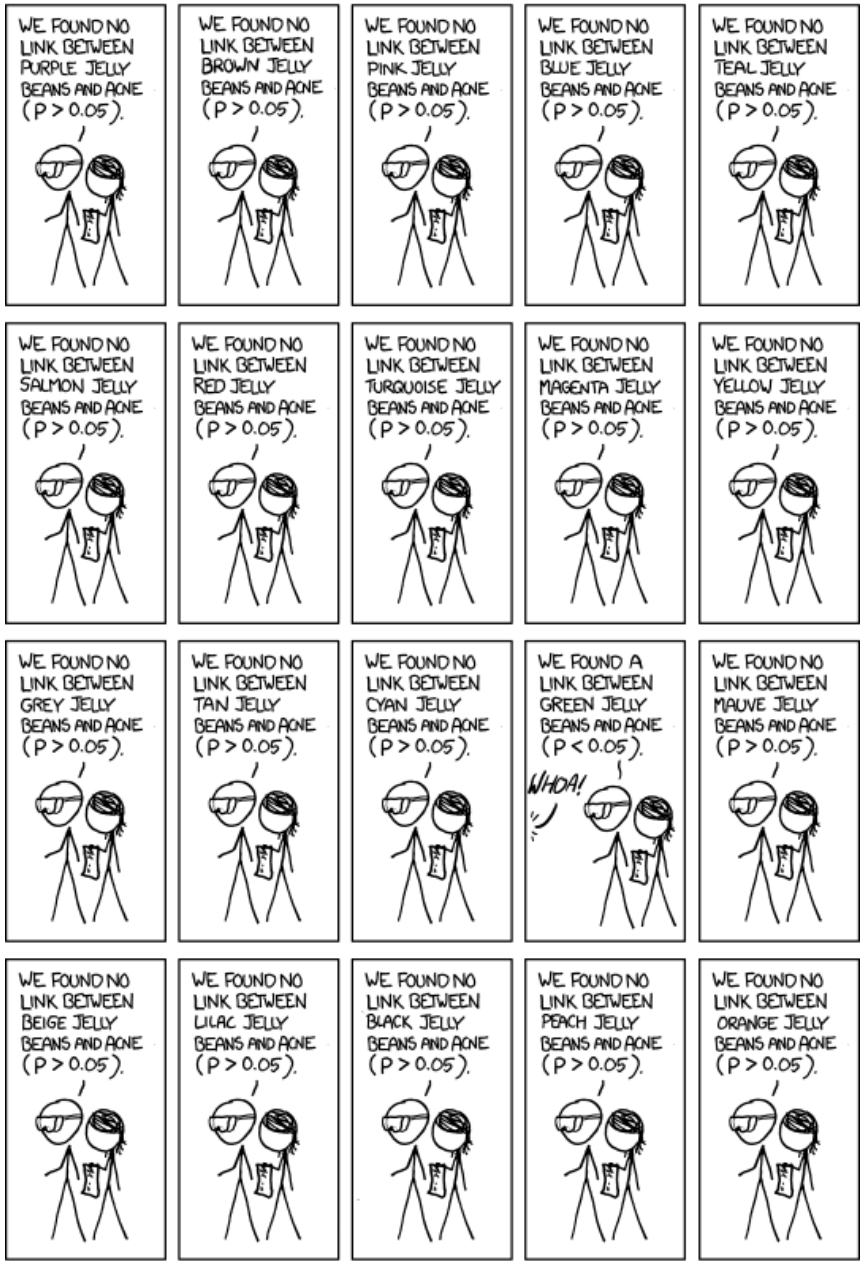

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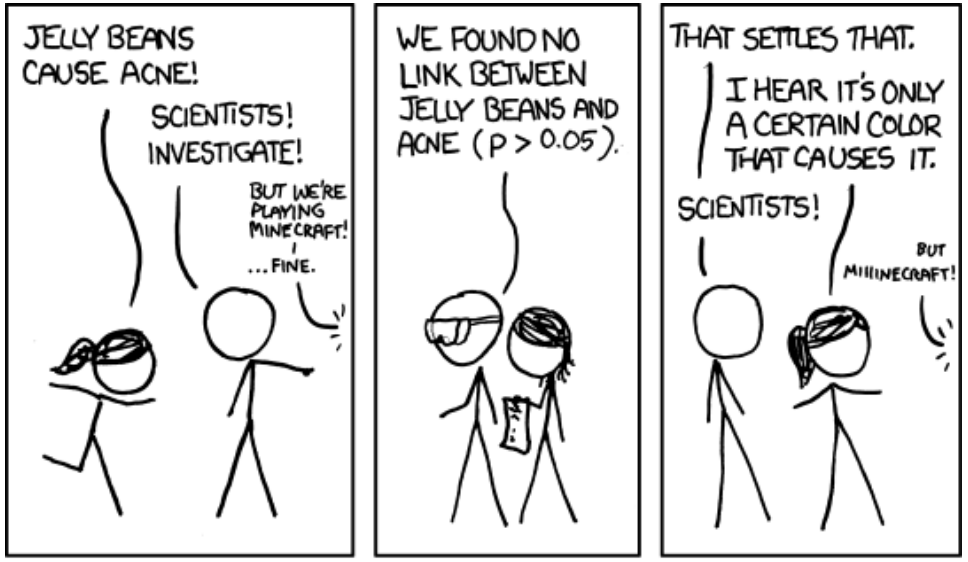

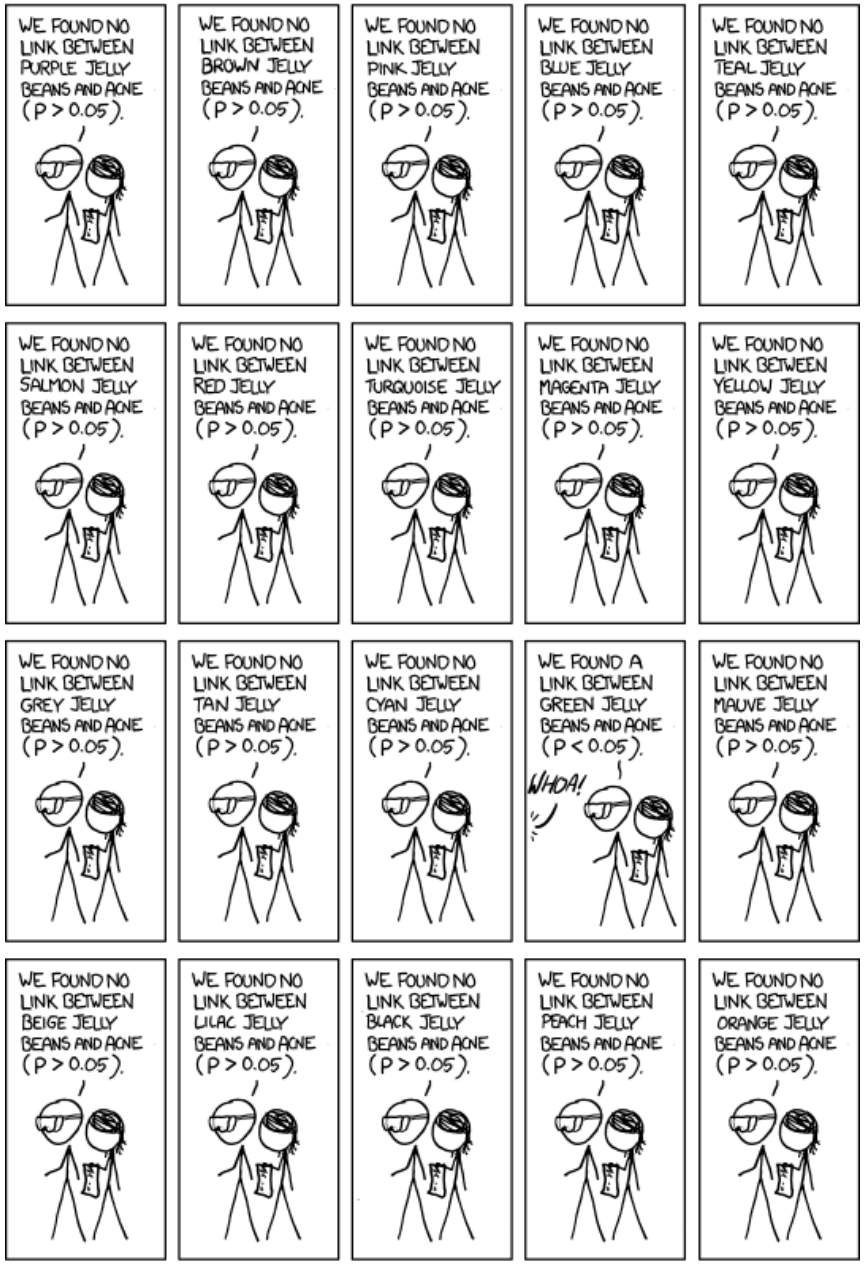

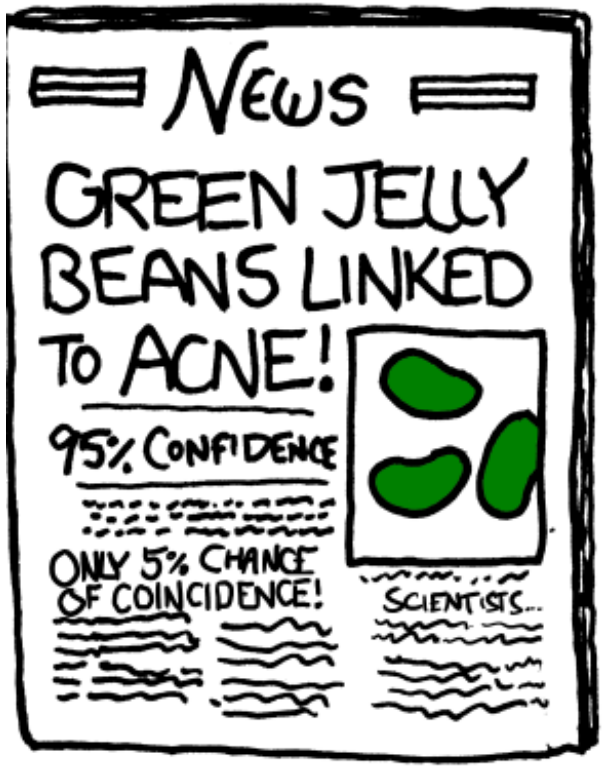

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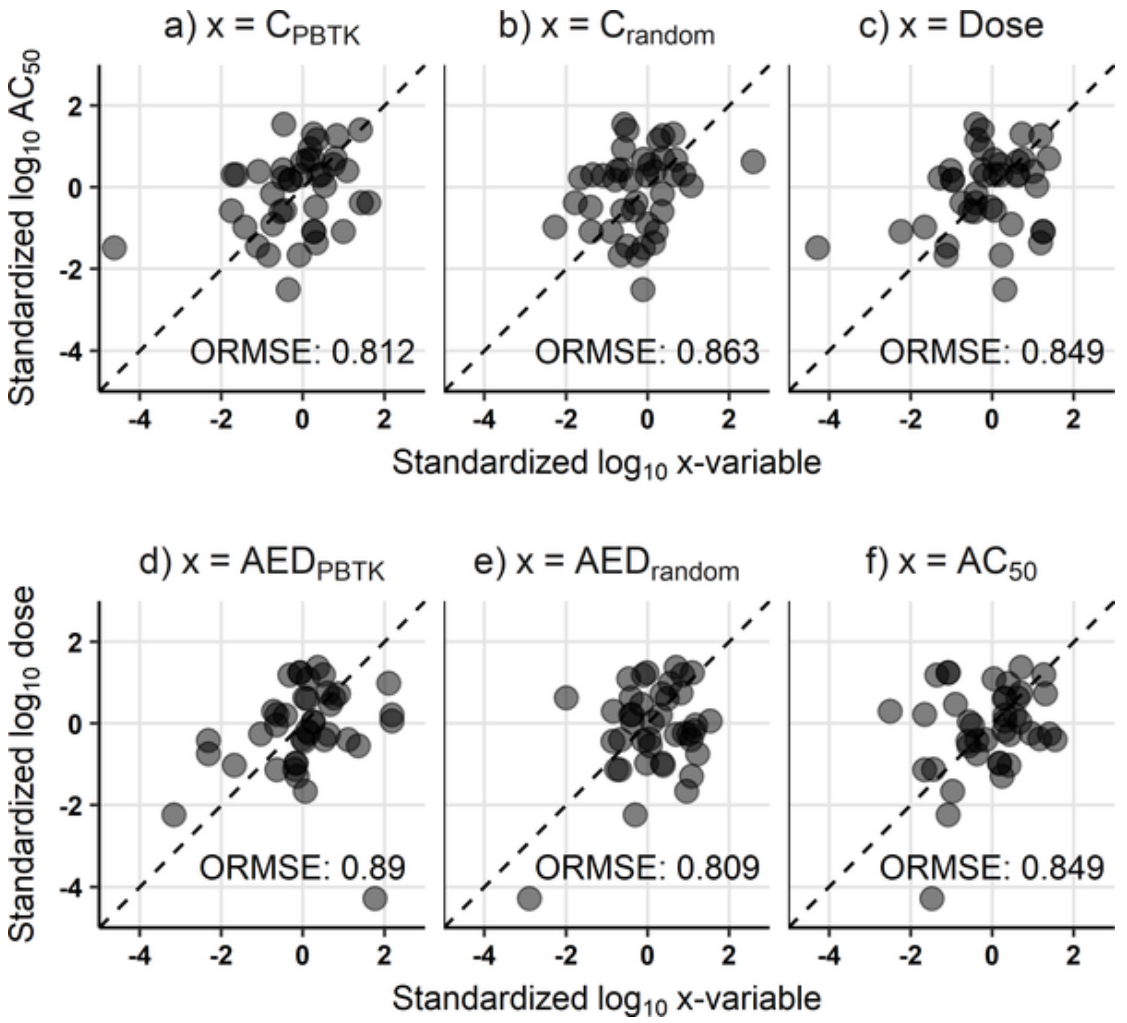
As a sanity check, we also performed IVIVE using PBTK for a randomly selected chemical:



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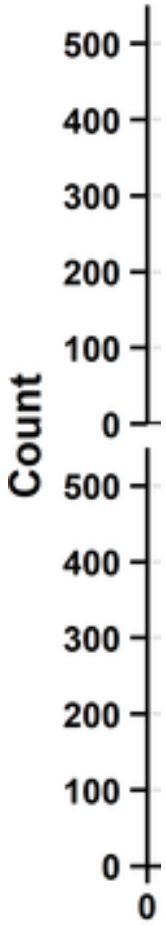
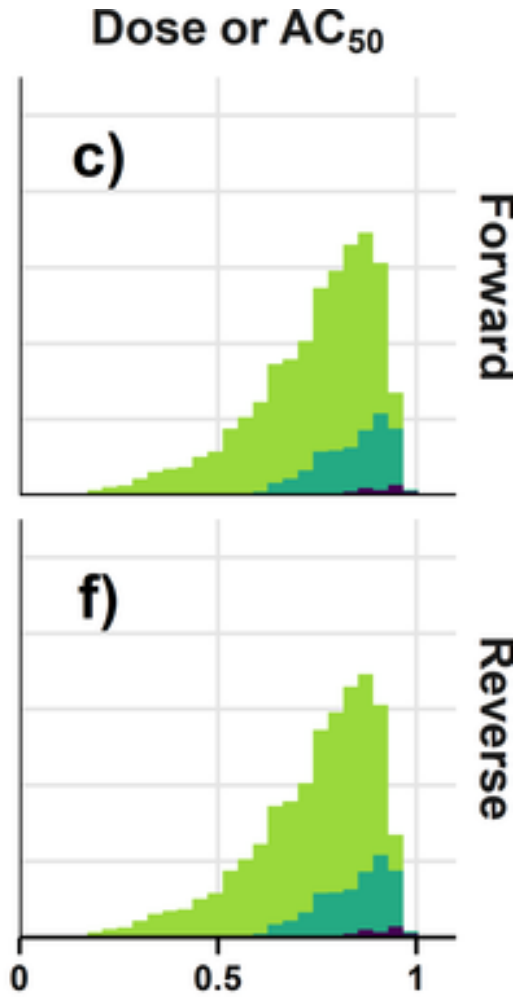
Distribution of ORMSE

For each *in vitro-in vivo* endpoint pair, we calculate the ORMSE across all available chemicals





Lower values indicate lesser error)

Number of Chemicals 5:10 10:20 ≥ 20

Honda *et al*. (2019)

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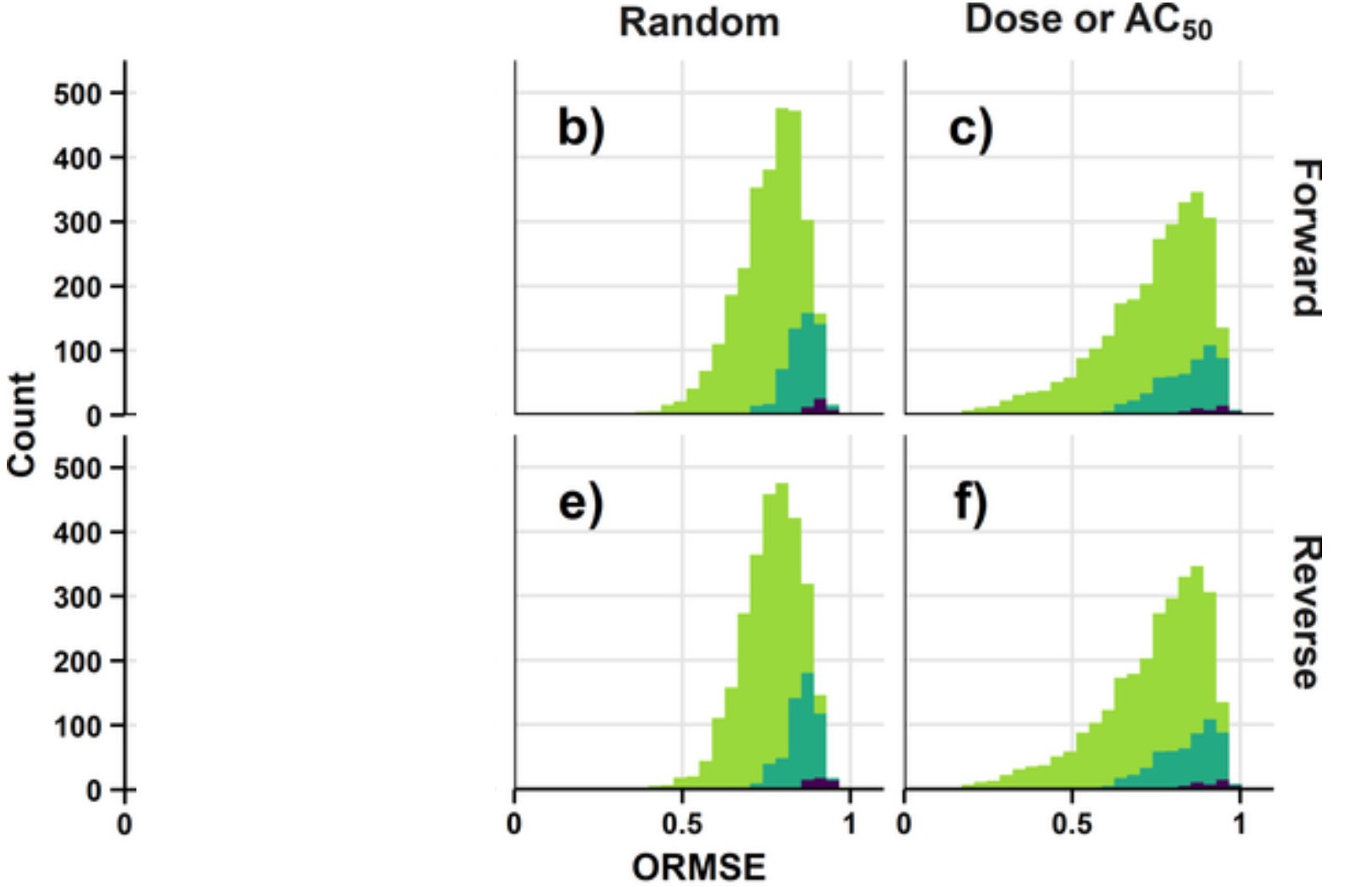

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Randomly selecting the chemical for the IVIVE increases error (on average)

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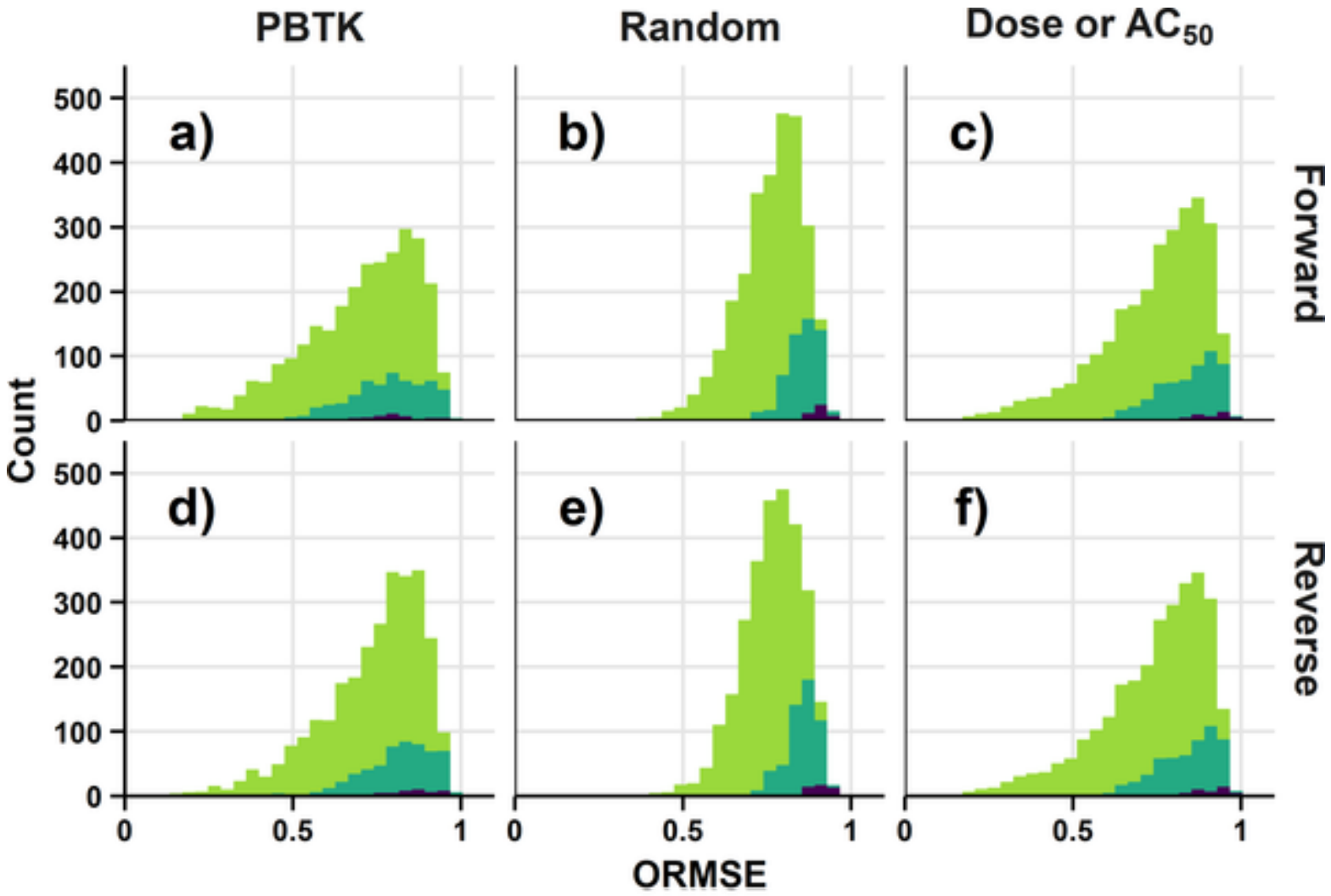

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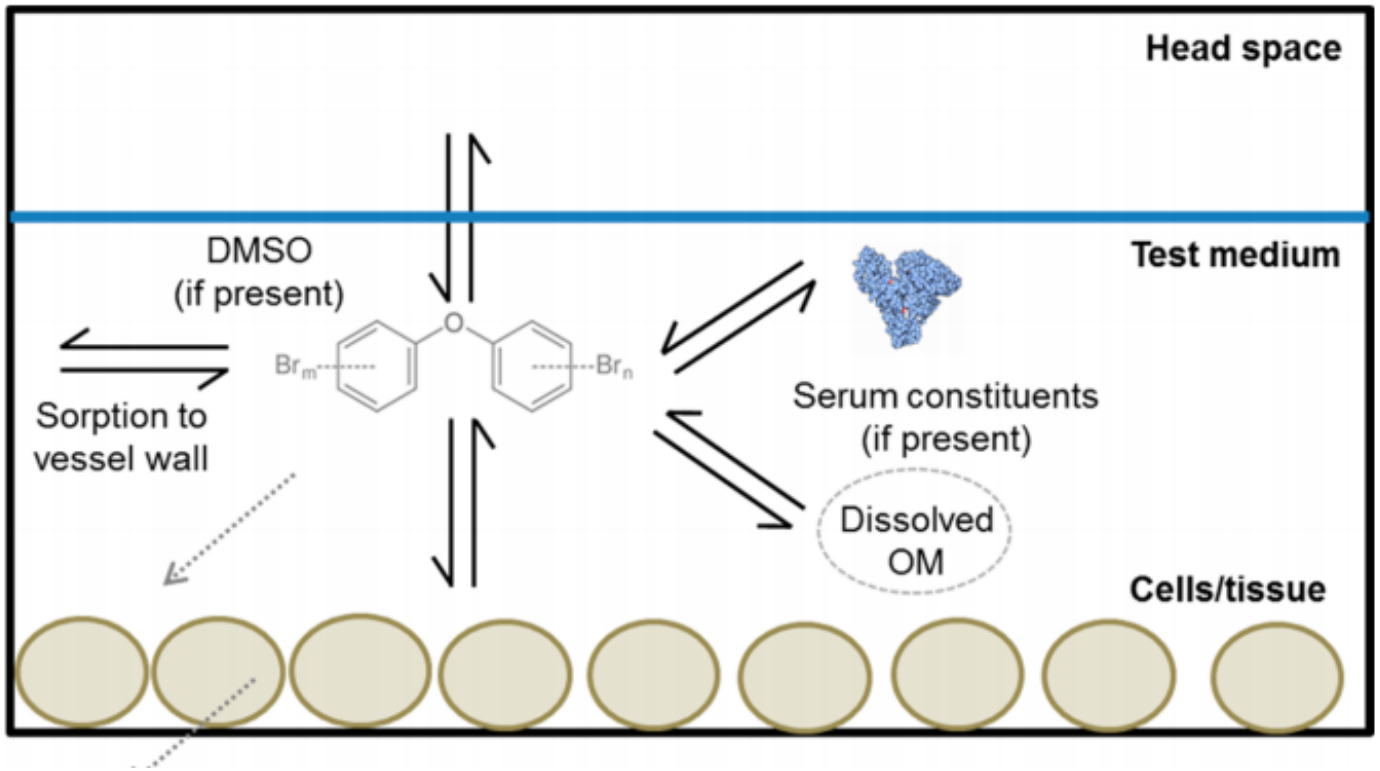
Using PBTK lowers the error

Honda *et al*. (2019)

What About *In Vitro* Distribution? *(Please Stop Discussing PBTK!)*

- Armitage et al. (2014) suggest that in vitro partitioning relates strongly to logKow and serum in the medium
- Sorption to plastic played a smaller role in determining the cellular concentration
- We can check to see if using an in vitro disposition model improves IVIVE (that is, reduces error in comparisons between *in vivo* and *in vitro* endpoints)
- Note, Armitage model expanded to ionizable compounds by Fischer et al. (2017)

Mass-balance model:
DMSO (dimethyl sulfoxide, a typical solvent), OM (organic matter)

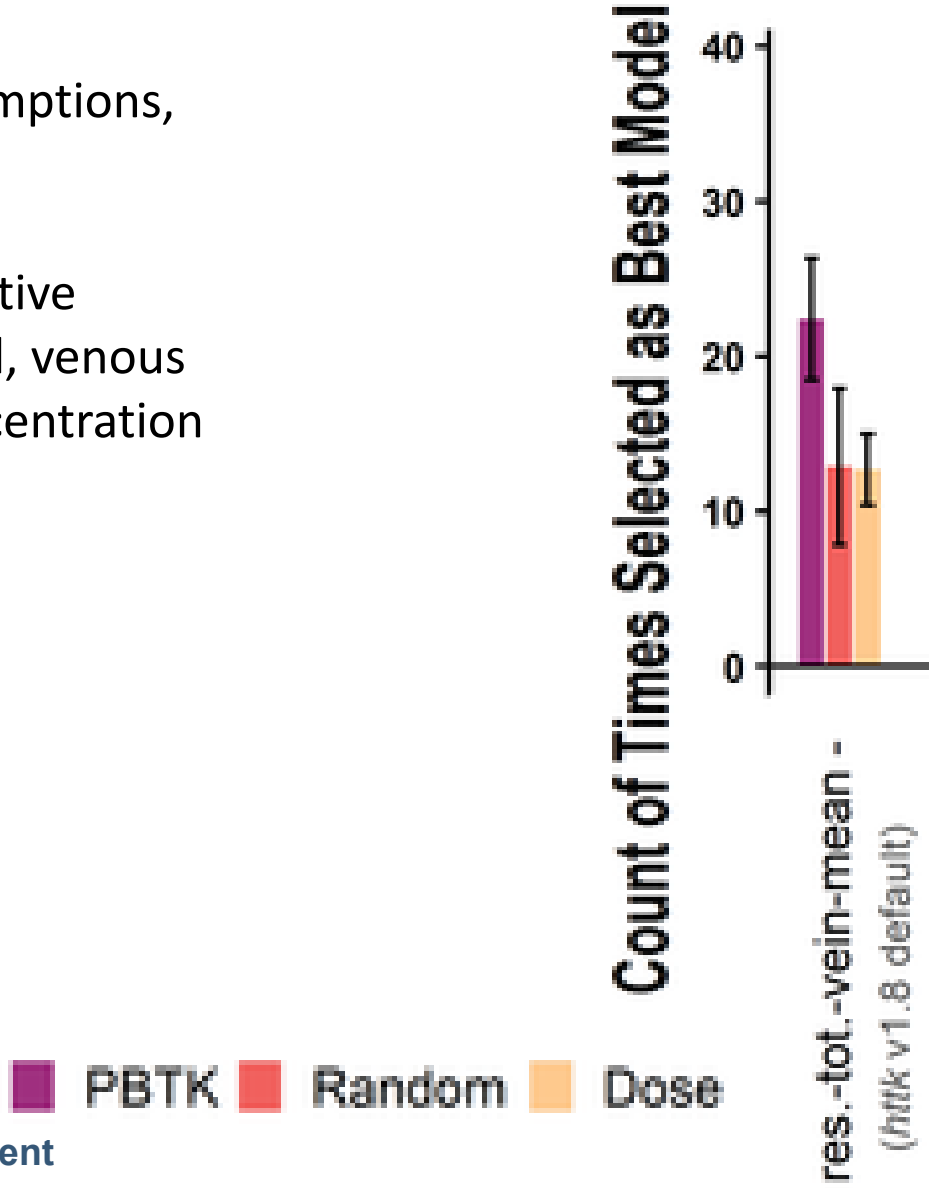


Armitage et al. (2014)

Impact of IVIVE Assumptions

Different combinations of assumptions, for example:

res-tot-vein-mean = restrictive metabolism, total chemical, venous concentrations, mean concentration during tox study



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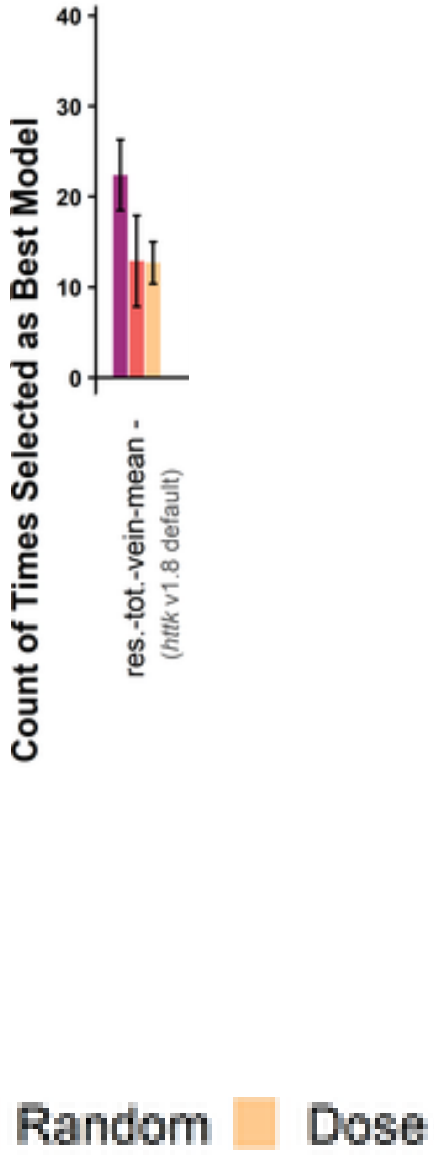

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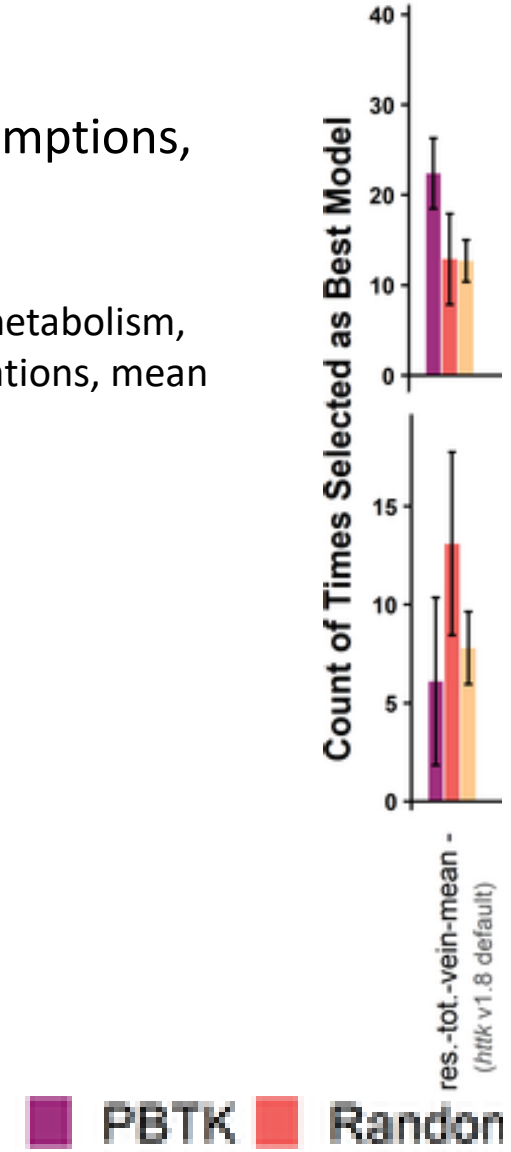

Forward Dosimetry

Reverse Dosimetry

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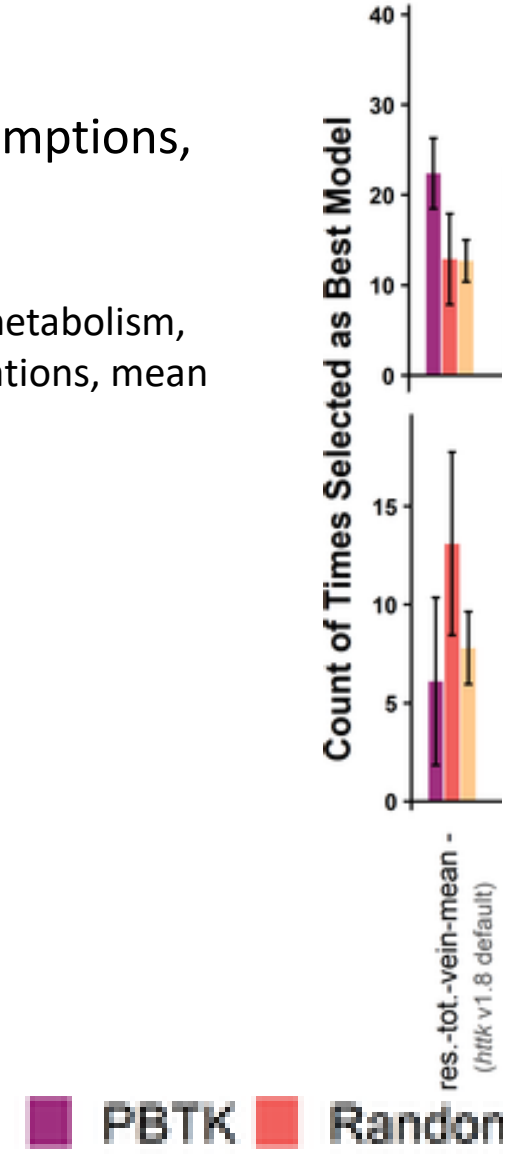
Different combinations of assumptions, for example:

res-tot-vein-mean = restrictive metabolism, total chemical, venous concentrations, mean concentration during tox study



Forward Dosimetry

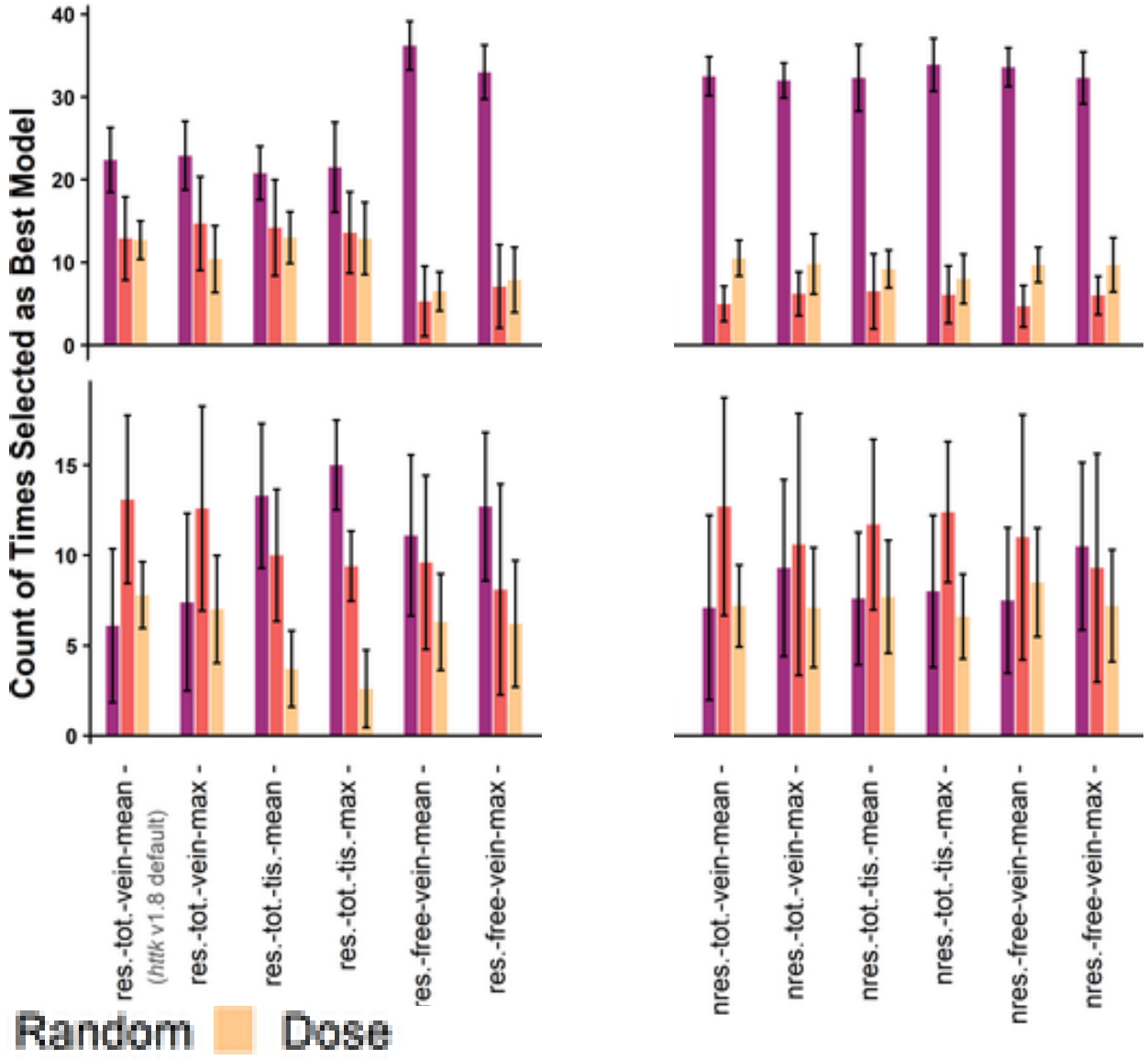
Reverse Dosimetry

Impact of IVIVE Assumptions

Different combinations of assumptions, for example:

res-tot-vein-mean = restrictive metabolism, total chemical, venous concentrations, mean concentration during tox study

nres-tot-tis-max = non-restrictive metabolism, total chemical, tissue concentrations, max conc. during tox study



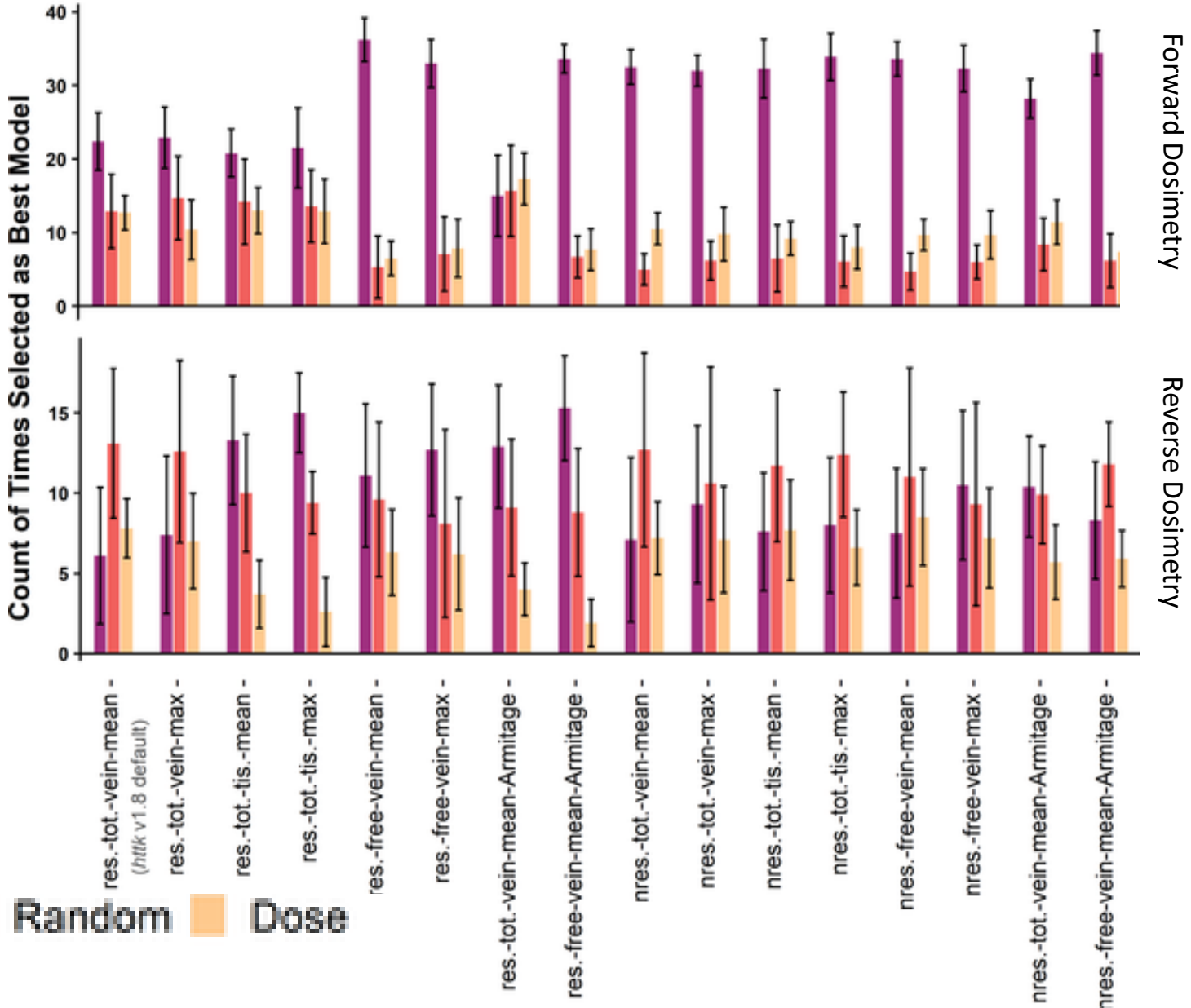
Impact of IVIVE Assumptions

Different combinations of assumptions, for example:

res-tot-vein-mean = restrictive metabolism, total chemical, venous concentrations, mean concentration during tox study

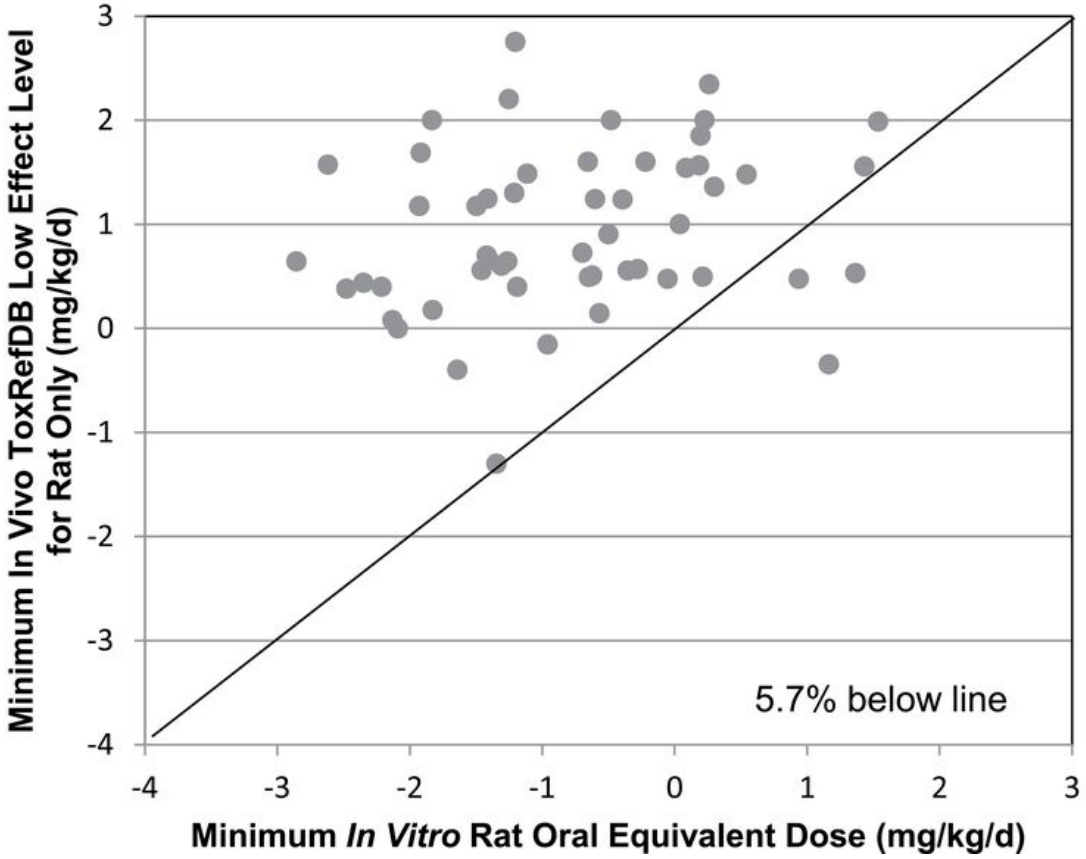
nres-tot-tis-max = non-restrictive metabolism, total chemical, tissue concentrations, max conc. during tox study

Several IVIVE combinations using the Armitage model decreased error, but no single ideal approach

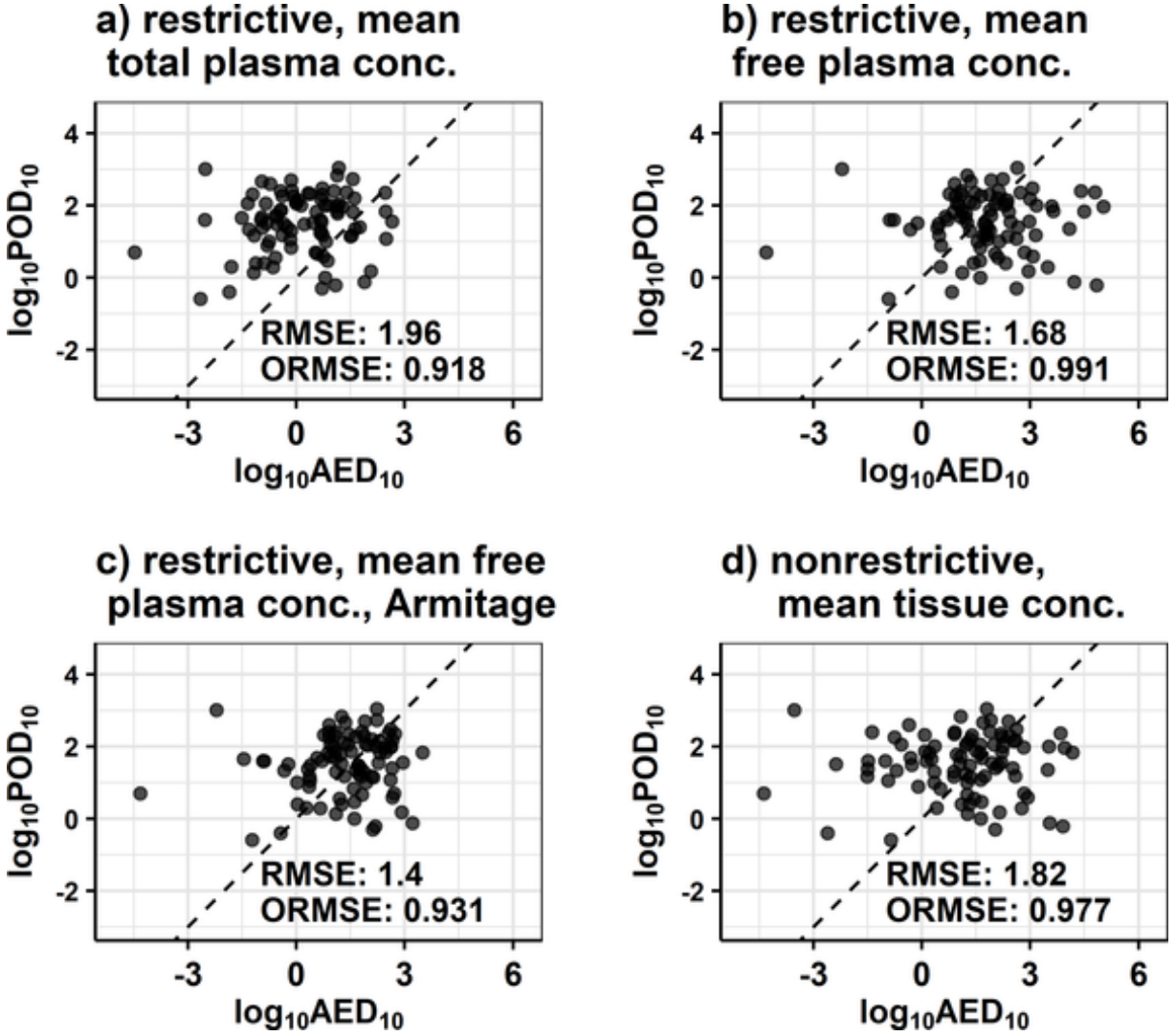


Comparing Points of Departure and IVIVE

Wetmore et al. (2013)

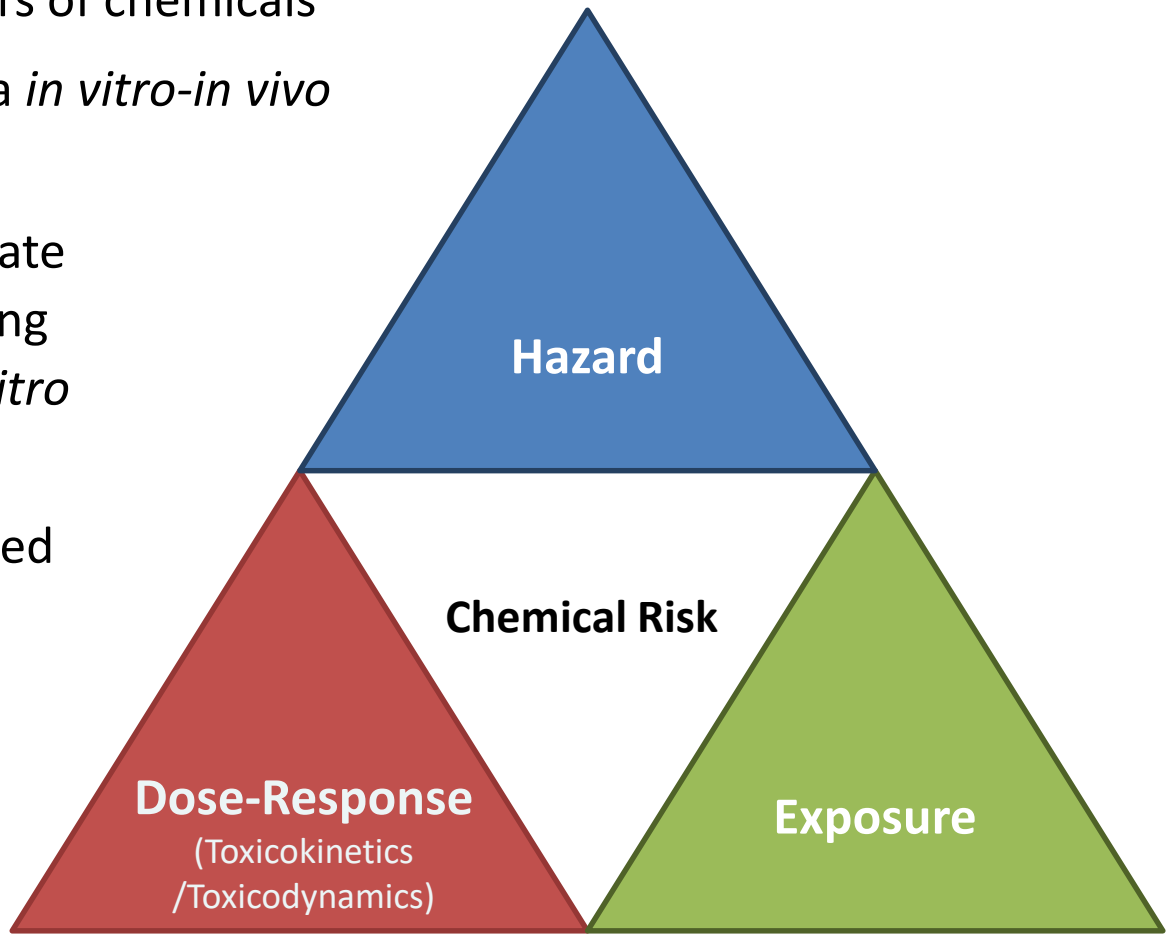


Honda et al. (2019)



Summary

- NAMs for TK allow risk-based prioritization of large numbers of chemicals
- *In vitro* disposition modeling and PBTK enable improved via *in vitro-in vivo* extrapolation (IVIVE)
- We tested various sets of IVIVE assumptions and demonstrate that the combination of PBTK and *in vitro* disposition modeling improves our ability to observe the association between *in vitro* bioactivity and *in vivo* toxicity data.
- Potency values from *in vitro* screening should be transformed IVIVE to build better machine learning and other statistical models for predicting *in vivo* toxicity in humans



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