Systemic Toxicity Predictions Using In Vitro and In Silico Approaches

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Big Questions

1. At what dose does a chemical cause adverse affects?

2. What effects does the chemical cause?

3. Can we answer 1 and 2 without using animals?

NAMs (New Approach Methodologies) attempt to answer these

New Approach Methods

- In silico (e.g. QSAR and Read-across)
  - Estimate effects and doses
- In vitro assays
  - Broad / screening (transcriptomics, cell painting)
  - Targeted (receptors, enzymes)
  - In vitro PODs, modes / mechanisms of action
- In vitro Toxicokinetics
  - Allow conversion of an in vitro POD to in vivo (IVIVE)
- Databases of existing traditional toxicology data
  - Enables training and validation of NMA models

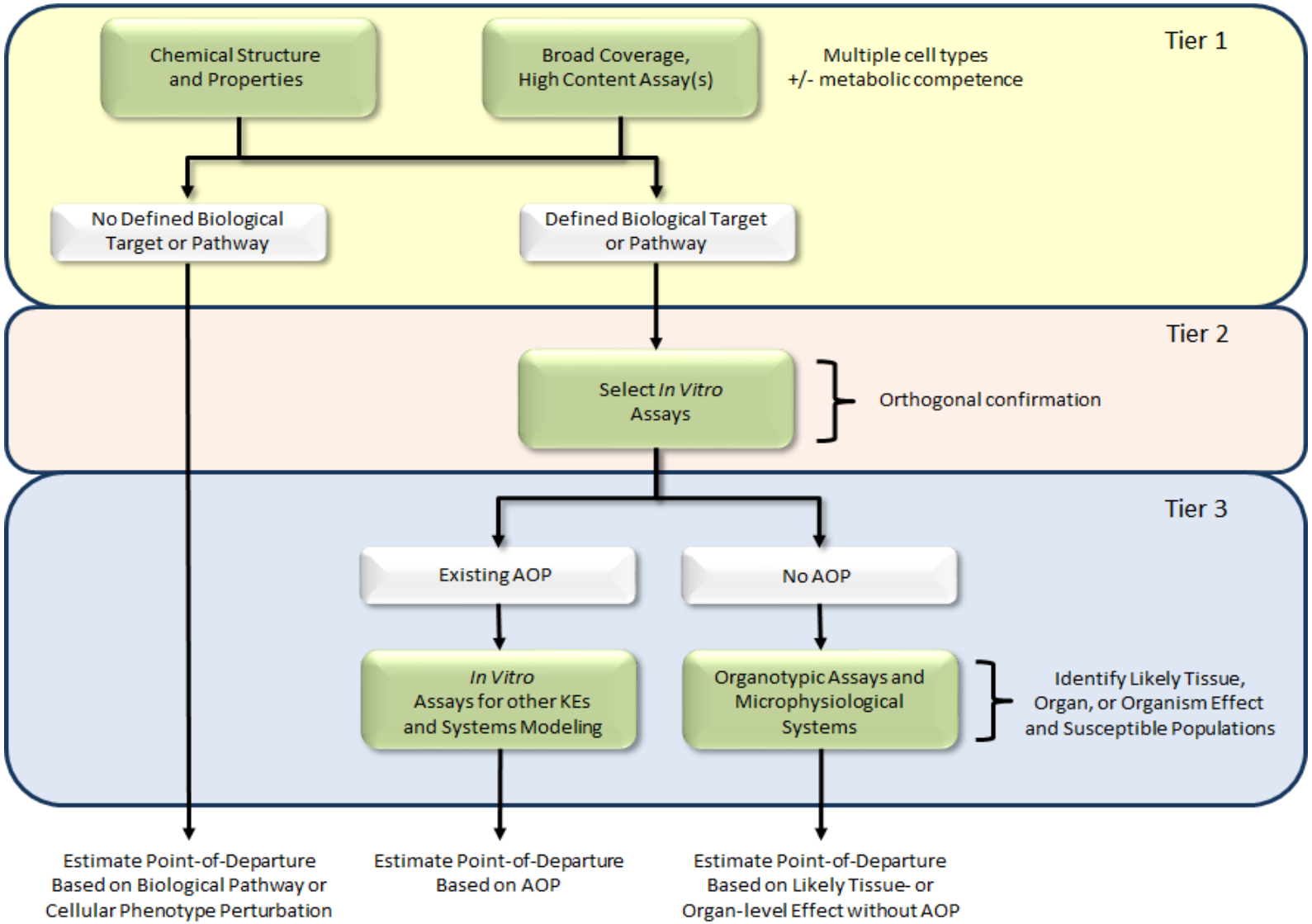
Overall Goals

- Predict in vivo points of departure without using animals (mg/kg/day)

- Approach 1: In vitro to in vivo (IVIVE)
  - Measure in vitro points of departure (μM)
  - Estimate toxicokinetics
  - Back-calculate oral dose that would lead to internal concentration=in vitro POD

- Approach 2: QSAR Extrapolation of Known In Vivo PODs
  - Make use of large data set of existing PODs
  - Build structure-based models to predict PODs for new chemicals

IVIVE Context



The "CompTox Blueprint"

Use in vitro methods to understand possible effects (MIE in AOP) and PODs

The NexGen Blueprint of CompTox at USEPA
Tox. Sci. 2019; 169(2):317-322

Two Screening Technologies

- High-Throughput Phenotypic Profiling(HTPP)
  - Also called Cell Painting
  - Visualize different cell compartments
  - Examine changes in size, shape, texture

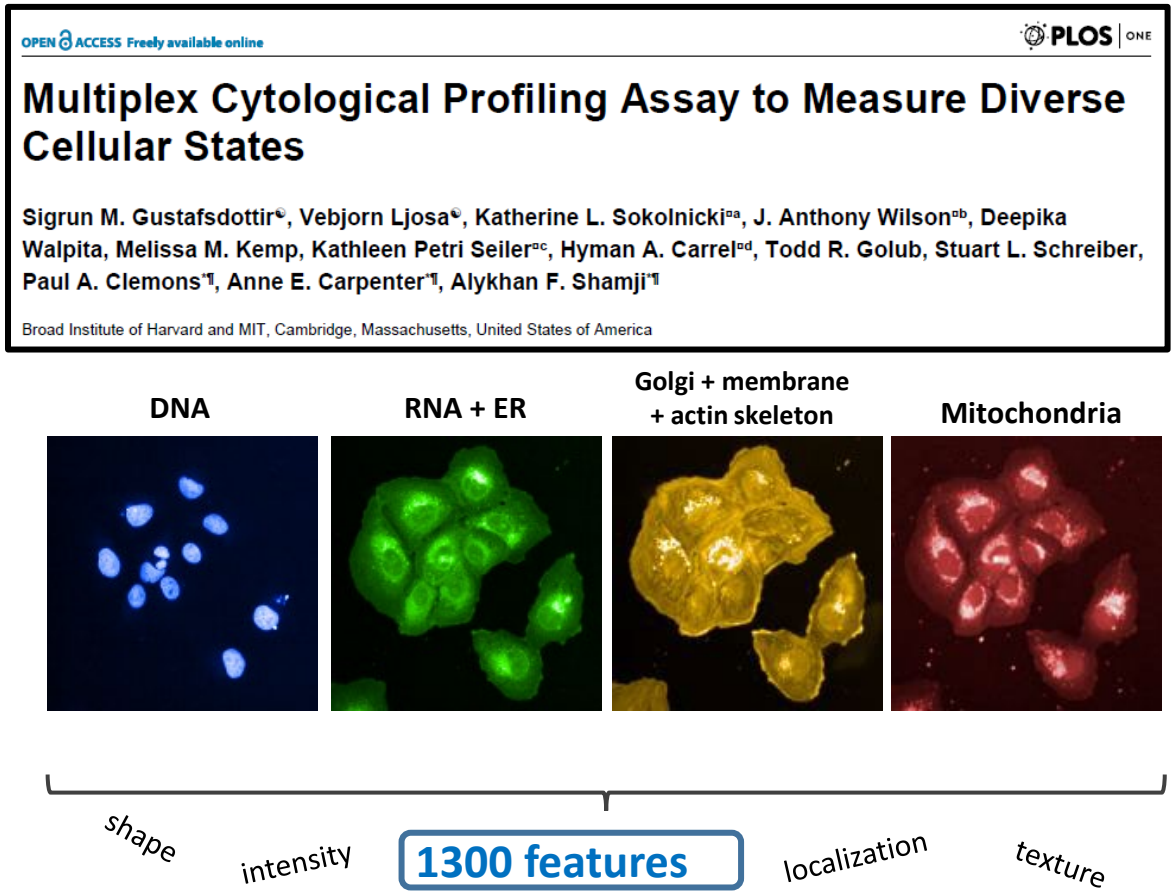
- High-throughput Transcriptomics (HTTr)
  - Measure changes in gene expression due to chemical exposure
  - Can run in whole genome or reduced coverage mode
  - We use the Temp-O-Seq Platform

IVIVE Tier 1: Cell Painting Assay (HTPP)

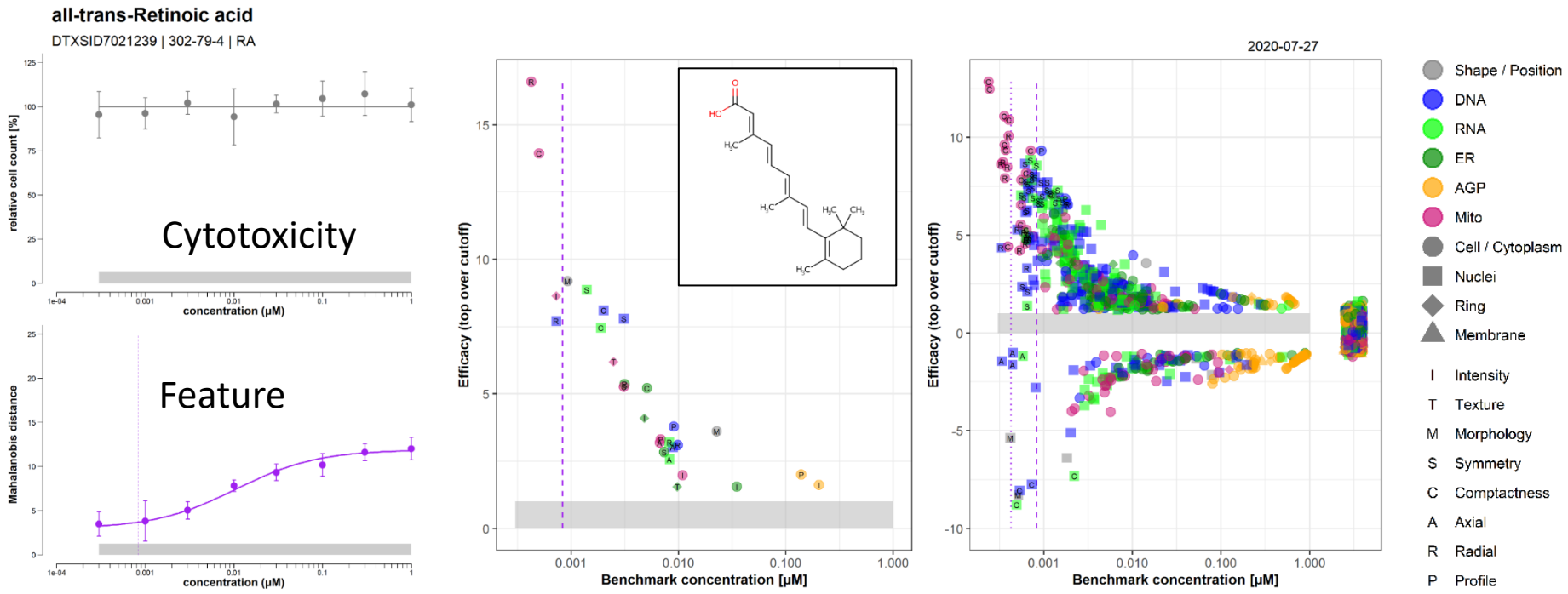
Cell Painting is a profiling method that measures a large variety of phenotypic features in fluoroprobe labeled cells *in vitro*.

- High-throughput
- Scalable
- Amenable to lab automation
- Deployable across multiple human-derived cell types.
- Reproducible
- Cost-effective (¢ / well)
- Infrastructure investment
- High volume data management

Laboratory & bioinformatics workflows for conduct of this assay have been established at CCTE.



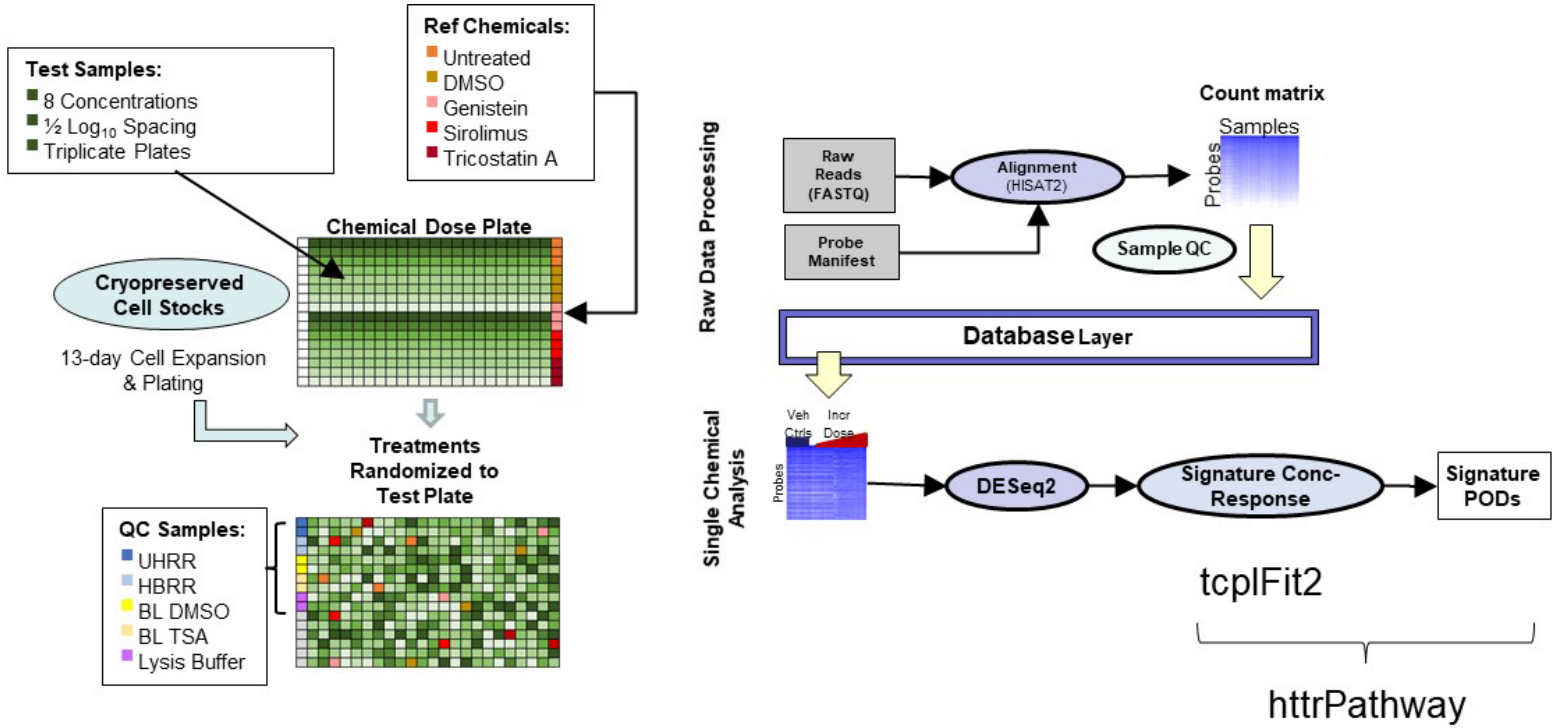
HTPP Concentration-Response Modeling Example



- At each concentration score each of 1300 features
- Do concentration-response analyses to get potency estimate
- Consolidate features into 49 categories for better interpretation

IVIVE Tier 1: Transcriptomics (HTTr)

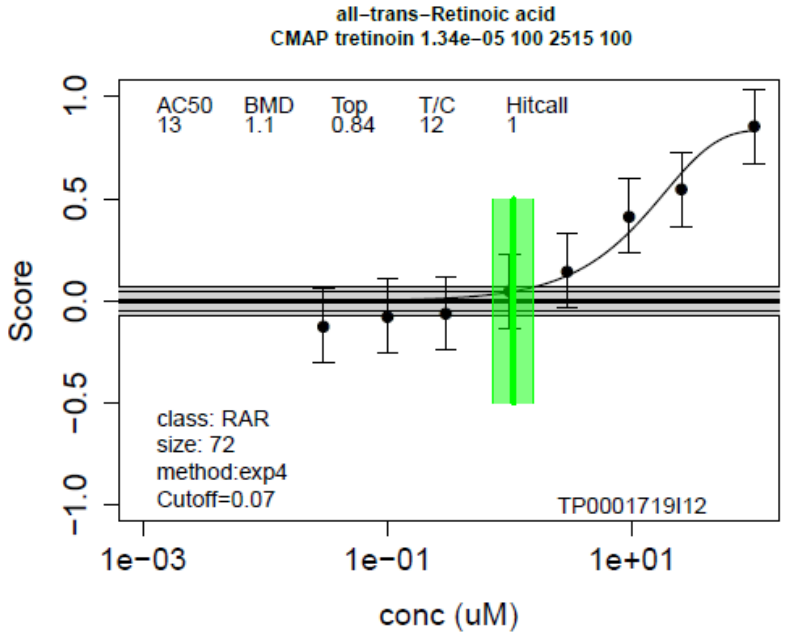
- Measure changes in gene expression across the whole genome
- Run in concentration-response
- Summarize data to the level of pathways (“signatures”, gene sets)



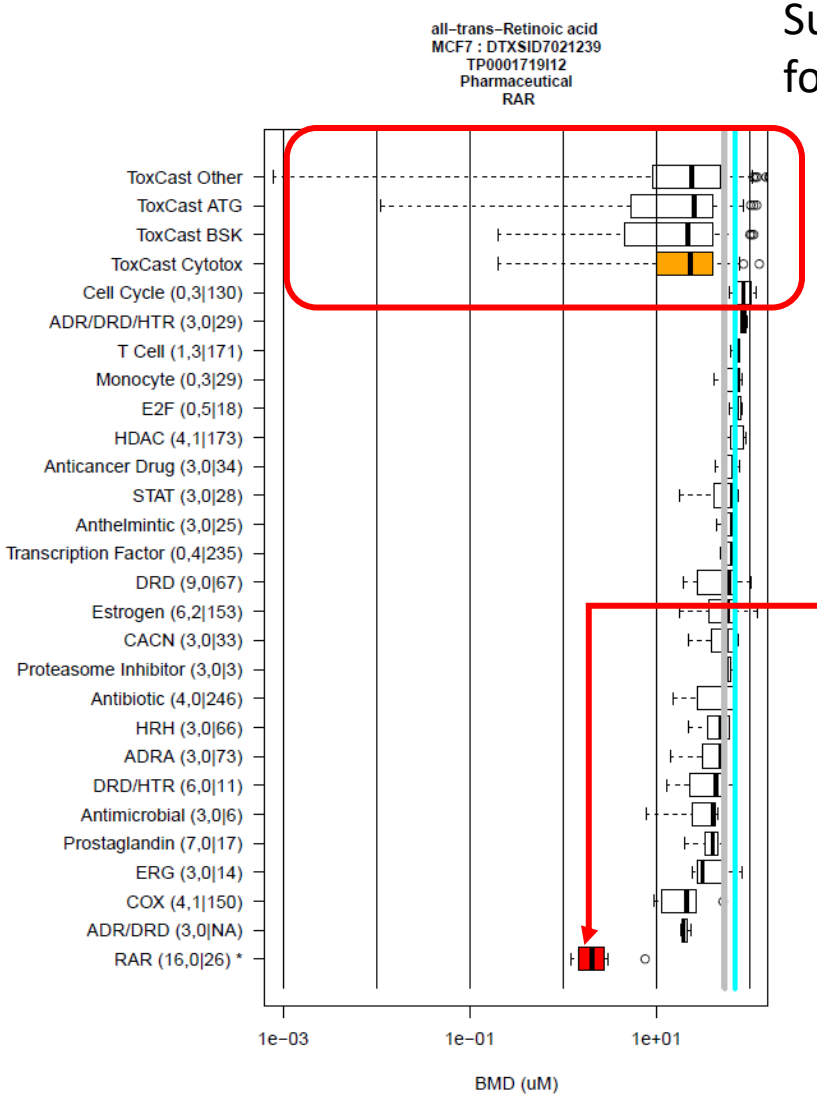
Tempo-Seq method is cost effect way to run 100s to 1000s of chemicals

Example HTTr Data

Single Concentration-Response Example



- Confidence Interval (CI) around points from the fitting error term
- Outer gray band is 95% CI of null dist.
- Inner lines are benchmark response
- Green vertical band is BMD and 95% CI



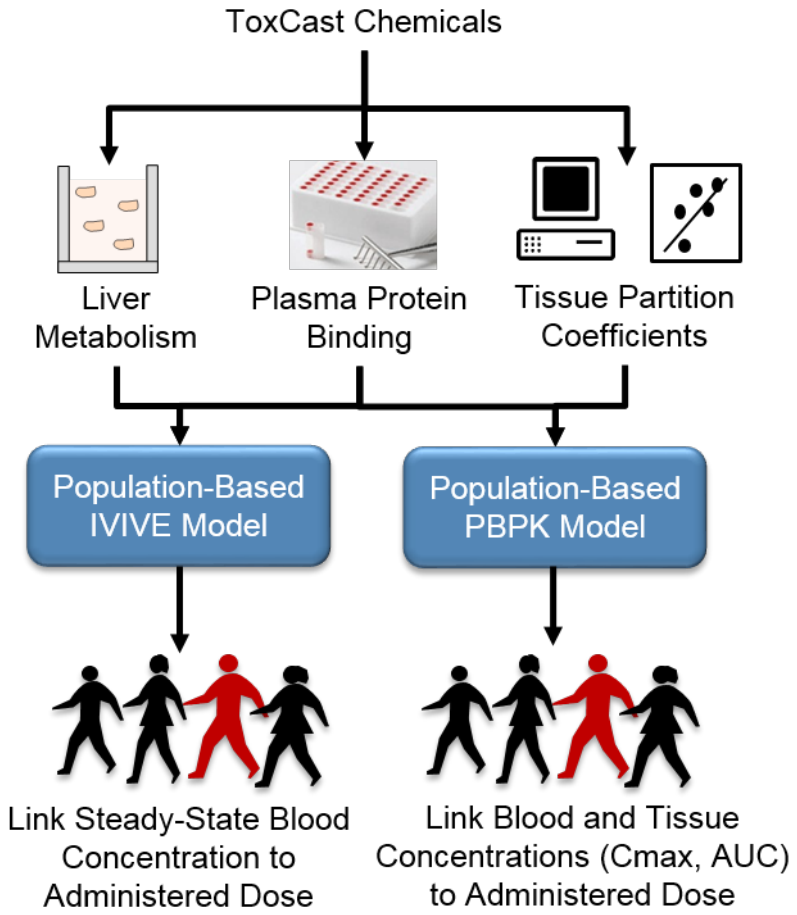
Summary across all pathways for one chemical

Data from other in vitro assays (“ToxCast”) may provide lower PODs

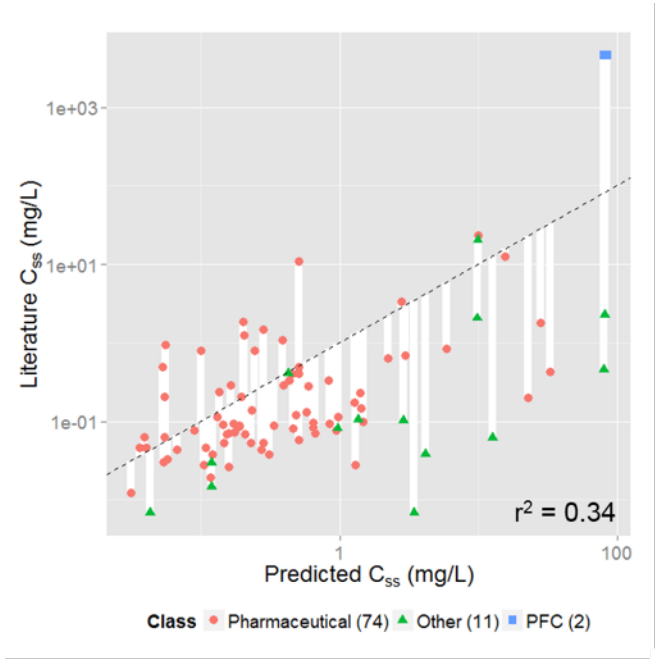
HTTr POD

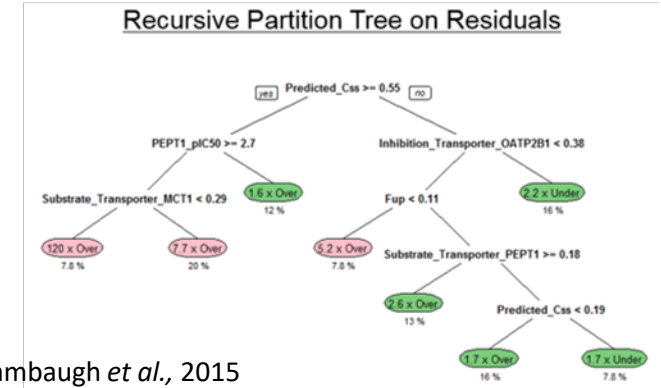
Toxicokinetics Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



Wetmore et al.





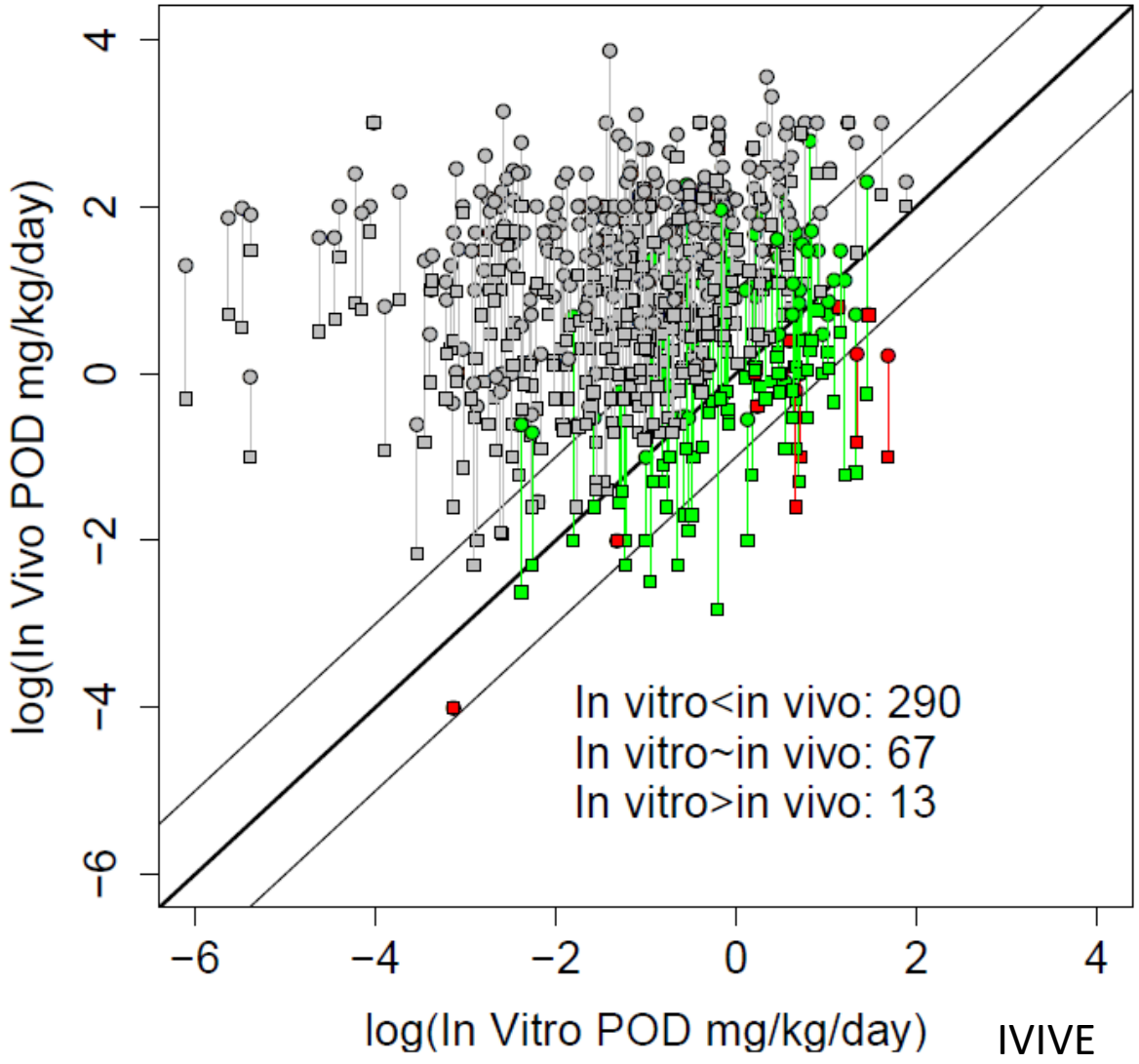
Wambaugh *et al.,* 2015

Putting it all together

- *In vitro* assays yield POD in μM
  - Select the minimum "relevant" *in vitro* POD
- TK yields *in vitro* to *in vivo* conversion factor
  - "Concentration at Steady State", C_{ss}
  - Blood concentration for a 1 mg/kg/day steady-state dose
- IVIVE POD ("oral equivalent dose") = *in vitro* POD / C_{ss}
- Exposure model yields estimate of exposure (mg/kg/day)

- BER: Bioactivity to Exposure Ratio
  - IVIVE POD / Exposure estimate
  - BER >> 1 implies low concern for risk

IVIVE PODs tend to provide low (protective) POD estimates: BERs are conservative



Only ~4% have *in vitro* POD consistently greater than *in vivo* values

Issue: what is the correct *in vitro* POD assay?

- Bioactivity vs. adversity

Work in progress: comparison of results taking into account both *in vivo* and *in vitro* uncertainties

PODs from QSAR models

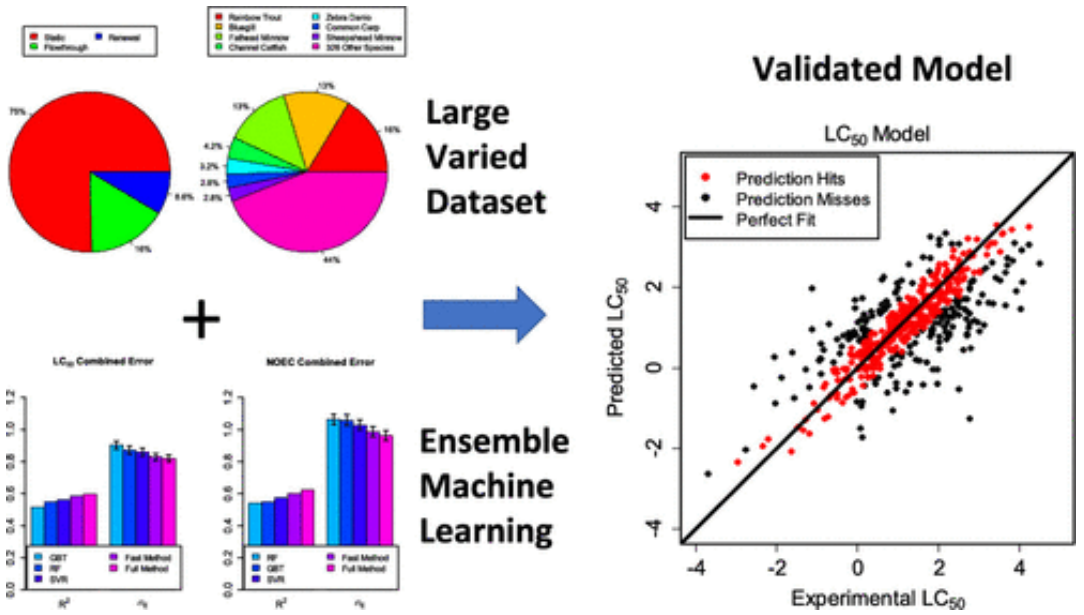
- Start with large database of historical in vivo PODs
- Use EPA ToxValDB
  - Collect in vivo data from >40 sources
  - Focus on public collections, supplemented with targeted literature searching
  - PODs from experimental studies, as well as reference doses, exposure limits and other kinds of quantitative values
  - Mammalian and ecological species
  - ~ 1,000,000 records
  - Available as an Excel file or through the EPA CompTox Chemicals Dashboard
    - https://comptox.epa.gov/dashboard

Basic QSAR modeling

- Matrix of chemical descriptors + experimental endpoint
- Use many different machine learning methods to predict quantitative values (e.g. PODs) or classes (e.g. genotoxic or not)
- Our models also incorporate uncertainty and variability in source in vivo data
- Output should also provide confidence intervals around values (e.g. PODs)

Fish QSAR Model

- Goal: Develop QSAR model to predict points of departure for fish acute and repeat-dose toxicity studies
- Model produces results at individual species level or at higher taxonomic levels
- Uses data from ToxValDB and ECOTOX
- Being evaluated against other EPA fish QSAR models



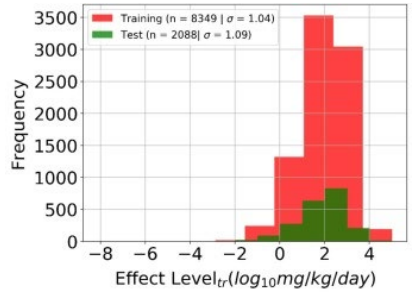
Environmental Science & Technology
Article
Cite This: Environ. Sci. Technol. 2019, 53, 12793−12802
pubs.acs.org/est

Ensemble QSAR Modeling to Predict Multispecies Fish Toxicity Lethal Concentrations and Points of Departure

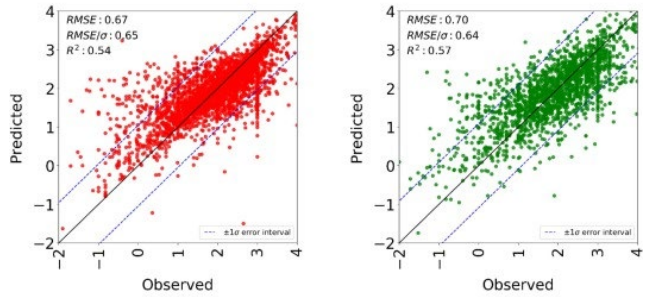
Thomas Y. Sheffield[†] and Richard S. Judson[*,‡]

Repeat Dose Mammalian QSAR Model

- Goal: Develop QSAR model to predict points of departure for repeat dose mammalian studies
  - Developed to support prioritization processes like the TSCA project
  - Compilation of the largest dataset of environmentally relevant chemicals for the development of POD models.
  - Assessment of underlying variability in the experimental data coming from a variety of in vivo studies.
  - Develop models to predict putative PODs along with 95% confidence intervals.
  - Incorporation of data variability to understand model uncertainty and derivation of confidence intervals.
  - Enrichment analysis to evaluate the suitability of these models from a screening level risk assessment perspective.



(a)



(b) (c)

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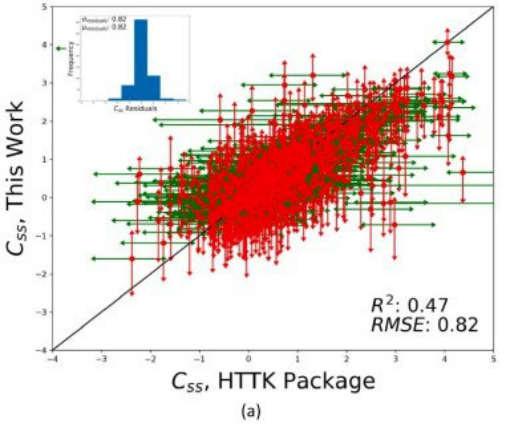
Computational Toxicology
Volume 16, November 2020, 100139

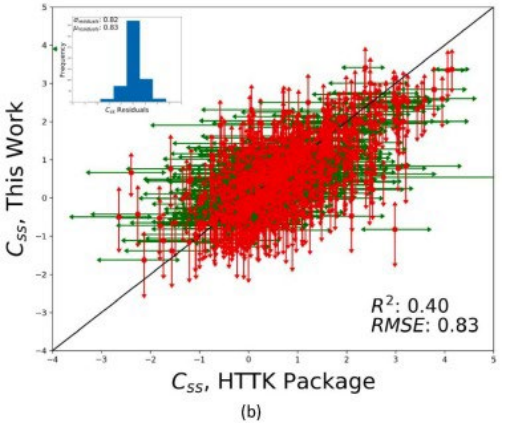
Structure-based QSAR models to predict repeat dose toxicity points of departure

Prachi Pradeep [a, b], Katie Paul Friedman [b], Richard Judson [b]

Toxicokinetics QSAR Model

- Goal: Predict In Vitro TK parameters to reduce testing requirements
  - Evaluation of the utility and ability of chemical structure information to predict TK parameters in silico.
  - Development of read-across and QSAR models of TK parameters using a dataset of 1487 environmental chemicals.
  - Demonstrating the utility of predicted TK parameters to estimate uncertainty in steady-state Css and IVIVE analyses.
  - Derivation of bioactivity-exposure ratio to compare human OEDs and exposure predictions for chemical prioritization.





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Computational Toxicology
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Using chemical structure information to develop predictive models for *in vitro* toxicokinetic parameters to inform high-throughput risk-assessment

Prachi Pradeep [a, b], Grace Patlewicz [b], Robert Pearce [a, b, 1], John Wambaugh [b], Barbara Wetmore [b], Richard Judson [b]

Summary

- Two major approaches for predicting in vivo PODs
  - In vitro- to-in vivo extrapolation
  - In vitro POD + in vitro TK
- QSAR
  - Use historical in vivo data to train machine learning models
- Both methods have uncertainty, often > 1 order of magnitude
  - Traditional in vivo testing also has such uncertainties due to study protocol, Species, strain, lab-to-lab variation
- Methods now being used mainly in priority setting contexts

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