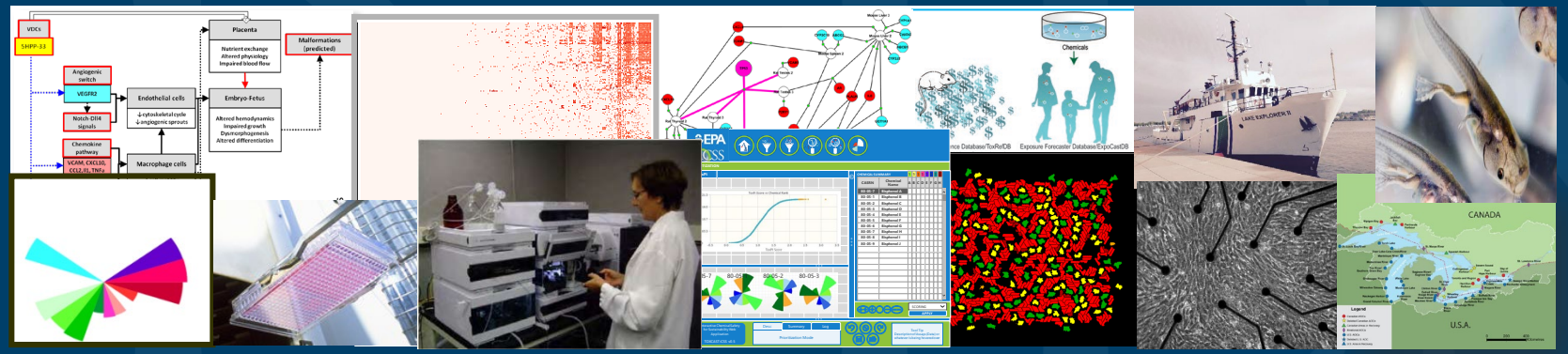
Update on Alternatives Research Activities at EPA



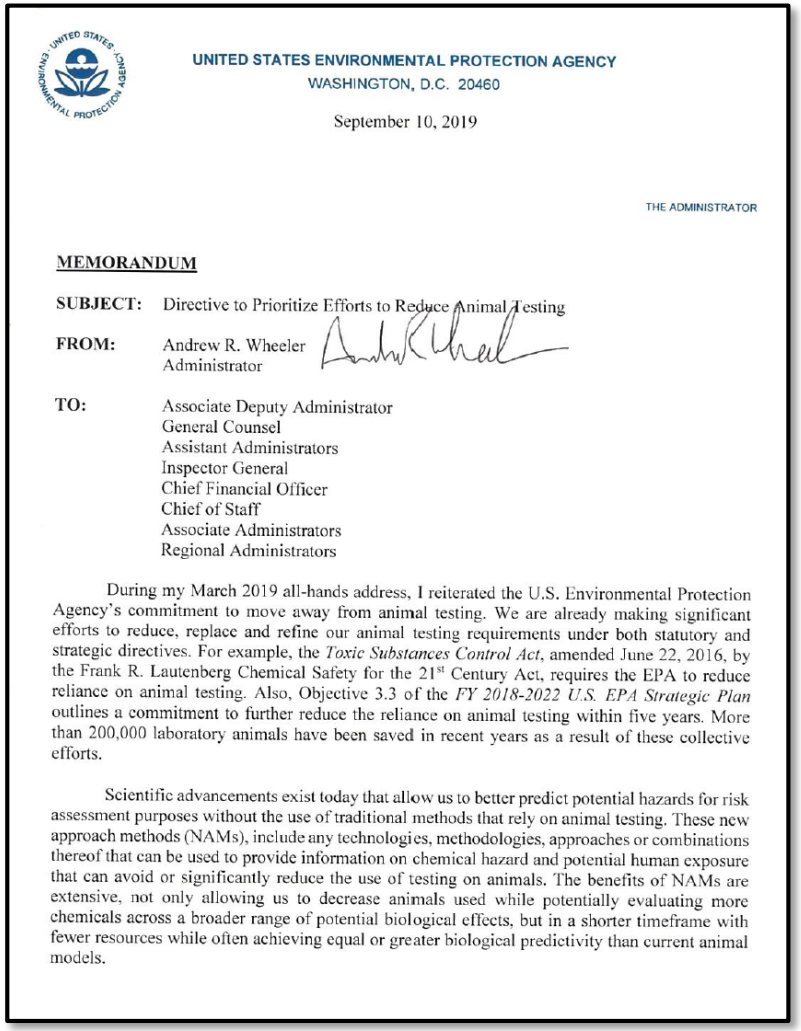
ICCVAM Public Forum

May 21, 2020

Rusty Thomas
Director
Center for Computational Toxicology and Exposure

The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA

The Release of the EPA Memo Provided Clear Agency Goals for Reduction in Animal Testing



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

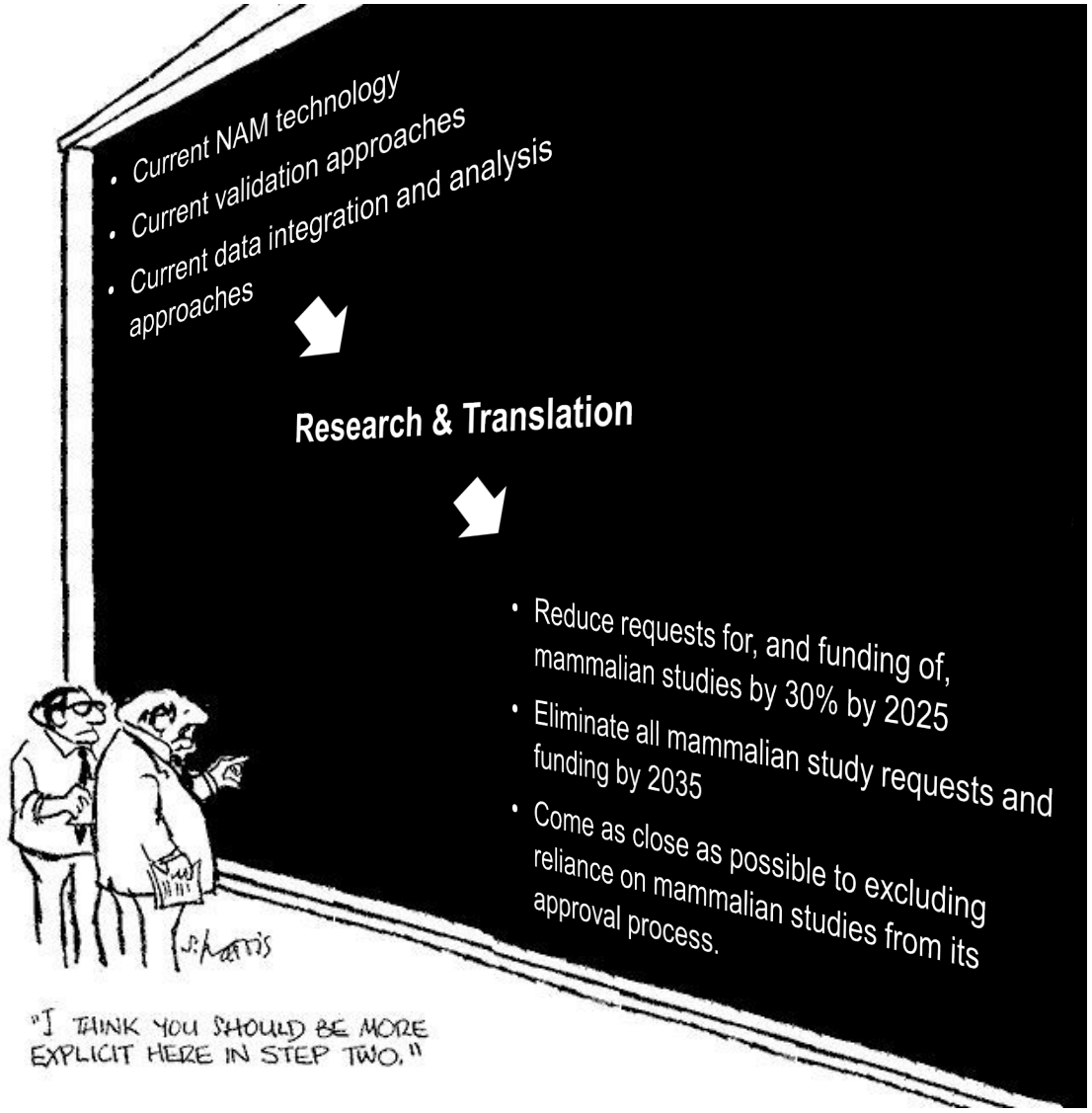
TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

During my March 2019 all-hands address, I reiterated the U.S. Environmental Protection Agency's commitment to move away from animal testing. We are already making significant efforts to reduce, replace and refine our animal testing requirements under both statutory and strategic directives. For example, the *Toxic Substances Control Act*, amended June 22, 2016, by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, requires the EPA to reduce reliance on animal testing. Also, Objective 3.3 of the *FY 2018-2022 U.S. EPA Strategic Plan* outlines a commitment to further reduce the reliance on animal testing within five years. More than 200,000 laboratory animals have been saved in recent years as a result of these collective efforts.

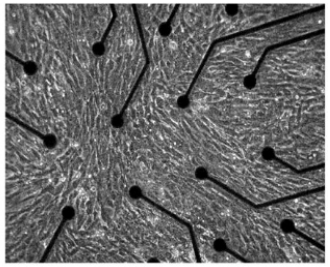
Scientific advancements exist today that allow us to better predict potential hazards for risk assessment purposes without the use of traditional methods that rely on animal testing. These new approach methods (NAMs), include any technologies, methodologies, approaches or combinations thereof that can be used to provide information on chemical hazard and potential human exposure that can avoid or significantly reduce the use of testing on animals. The benefits of NAMs are extensive, not only allowing us to decrease animals used while potentially evaluating more chemicals across a broader range of potential biological effects, but in a shorter timeframe with fewer resources while often achieving equal or greater biological predictivity than current animal models.

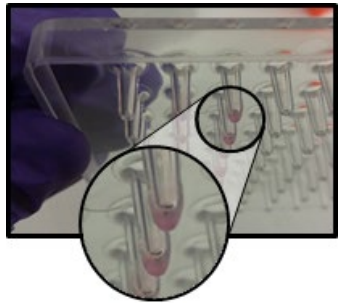
- Goals:
  - Reduce requests for, and funding of, mammalian studies by 30% by 2025
  - Eliminate all mammalian study requests and funding by 2035
  - Come as close as possible to excluding reliance on mammalian studies from its approval process (subject to applicable legal requirements).
- Objectives:
  - Evaluate regulatory flexibility for accommodating the use of NAMs
  - Develop baselines and metrics for assessing progress
  - Validation to ensure NAMs are equivalent to or better than the animal tests
  - Demonstration that NAMs are applicable for use in risk assessment and protective of human health and environment
  - Engage and communicate with stakeholders

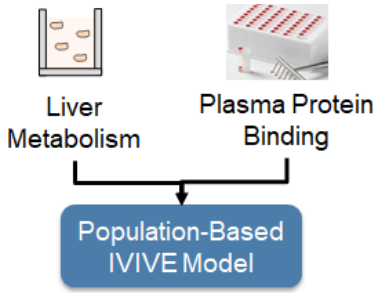
The Challenge…



ORD Research to Fill in “Step 2”





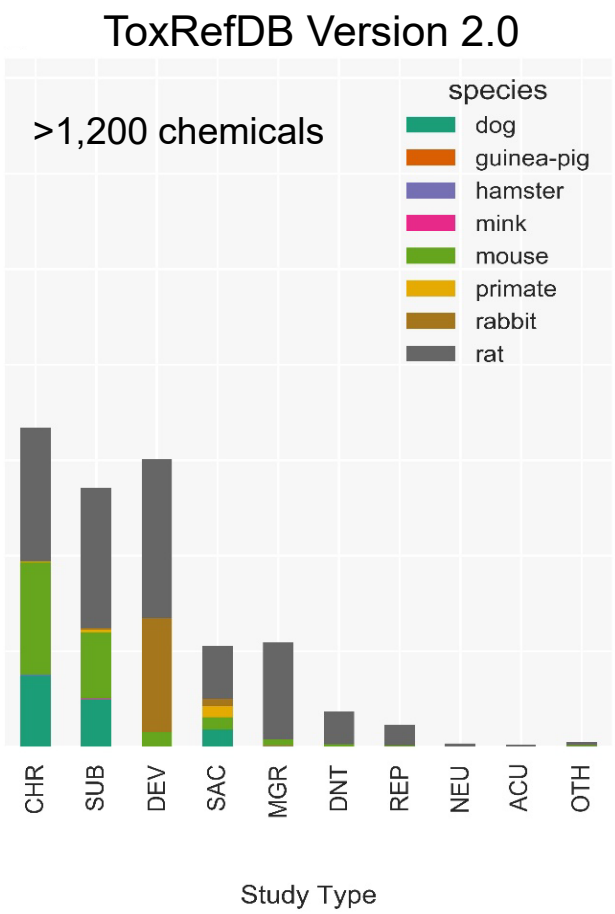


- Establish expectations on the variability of current toxicity studies
- Incorporate technological and data analysis advances to developing new alternatives
- Address limitations of *in vitro* test systems
- Build confidence through case studies

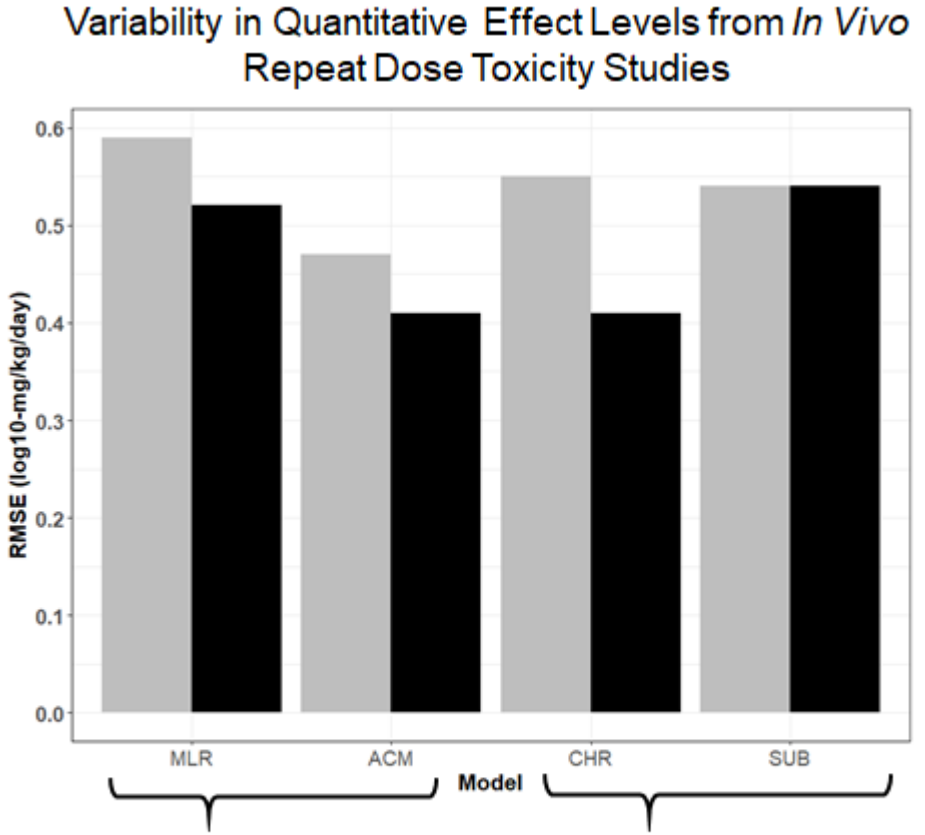
Mandate to Evaluate the Reliability and Relevance of Traditional Toxicity Testing Models

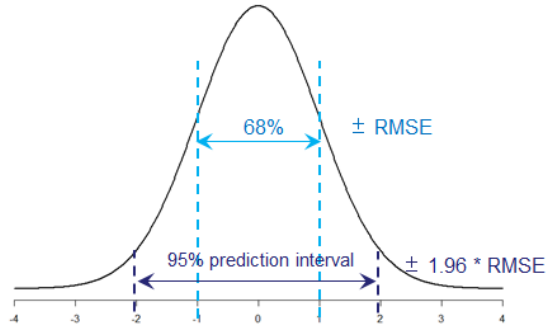
- Section 4(h) in the new TSCA legislation requires –
  - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - Alternative approaches need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models

Evaluating Reproducibility of Traditional Toxicity Studies



Watford *et al., Repro Toxicol*, 2019

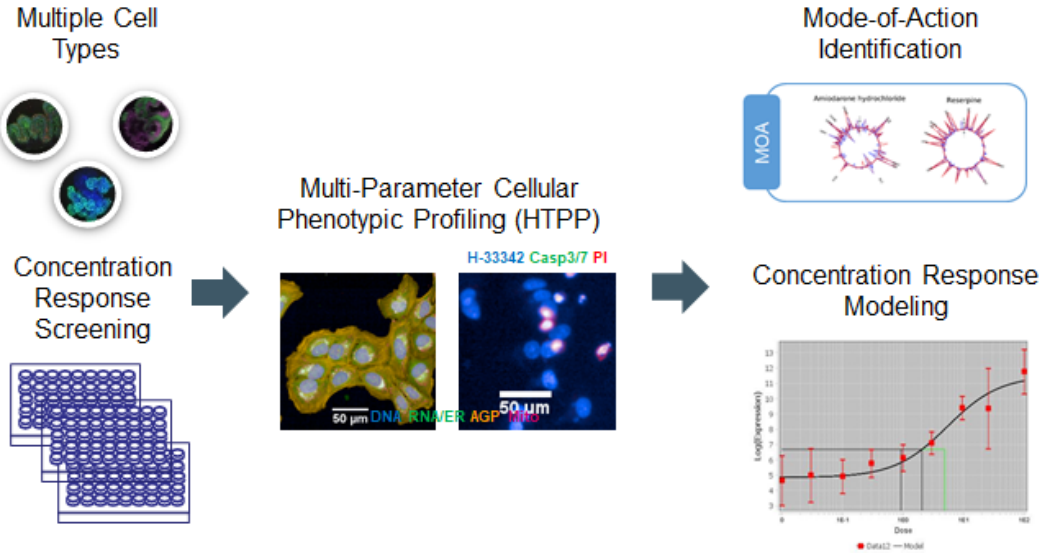


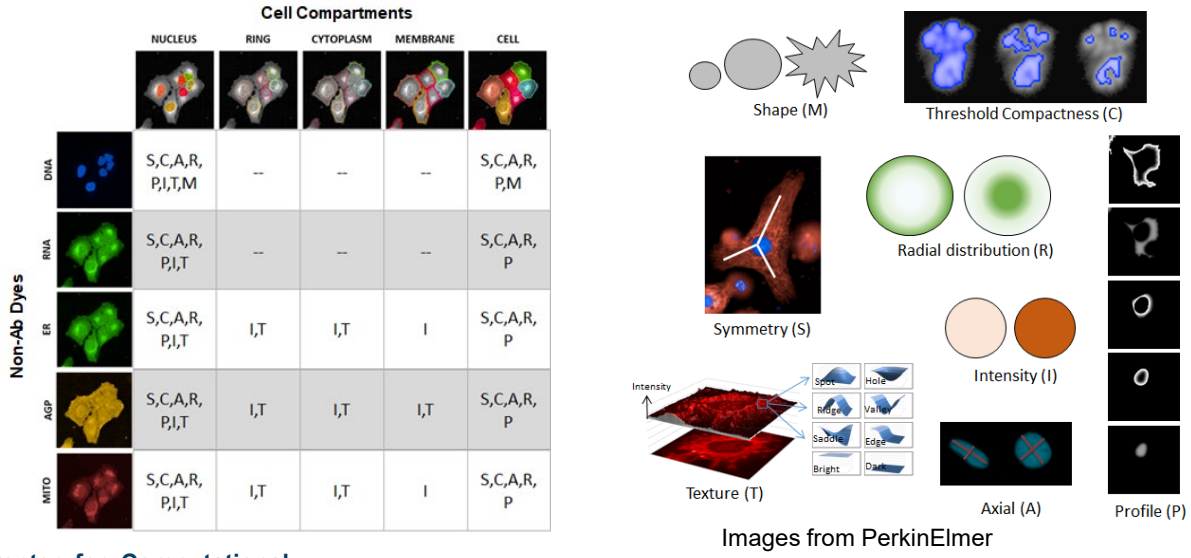


Using an RMSE=0.59, the minimum 95% PI of an LEL/LOAEL is:
1 mg/kg/day ➔ 0.07 – 14 mg/kg/day.
10 mg/kg/day ➔ 0.7 – 143 mg/kg/day.

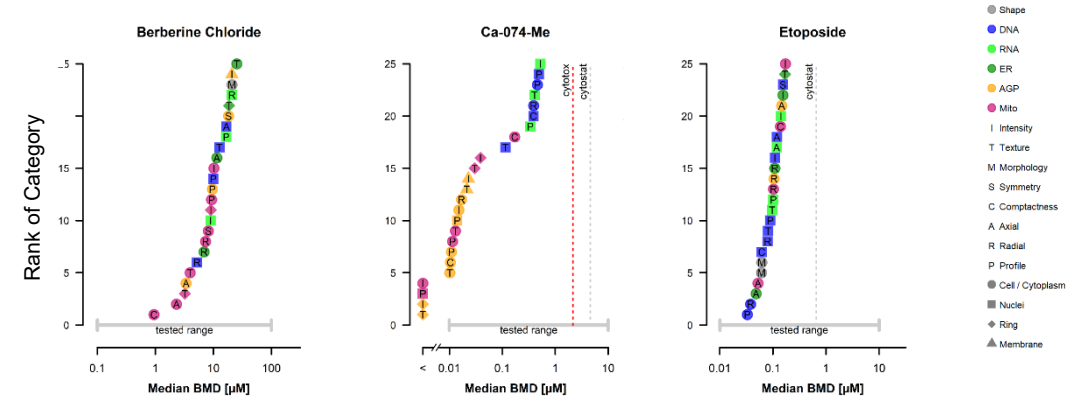
Pham *et al., Comp Toxicol.,* In Press

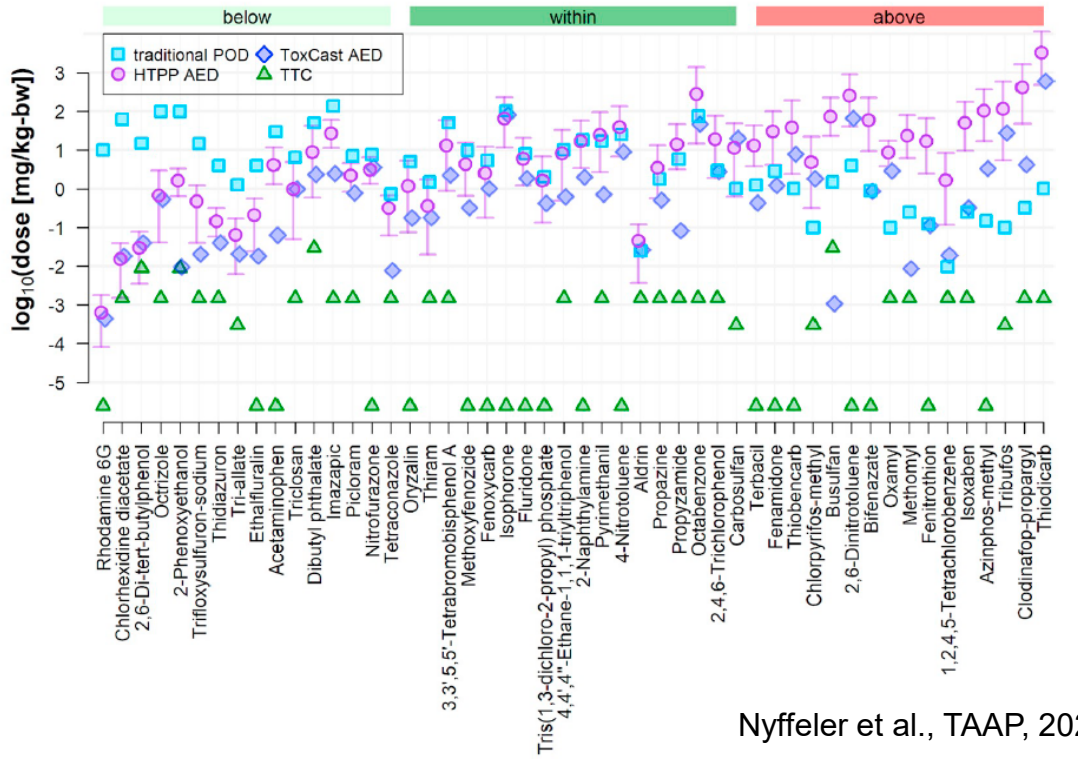
Comparing 'Cellular Pathology' With *In Vivo* Pathology Responses





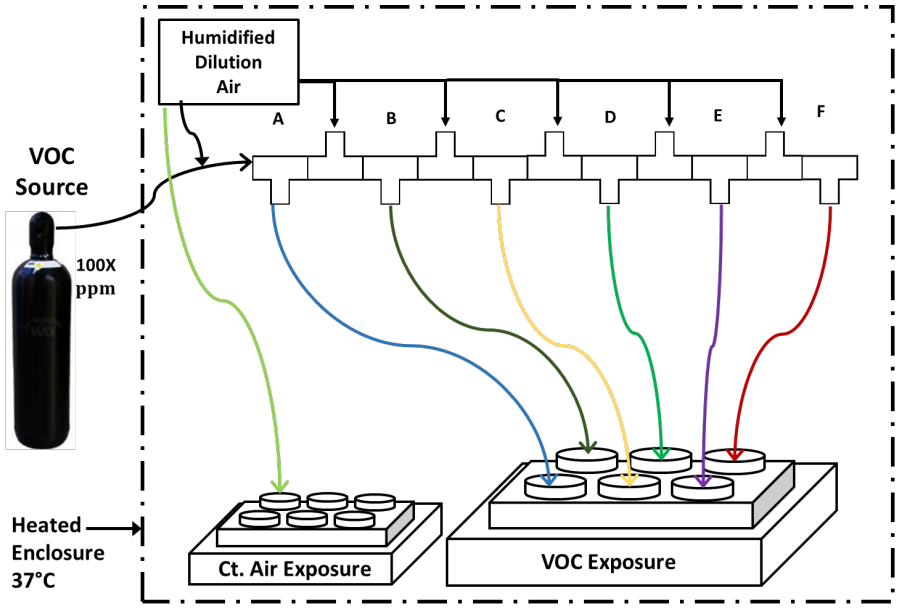
Images from PerkinElmer

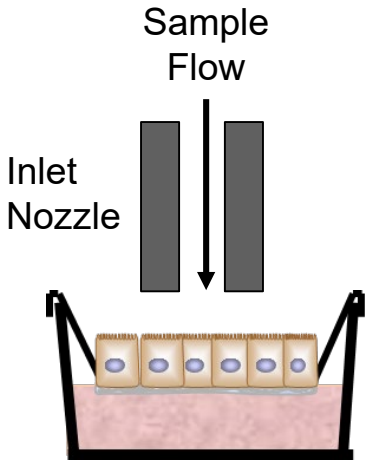




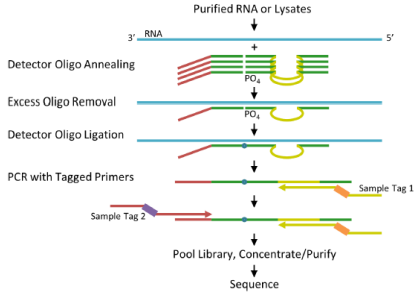
Nyffeler et al., TAAP, 2020

Adapting *In Vitro* Assays to Test Volatile Chemicals

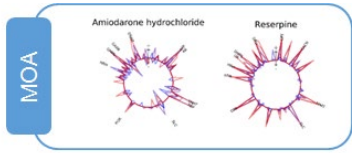




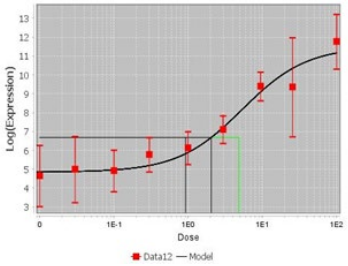
Whole Genome Transcriptomics (HTTr)



Mode-of-Action Identification



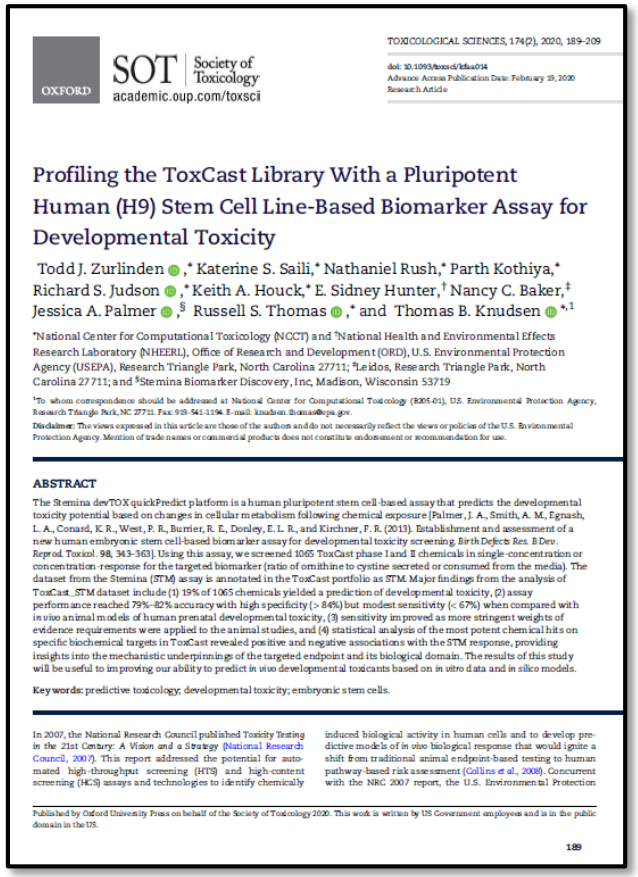
Concentration Response Modeling



Chemical Name	Gene Set Collection	BEAS-2B, BMC of most sensitive gene set (ppm)	HBEC, BMC of most sensitive gene set (ppm)	ACGIH TLV (ppm)
1-Bromopropane	MSigDB_C2	2.49302	9.93639	
1-Bromopropane	MSigDB_H	2.97983	NA	0.1
1-Bromopropane	Reactome	2.664425	NA	
Carbon Tetrachloride	MSigDB_C2	9.23691	NA	
Carbon Tetrachloride	MSigDB_H	16.91345	NA	10
Carbon Tetrachloride	Reactome	11.0172	NA	
Trichloroethylene	MSigDB_C2	48.9539	27.9907	
Trichloroethylene	MSigDB_H	NA	36.4984	50
Trichloroethylene	Reactome	69.6447	32.0725	
Dichloromethane	MSigDB_C2	136.124	269.865	
Dichloromethane	MSigDB_H	231.7465	394.894	100
Dichloromethane	Reactome	136.124	355	

A.Speen (CPHEA), M. Higuchi (CPHEA), and J. Harrill, Unpublished

Integrating *In Vitro* Assays to Predict Developmental Toxicity



TOXICOLOGICAL SCIENCES, 174(2), 2020, 189–209

doi: 10.1093/toxsci/kfaa014
Advance Access Publication Date: February 19, 2020
Research Article

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity

Todd J. Zurlinden, Katerine S. Saili, Nathaniel Rush, Parth Kothiya, Richard S. Judson, Keith A. Houck, E. Sidney Hunter, Nancy C. Baker, Jessica A. Palmer, Russell S. Thomas, and Thomas B. Knudsen

National Center for Computational Toxicology (NCCT) and National Health and Environmental Effects Research Laboratory (NHEERL), Office of Research and Development (ORD), U.S. Environmental Protection Agency (USEPA), Research Triangle Park, North Carolina 27711; Leidos, Research Triangle Park, North Carolina 27711; and Stemina Biomarker Discovery, Inc, Madison, Wisconsin 53719

To whom correspondence should be addressed at National Center for Computational Toxicology (B205-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711. Fax: 919-541-1194. E-mail: knudsen.thomas@epa.gov.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

The Stemina devTOX quickPredict platform is a human pluripotent stem cell-based assay that predicts the developmental toxicity potential based on changes in cellular metabolism following chemical exposure [Palmer, J. A., Smith, A. M., Egnash, L. A., Conard, K. R., West, P. R., Burrier, R. E., Donley, E. L. R., and Kirchner, F. R. (2013). Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. *Birth Defects Res. B Dev. Reprod. Toxicol.* 98, 343–363]. Using this assay, we screened 1065 ToxCast phase I and II chemicals in single-concentration or concentration-response for the targeted biomarker (ratio of ornithine to cystine secreted or consumed from the media). The dataset from the Stemina (STM) assay is annotated in the ToxCast portfolio as STM. Major findings from the analysis of ToxCast_STM dataset include (1) 19% of 1065 chemicals yielded a prediction of developmental toxicity, (2) assay performance reached 79%–82% accuracy with high specificity (> 84%) but modest sensitivity (< 67%) when compared with *in vivo* animal models of human prenatal developmental toxicity, (3) sensitivity improved as more stringent weights of evidence requirements were applied to the animal studies, and (4) statistical analysis of the most potent chemical hits on specific biochemical targets in ToxCast revealed positive and negative associations with the STM response, providing insights into the mechanistic underpinnings of the targeted endpoint and its biological domain. The results of this study will be useful to improving our ability to predict *in vivo* developmental toxicants based on *in vitro* data and *in silico* models.

Key words: predictive toxicology; developmental toxicity; embryonic stem cells.

In 2007, the National Research Council published Toxicity Testing in the 21st Century: A Vision and a Strategy (National Research Council, 2007). This report addressed the potential for automated high-throughput screening (HTS) and high-content screening (HCS) assays and technologies to identify chemically induced biological activity in human cells and to develop predictive models of *in vivo* biological response that would ignite a shift from traditional animal endpoint-based testing to human pathway-based risk assessment (Collins et al., 2008). Concurrent with the NRC 2007 report, the U.S. Environmental Protection

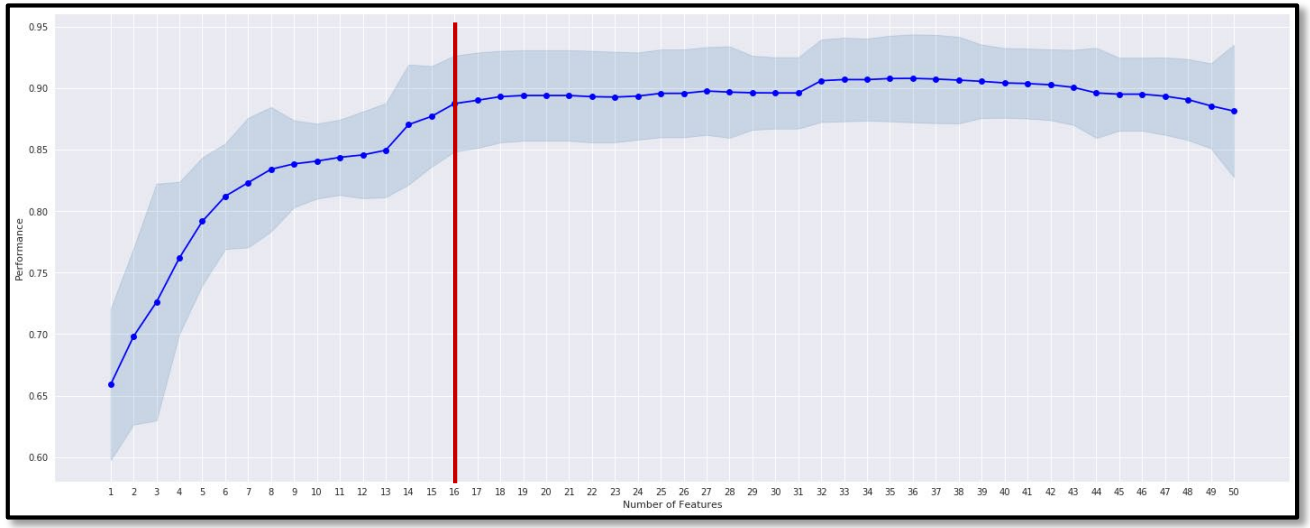
Published by Oxford University Press on behalf of the Society of Toxicology 2020. This work is written by US Government employees and is in the public domain in the US.

189

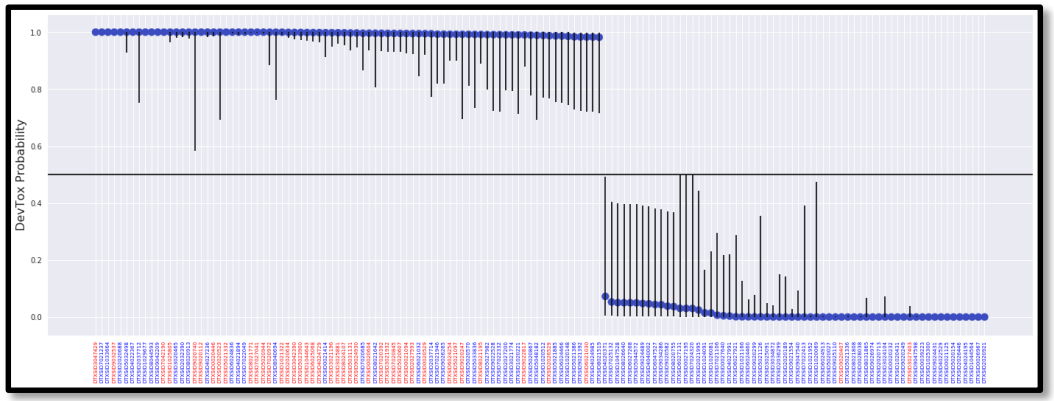
Augmented DevTox prediction model uses Stemina + ToxCast assays

Metric*	mean +/- sdev
ROC_AUC	0.91 +/- 0.03
Balanced Accuracy	0.82 +/- 0.04
NPV	0.80 +/- 0.05
PPV	0.90 +/- 0.08

*80/20 split (train/test) of the "Med_plus" data set (CLEAR rat OR rabbit, NO rat AND rabbit)



- Bayesian logisitic regression to determine probabilistic model for DevTox
- Capability to tune model for increased sensitivity OR specificity



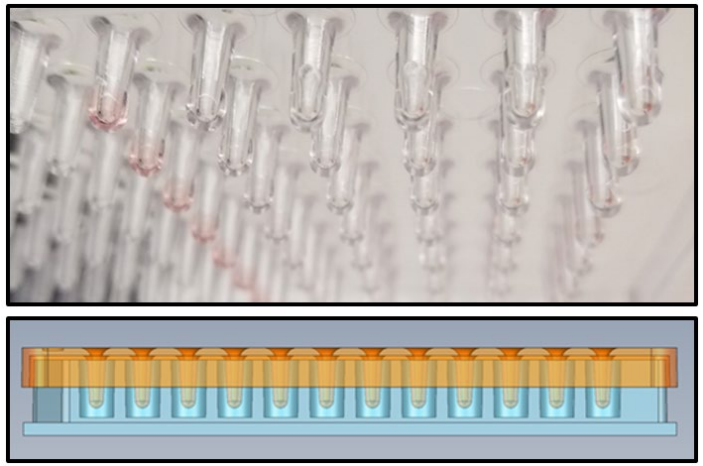
- Application of the "high specificity" model to ~580 chemicals on TSCA non-confidential inventory
- 144 chemicals predicted with confidence to fall into DevTox positive or negative domains

Zurlinden *et al., Toxicol Sci.*, 2020
T. Zurlinden, T. Knudsen, Unpublished

Incorporating Xenobiotic Metabolism Into *In Vitro* Assays

United States Environmental Protection Agency

AIME Method: S9 Fraction Immobilization in Alginate Microspheres on 96- or 384-well peg

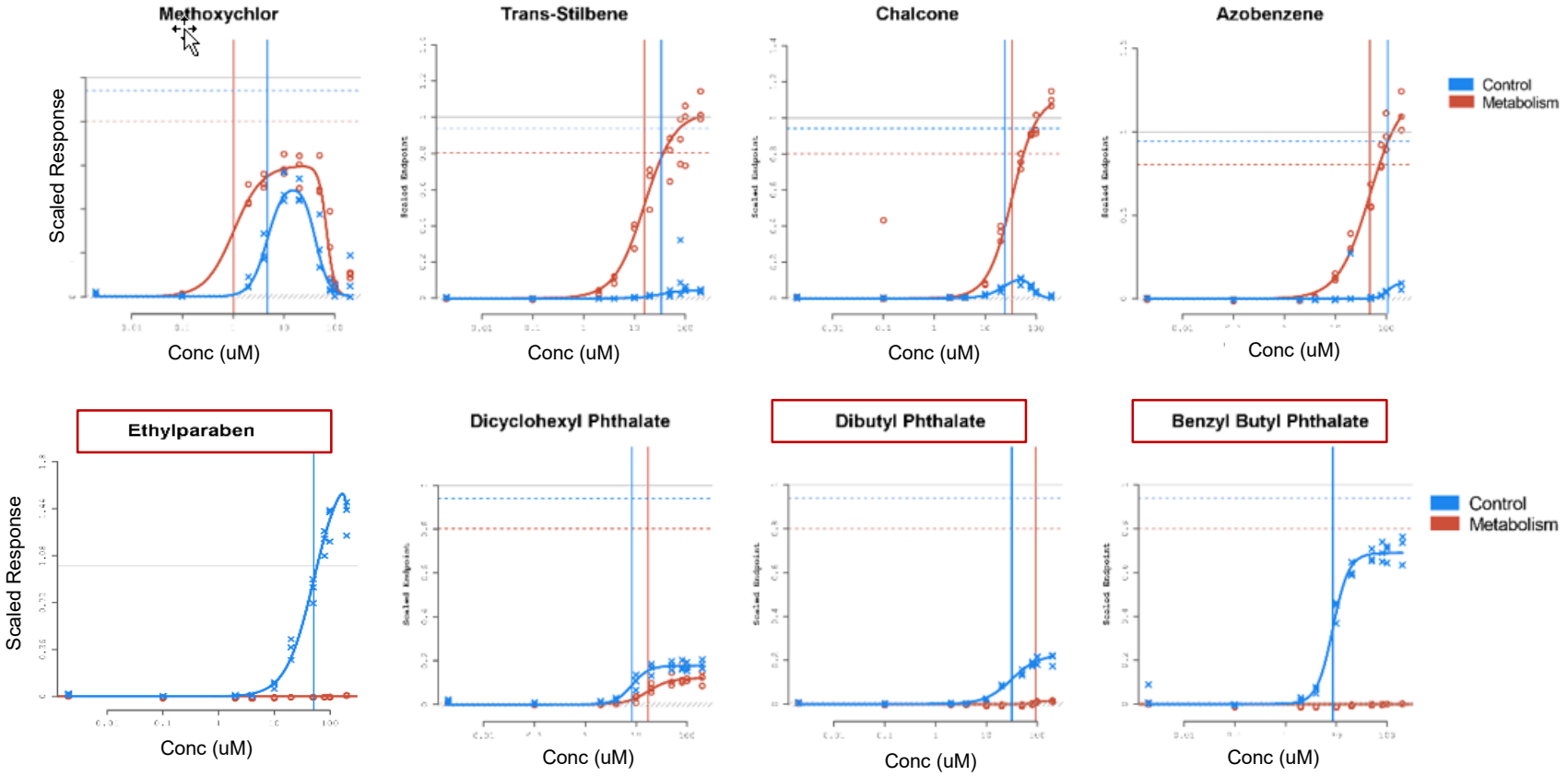


Screening Window of VM7 (formerly BG1) ER Transactivation Assay

		Metabolism	
		Neg	Pos
NRS	Neg	0.91	0.89
	Pos	0.91	0.71
		Z'	

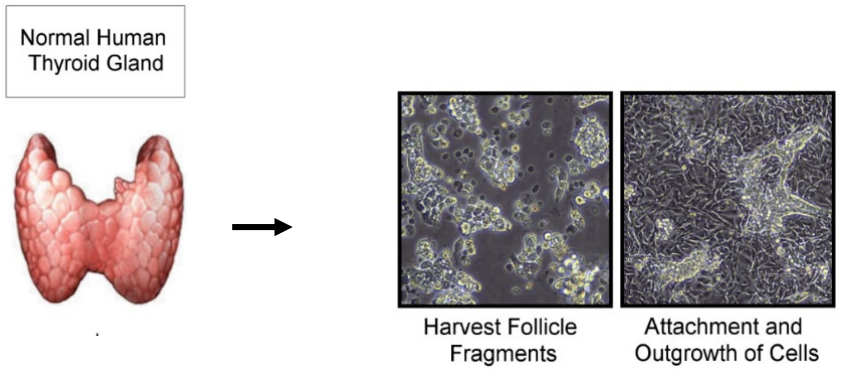
Application to ER Transactivation Assay (ERTA)

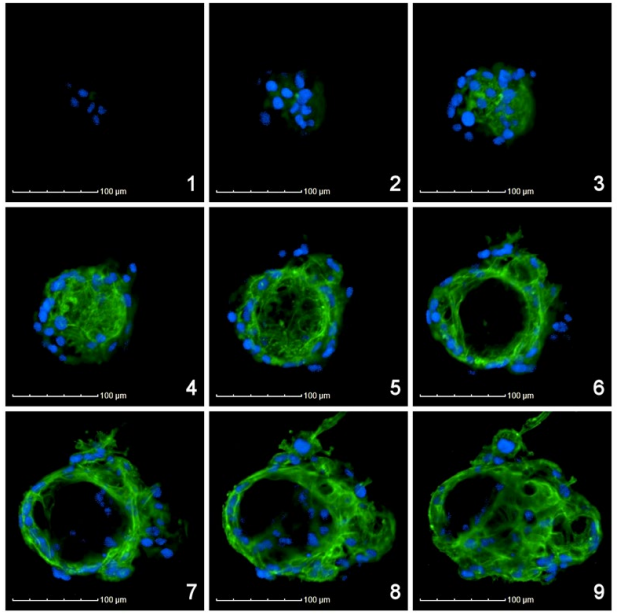
Pilot Screening Results of Pinto et al., 2016 Library



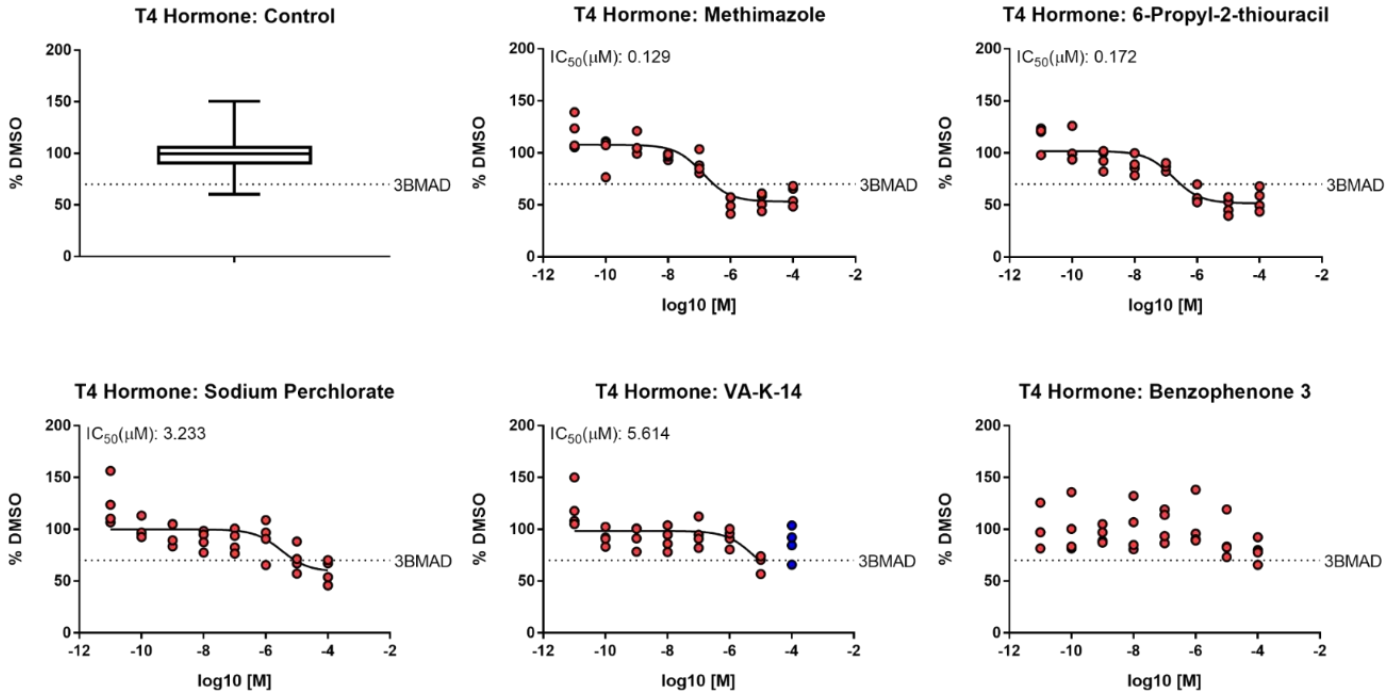
C. Deisenroth, In Review
Collaboration with Unilever

Developing Organotypic Culture Models to Identify Tissue/Organ Effects



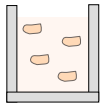


Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin



Deisenroth *et al.*, *Toxicol Sci*, 2020

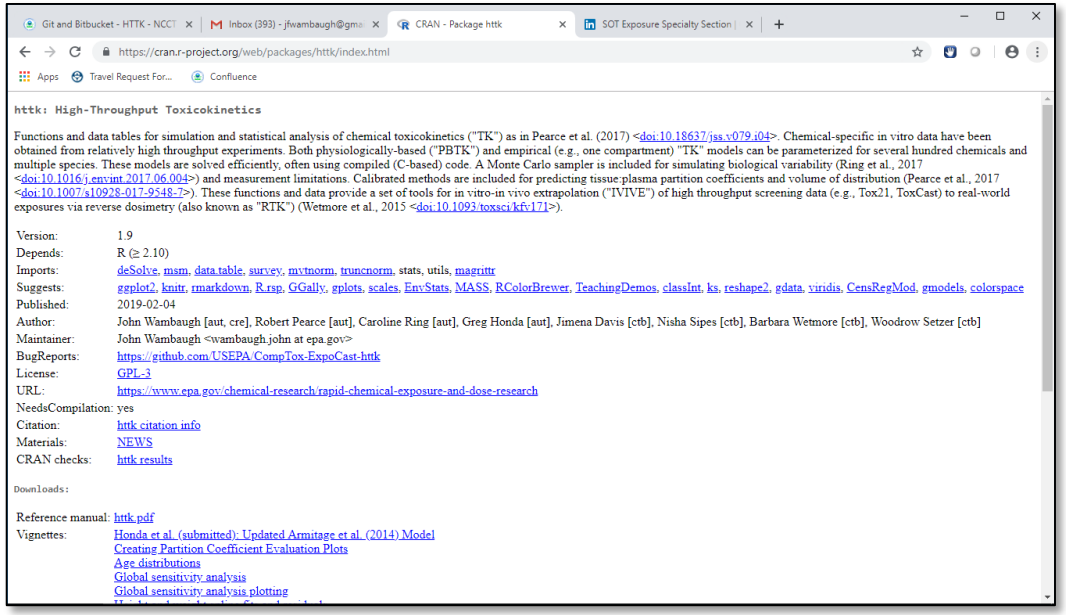
Putting Alternative Test Results in a Dose Context



Liver Metabolism | Tissue Partitioning | Plasma Protein Binding

Population-Based IVIVE Model

Oral Dose Required to Achieve Concentrations Equivalent to *In Vitro* Bioactivity



httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") as in Pearce et al. (2017) <doi:10.18637/jss.v079.i04>. Chemical-specific in vitro data have been obtained from relatively high throughput experiments. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability (Ring et al., 2017 <doi:10.1016/j.envint.2017.06.004>) and measurement limitations. Calibrated methods are included for predicting tissue:plasma partition coefficients and volume of distribution (Pearce et al., 2017 <doi:10.1007/s10928-017-9548-7>). These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., Tox21, ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK") (Wetmore et al., 2015 <doi:10.1093/toxsci/kfv171>).

Version:	1.9
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, utils, magrittr
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, reshape2, gdata, viridis, CensRegMod, gmodels, colorspace
Published:	2019-02-04
Author:	John Wambaugh [aut, cre], Robert Pearce [aut], Caroline Ring [aut], Greg Honda [aut], Jimena Davis [ctb], Nisha Sipes [ctb], Barbara Wetmore [ctb], Woodrow Setzer [ctb]
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
BugReports:	https://github.com/USEPA/CompTox-ExpoCast-httk
License:	GPL-3
URL:	https://www.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research
NeedsCompilation:	yes
Citation:	httk citation info
Materials:	NEWS
CRAN checks:	httk results

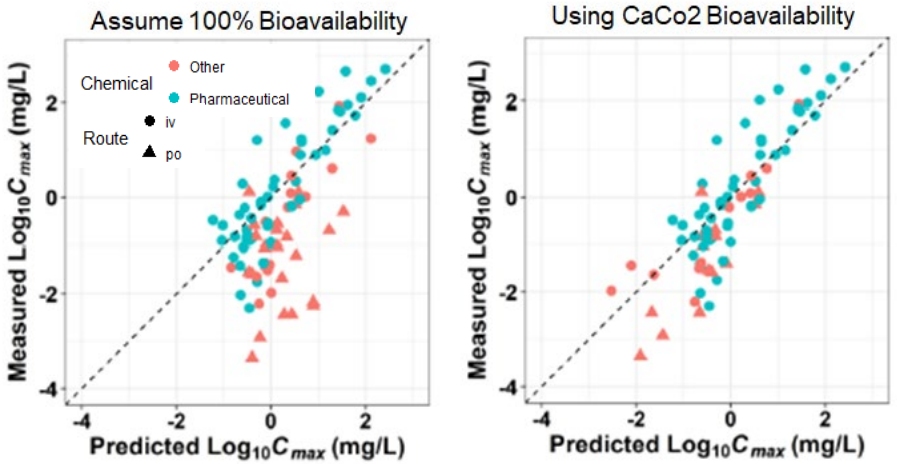
Downloads:

Reference manual:	httk.pdf
Vignettes:	Honda et al. (submitted): Updated Armitage et al. (2014) Model Creating Partition Coefficient Evaluation Plots Age distributions Global sensitivity analysis Global sensitivity analysis plotting

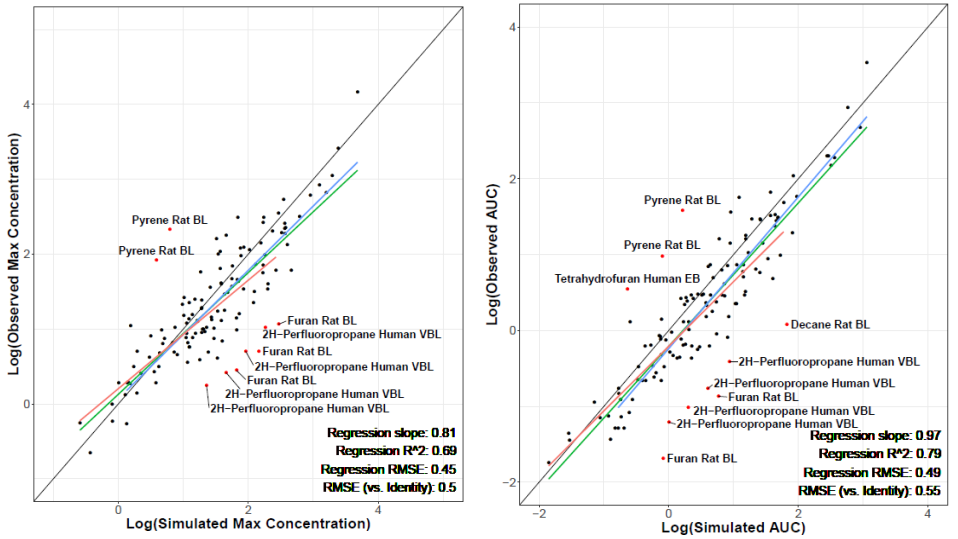
R package "httk"

- Open source, transparent, and peer-reviewed tools and data for **high throughput toxicokinetics (httk)**
- Allows *in vitro-in vivo* extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)
- Human-specific data for 987 chemicals
- Allows propagation of uncertainty

Improving Oral PK Models



Incorporating Generic Inhalation PBPK Model



Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al., Tox Sci.,* 2015
Wambaugh *et al.,Tox Sci.,* 2018
Wambaugh *et al.,Tox Sci.,* 2019
Linakis *et al.,* In Press.
G. Honda and J. Wambaugh, Unpublished

Case Studies to Build Confidence and Help Translate to Regulatory Application

Recently completed case studies

Ongoing and New Case Studies

- OPP/ORD case study to use NAMs on selected pesticides with established MOAs
- OPP/ORD case study to develop a NAM for evaluating developmental neurotoxicity
- OCSPP/ORD case study on integrating NAM to screen candidates for prioritization under TSCA
- OW/ORD case study on application of *in vitro* bioactivity and HTTK for screening-level assessments
- APCRA prospective case study on application of *in vitro* assays for hazard characterization
- APCRA case study on using NAMs to update chemical categories
- APCRA case study on computational approaches for rapid exposure estimates
- APCRA case study on modular integration of NAMs for identifying endocrine activity
- APCRA case study on using *in vitro* bioactivity to inform quantitative ecological hazard assessments
- APCRA case study on evaluating predictivity of HTTK methods

EPA United States Environmental Protection Agency

Take Home Messages…

- ORD is working on a diverse portfolio of research activities to meet the Agency's animal testing reduction goals
- Characterizing the variability and relevance of existing models will aid in establishing expectations for the performance of alternative methods
- Continued development and refinement of new technologies and analysis approaches will help comprehensively evaluate potential toxicological effects
- Systematically addressing technical limitations such as a lack of metabolism, testing challenging chemicals, and identifying organ/tissue effects will enable important information gaps to be filled
- Partnering with regulators and national and international partners on case studies will increase confidence in alternatives and accelerate application for a range of decision contexts

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