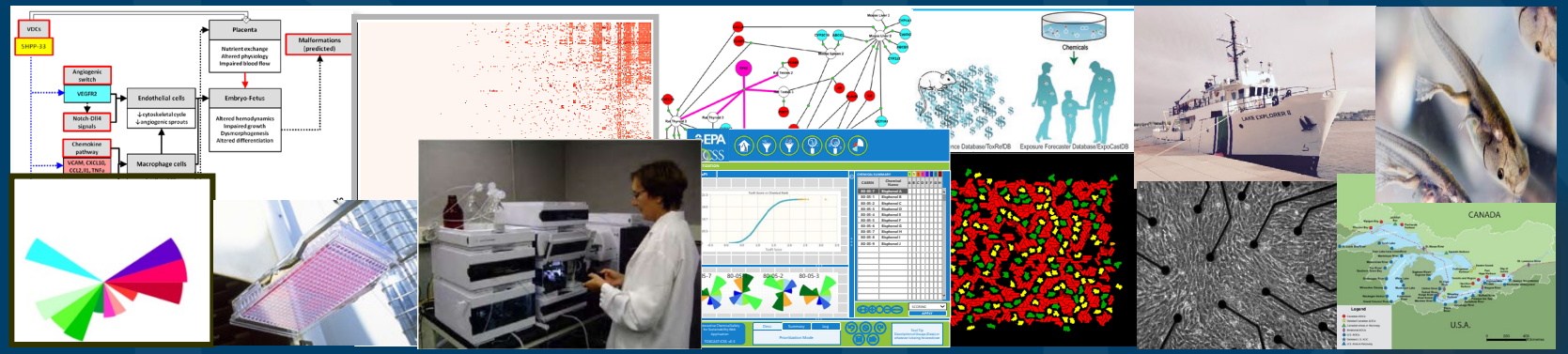
NAMs in TSCA Implementation

Perspectives from EPA ORD



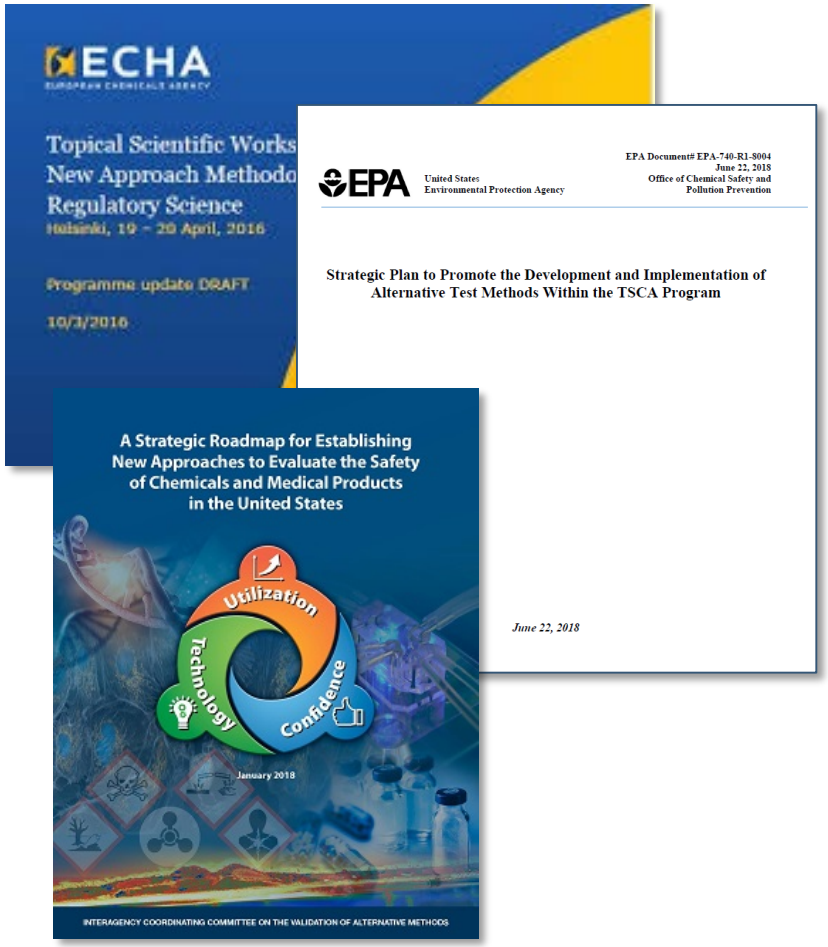
Global Chem Webinar

June 10, 2020

Rusty Thomas
Director
Center for Computational Toxicology and Exposure

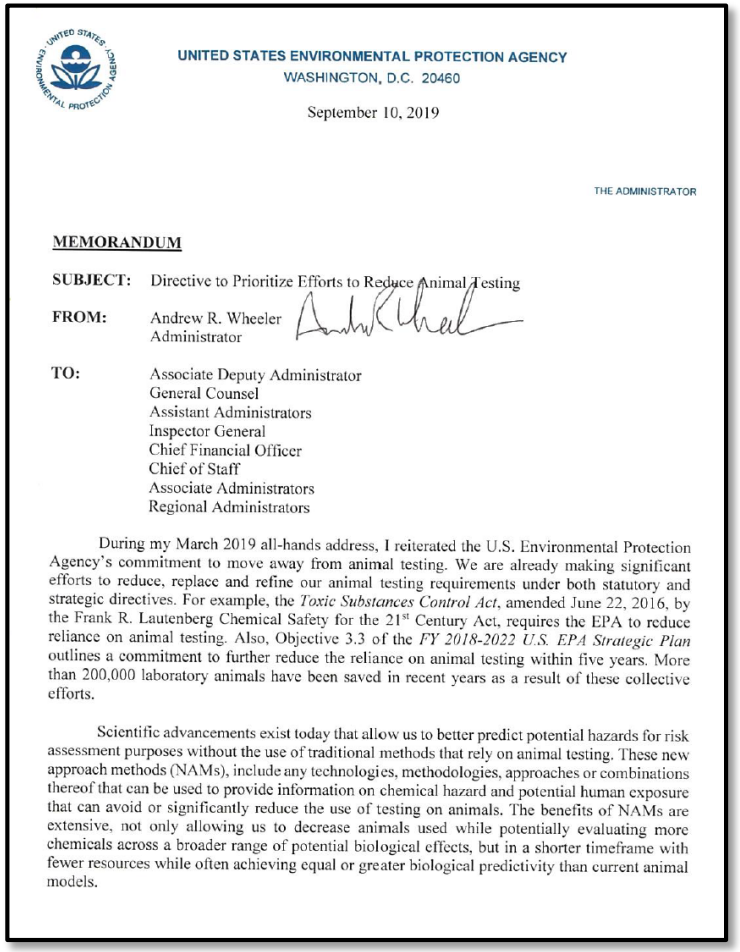
The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA

What is a New Approach Method?



- Coined in ~2014, but the definition of a New Approach Method (NAM) has evolved over time
- Currently, it is broadly descriptive reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment
- Functionally equivalent to “alternatives”, but can include exposure NAMs, eco NAMs, toxicokinetic NAMs, etc.

The Release of the EPA Memo Provided Clear 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

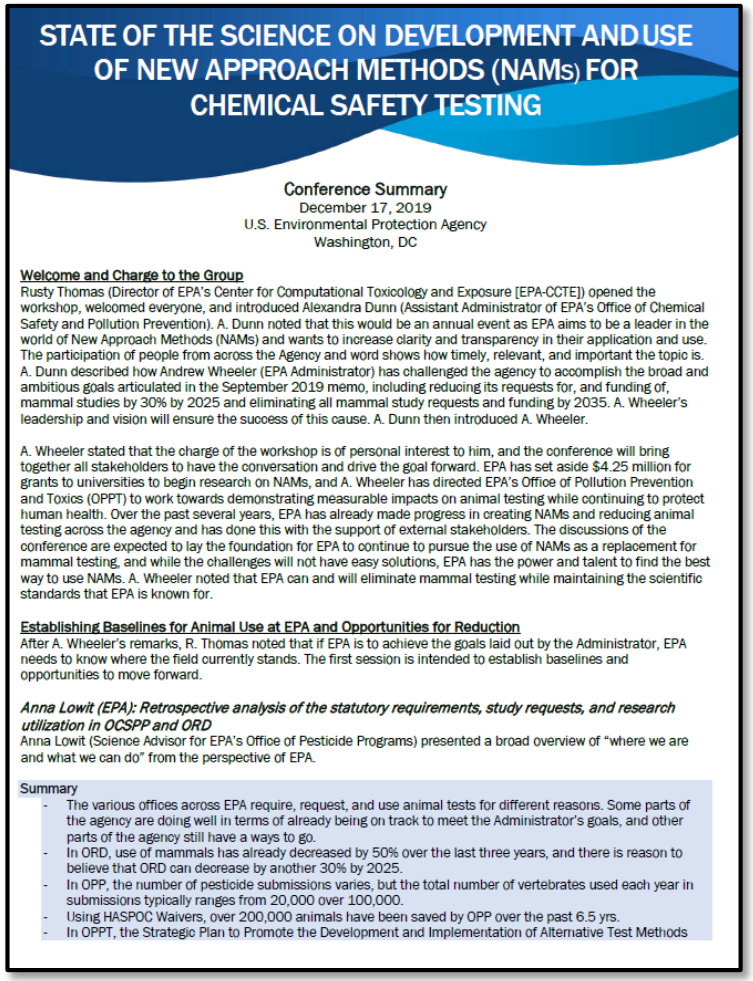
TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

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
- Other Requirements
  - Annual conference on the state of the science
  - Form a group of Agency experts to develop a work plan

First Annual NAM Conference Outlined the State of the Science in NAM Development and Application



STATE OF THE SCIENCE ON DEVELOPMENT AND USE OF NEW APPROACH METHODS (NAMs) FOR CHEMICAL SAFETY TESTING

Conference Summary
December 17, 2019
U.S. Environmental Protection Agency
Washington, DC

Welcome and Charge to the Group

Rusty Thomas (Director of EPA's Center for Computational Toxicology and Exposure [EPA-CCTE]) opened the workshop, welcomed everyone, and introduced Alexandra Dunn (Assistant Administrator of EPA's Office of Chemical Safety and Pollution Prevention). A. Dunn noted that this would be an annual event as EPA aims to be a leader in the world of New Approach Methods (NAMs) and wants to increase clarity and transparency in their application and use. The participation of people from across the Agency and word shows how timely, relevant, and important the topic is. A. Dunn described how Andrew Wheeler (EPA Administrator) has challenged the agency to accomplish the broad and ambitious goals articulated in the September 2019 memo, including reducing its requests for, and funding of, mammal studies by 30% by 2025 and eliminating all mammal study requests and funding by 2035. A. Wheeler's leadership and vision will ensure the success of this cause. A. Dunn then introduced A. Wheeler.

A. Wheeler stated that the charge of the workshop is of personal interest to him, and the conference will bring together all stakeholders to have the conversation and drive the goal forward. EPA has set aside $4.25 million for grants to universities to begin research on NAMs, and A. Wheeler has directed EPA's Office of Pollution Prevention and Toxics (OPPT) to work towards demonstrating measurable impacts on animal testing while continuing to protect human health. Over the past several years, EPA has already made progress in creating NAMs and reducing animal testing across the agency and has done this with the support of external stakeholders. The discussions of the conference are expected to lay the foundation for EPA to continue to pursue the use of NAMs as a replacement for mammal testing, and while the challenges will not have easy solutions, EPA has the power and talent to find the best way to use NAMs. A. Wheeler noted that EPA can and will eliminate mammal testing while maintaining the scientific standards that EPA is known for.

Establishing Baselines for Animal Use at EPA and Opportunities for Reduction

After A. Wheeler's remarks, R. Thomas noted that if EPA is to achieve the goals laid out by the Administrator, EPA needs to know where the field currently stands. The first session is intended to establish baselines and opportunities to move forward.

Anna Lowit (EPA): Retrospective analysis of the statutory requirements, study requests, and research utilization in OCSPP and ORD

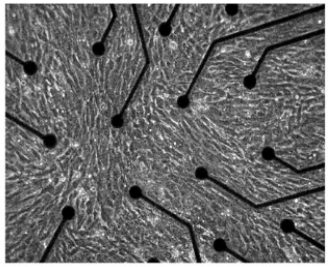
Anna Lowit (Science Advisor for EPA's Office of Pesticide Programs) presented a broad overview of "where we are and what we can do" from the perspective of EPA.

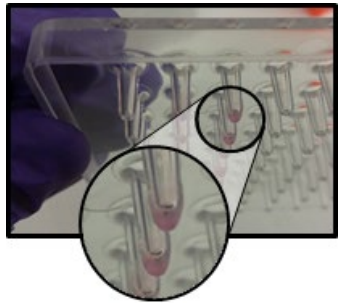
Summary

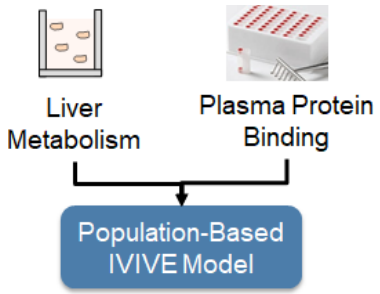
- The various offices across EPA require, request, and use animal tests for different reasons. Some parts of the agency are doing well in terms of already being on track to meet the Administrator's goals, and other parts of the agency still have a ways to go.
- In ORD, use of mammals has already decreased by 50% over the last three years, and there is reason to believe that ORD can decrease by another 30% by 2025.
- In OPP, the number of pesticide submissions varies, but the total number of vertebrates used each year in submissions typically ranges from 20,000 over 100,000.
- Using HASPOC Waivers, over 200,000 animals have been saved by OPP over the past 6.5 yrs.
- In OPPT, the Strategic Plan to Promote the Development and Implementation of Alternative Test Methods

- Location: Washington, DC
- Date: December 17, 2019
- Agenda Topics
  - Establishing Baselines for Animal Use at EPA and Opportunities for Reduction Objectives
  - Variability and Relevance of Current Animal Tests and Expectations for NAMs
  - State of the Science in Development and Application of NAMs
  - Developing Scientific Confidence in NAMs
  - Breakout Groups
- Attendees: 413 on-line, 94 in-person
- Summary report available: https://www.epa.gov/chemical-research/first-annual-conference-state-science-development-and-use-new-approach-methods-0

ORD Focus Areas to Fulfill Agency Goals

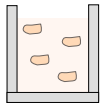






- Characterize performance of new and existing model systems
- Incorporate new technological and data analysis advances to developing NAMs
- Systematically address known limitations of NAM test systems
- Build confidence through case studies

Characterizing the Performance of NAMs for Toxicokinetics



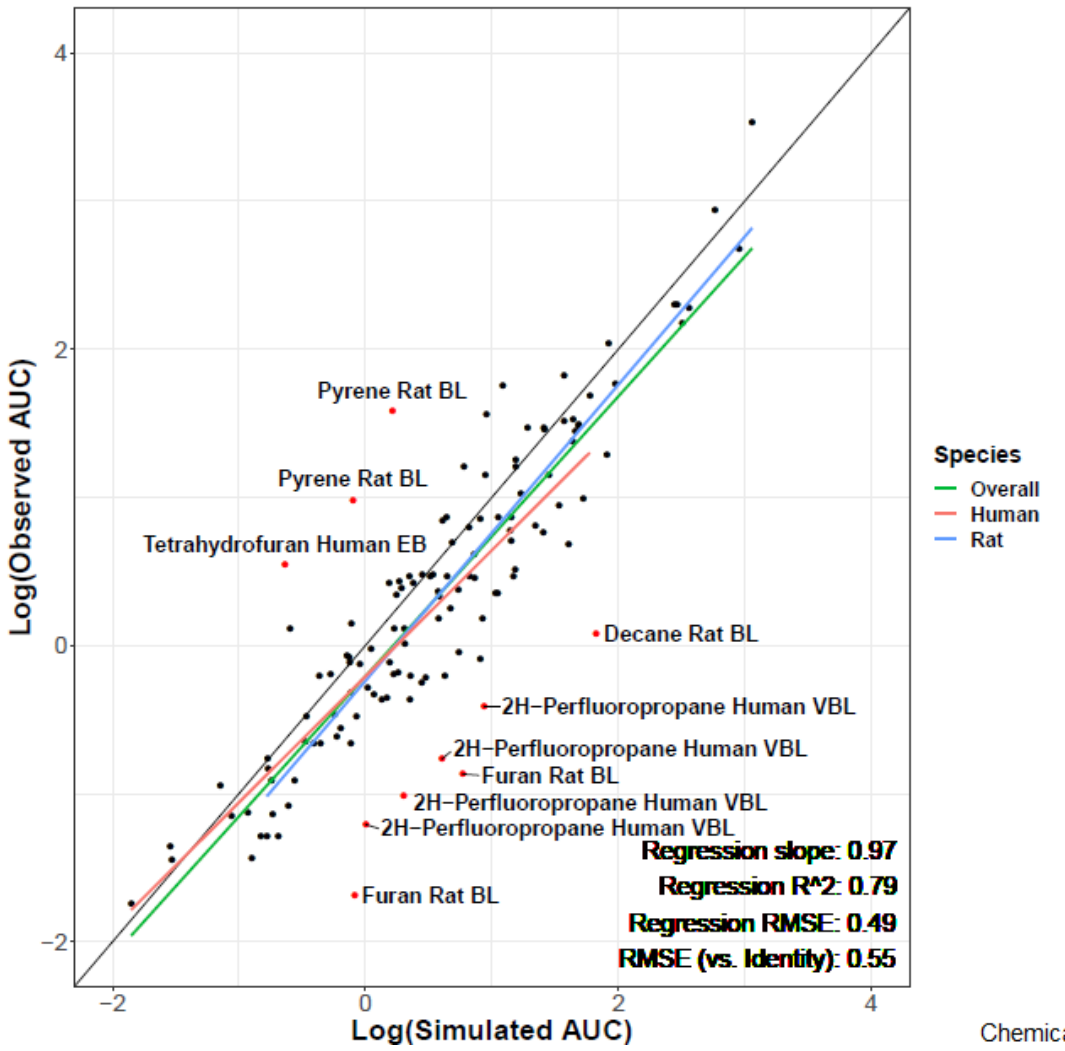
Liver Metabolism | Tissue Partitioning | Plasma Protein Binding

Population-Based IVIVE Model

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

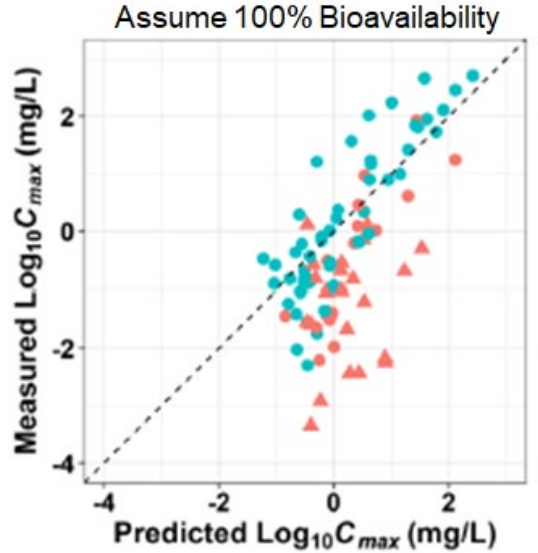
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al., Tox Sci.,* 2015
Wambaugh *et al.,Tox Sci.,* 2018
Wambaugh *et al.,Tox Sci.,* 2019
Linakis *et al.,* In Press.
G. Honda and J. Wambaugh, Unpublished

Generic Inhalation PBPK Model

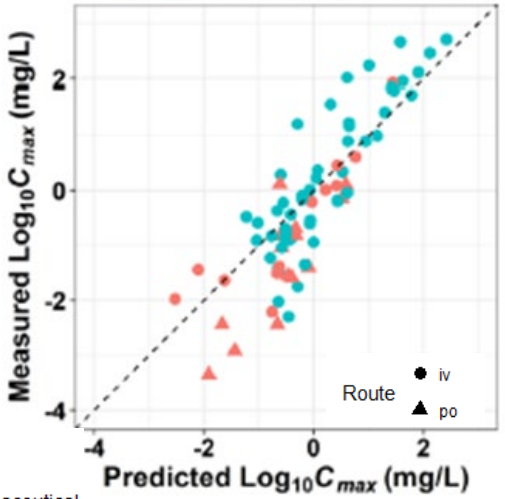


Oral PK Models

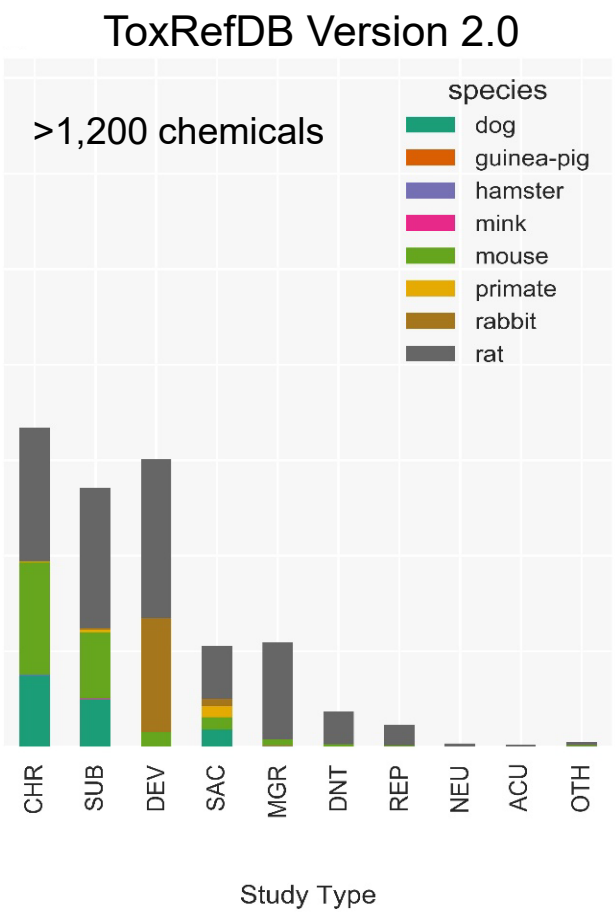
Assume 100% Bioavailability



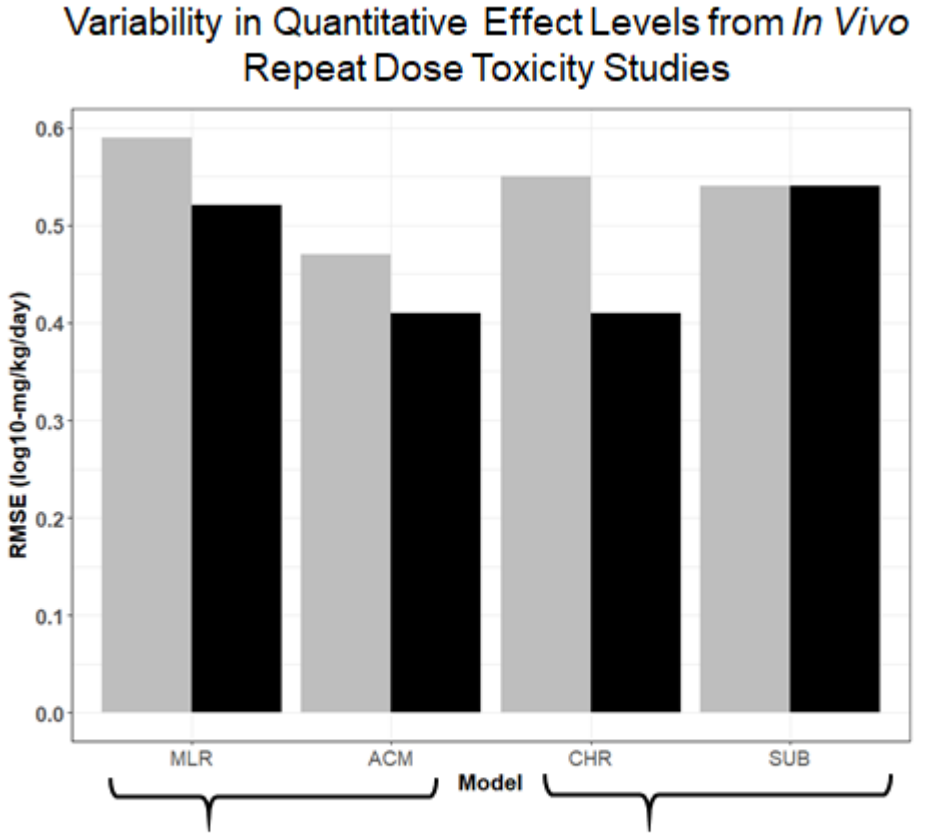
Using CaCo2 Bioavailability

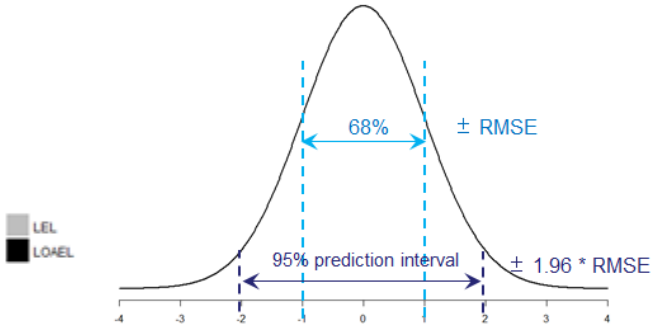


Characterizing the Quantitative Reproducibility of Traditional Toxicity Studies



Watford *et al., Repro Toxicol*, 2019

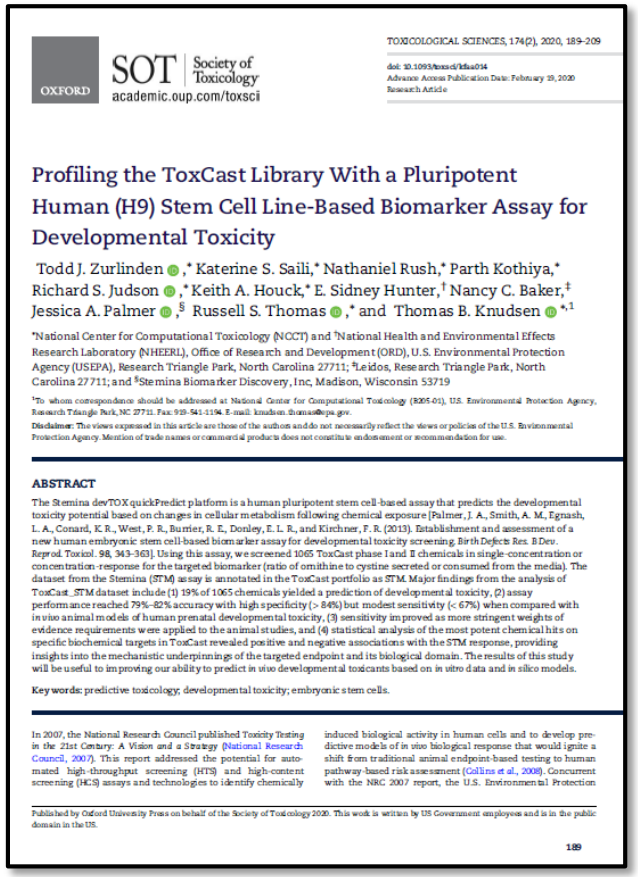




Using an RMSE=0.59, the minimum 95% PI of an LEL/LOAEL is:
1 mg/kg/day ➔ 0.07 – 14 mg/kg/day.
10 mg/kg/day ➔ 0.7 – 143 mg/kg/day.

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

Integrating *In Vitro* Assays and Computational Modeling to Predict Developmental Toxicity



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

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

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

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.

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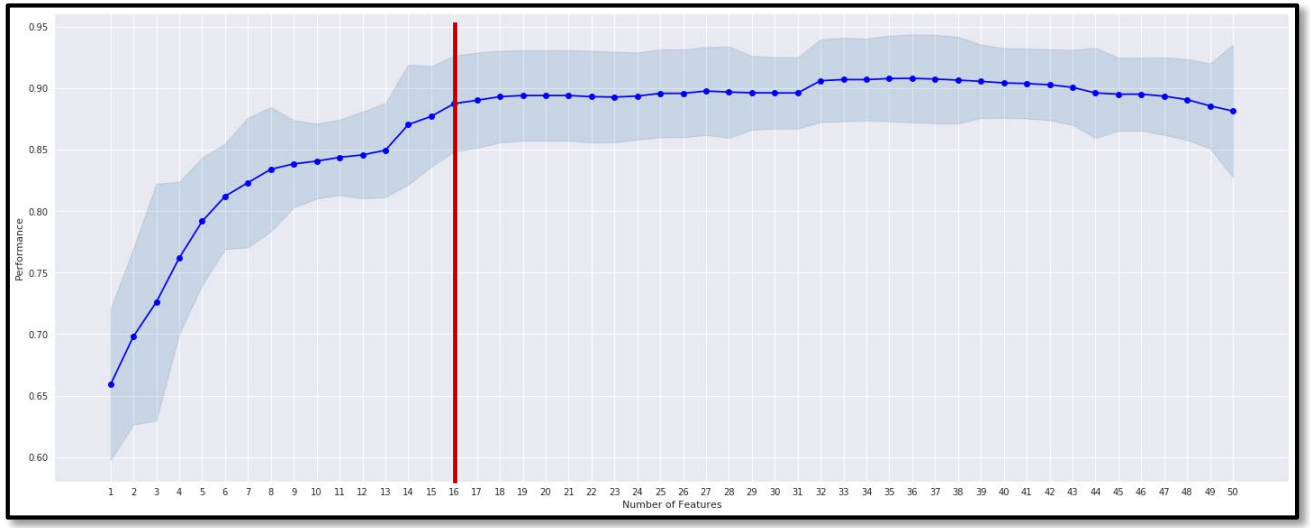
Zurlinden *et al., Toxicol Sci.*, 2020
T. Zurlinden, T. Knudsen, Unpublished

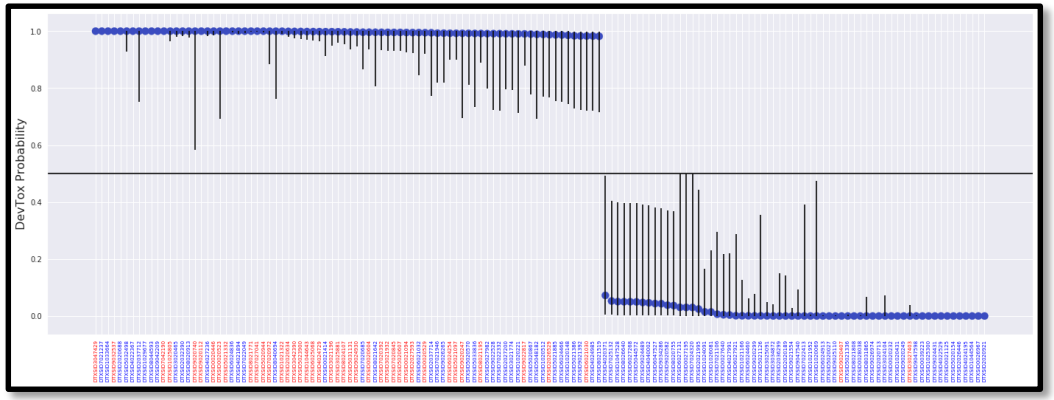
Integrated Computational Model Uses Stemina + ToxCast assays

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)

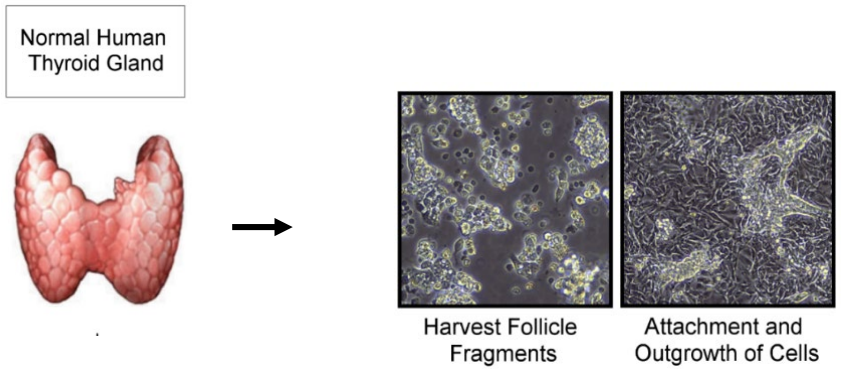


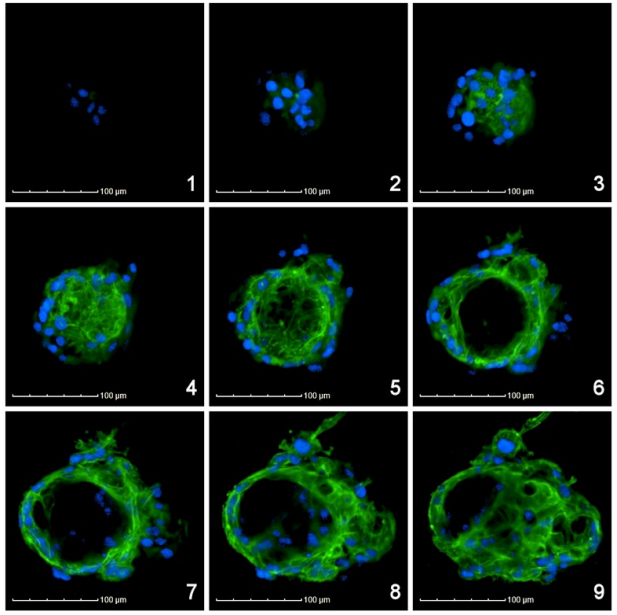
- Bayesian logistic regression to determine probabilistic model for DevTox
- Capability to tune model for increased sensitivity OR specificity



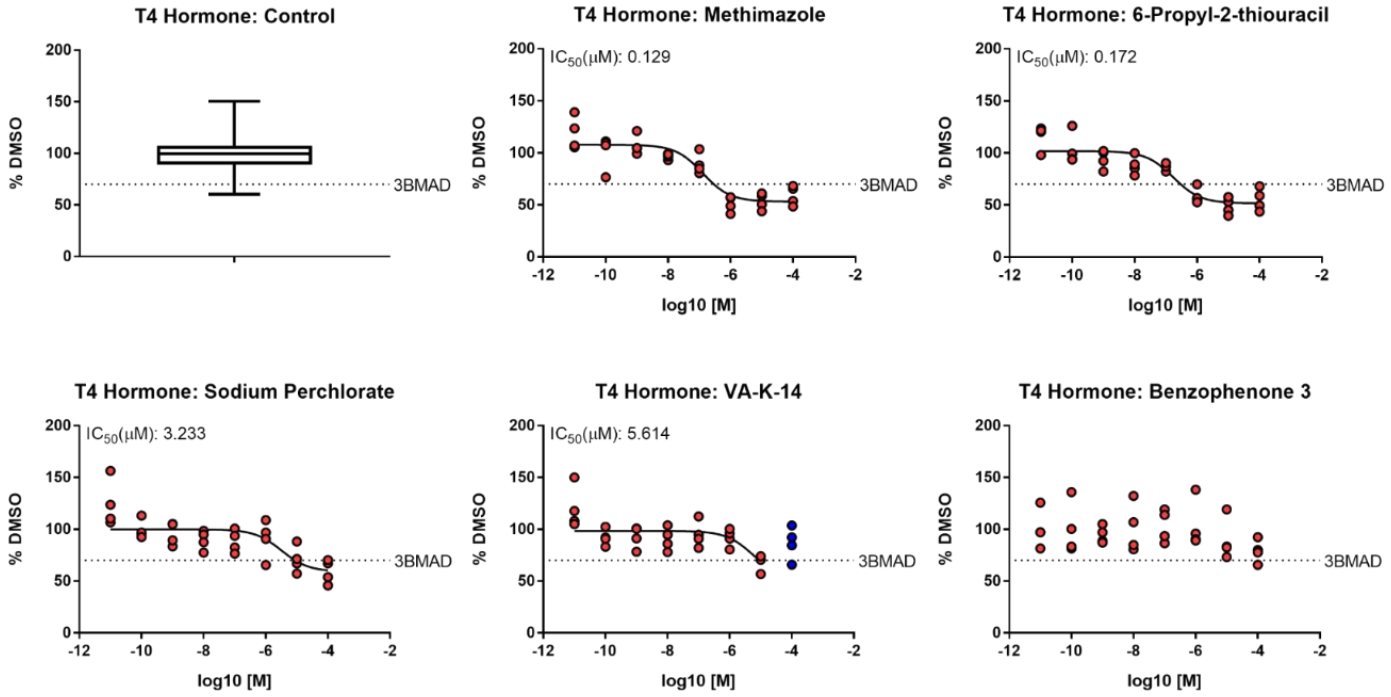
- Application of the "high specificity" model to ~580 chemicals on TSCA non-confidential inventory
- 144 chemicals predicted with confidence to fall into DevTox positive or negative domains

Develop 3D Culture Models to More Accurately Model Tissue/Organ Effects





Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin



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

Case Studies Being Used to Build Confidence and Help Translate NAMs for Regulatory Application

TOXICOLOGICAL SCIENCES, 2019, 1–24

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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

OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2019)28

Unclassified — English - Or. English
29 August 2019

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR ESTROGEN RECEPTOR ACTIVE CHEMICALS

Series on Testing and Assessment
No. 300

The corresponding annexes are available under the following cotes: ENV/JM/MONO(2019)28/ANN1

JT03450456

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 while ensuring protection of public health and the environment
- Characterizing the performance of both new and existing model systems will enable data-driven assessment of "information of equivalent or better scientific quality and relevance"
- Continued development and refinement of new technologies while systematically addressing technical limitations will help fill important information gaps, improve predictive performance, and broaden applicability of NAMs
- 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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- FDA
- NCATS

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- CPHEA
- CESER

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- A*STAR
- ECHA
- EFSA
- Health Canada

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