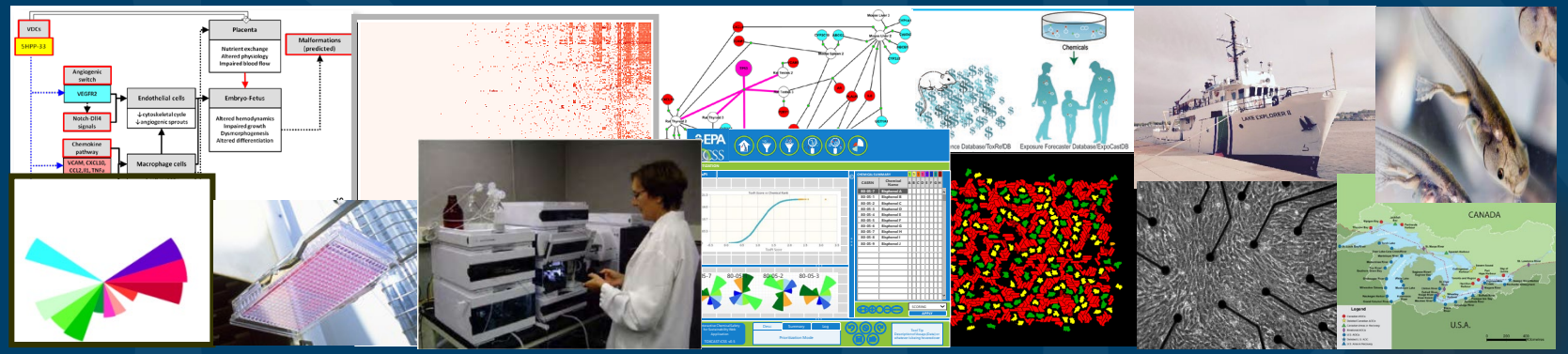
Growing Up and Leaving Home

Emerging from the Teenage Years in 21st Century Computational Toxicology

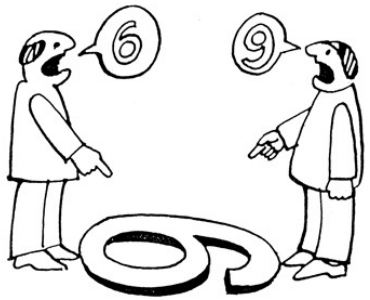


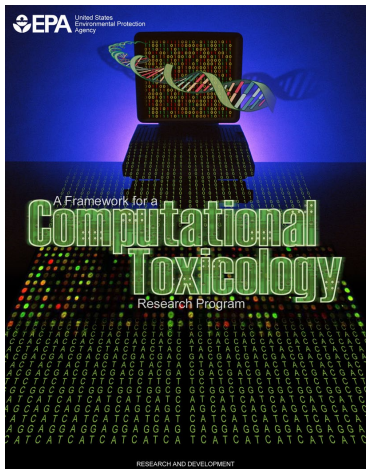
NCSOT Webinar

Sept 17, 2020

Rusty Thomas
Director
Center for Computational Toxicology and Exposure

So… When Was Computational Toxicology Actually Born?



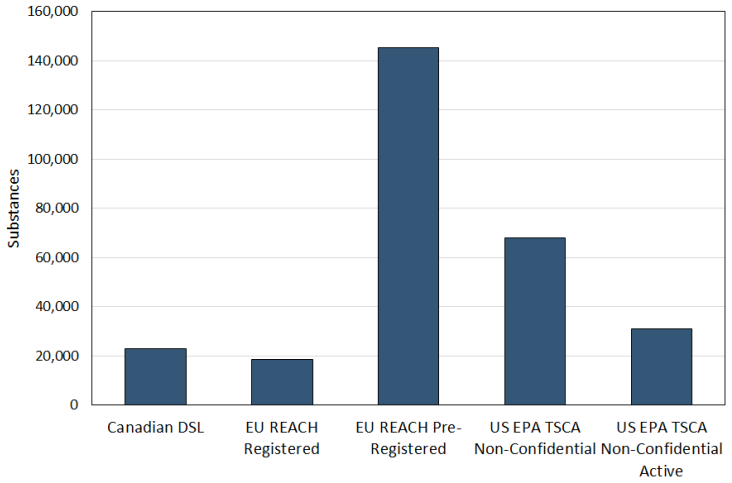


- Short answer… It depends.
- Application of computational modeling to toxicological endpoints began with development of QSARs in 1970s.
- In early 1970s, first physiologically-based pharmacokinetic (PBPK) models were developed
- In 1980s, significant growth in computer modeling in QSAR and PBPK modeling
- In 1990s, development of physiologically-based pharmacodynamic (PBPD) models for AChE inhibition and cell death/proliferation/mutation
- In the late 1990s, use of the term ‘computational toxicology’ appeared in the literature
- Strategic plan for Computational Toxicology research at EPA released in 2003
- National Academy of Sciences report on transforming toxicity testing released in 2007

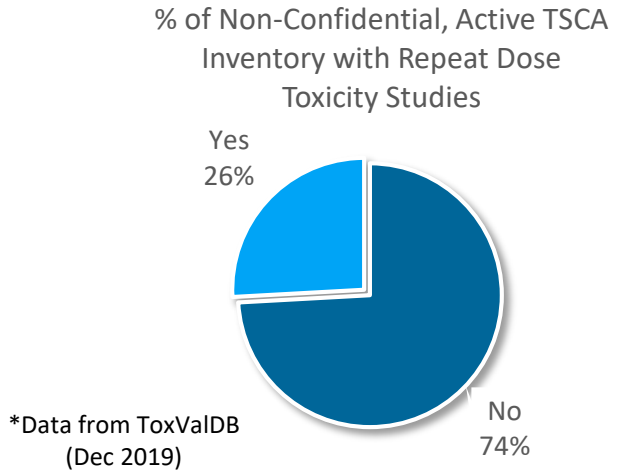
Multiple Factors Contributed to the Formation and Development of Computational Toxicology

United States Environmental Protection Agency

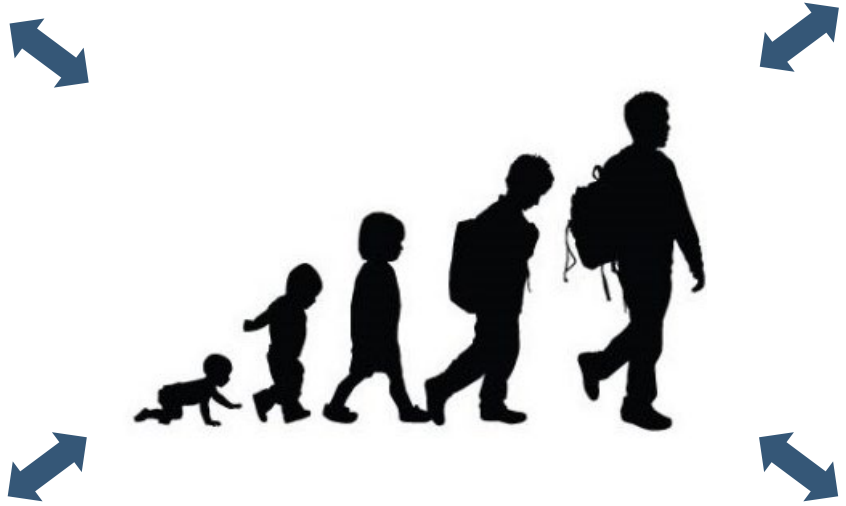
Number of Substances



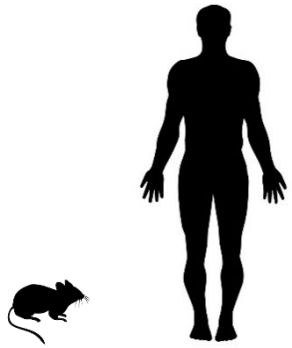
Amount of Data



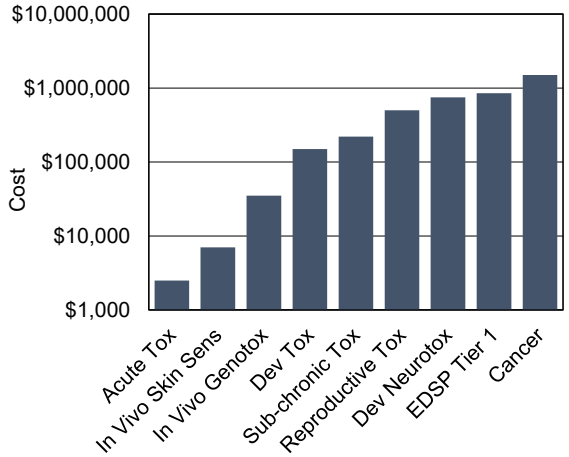
*Data from ToxValDB (Dec 2019)



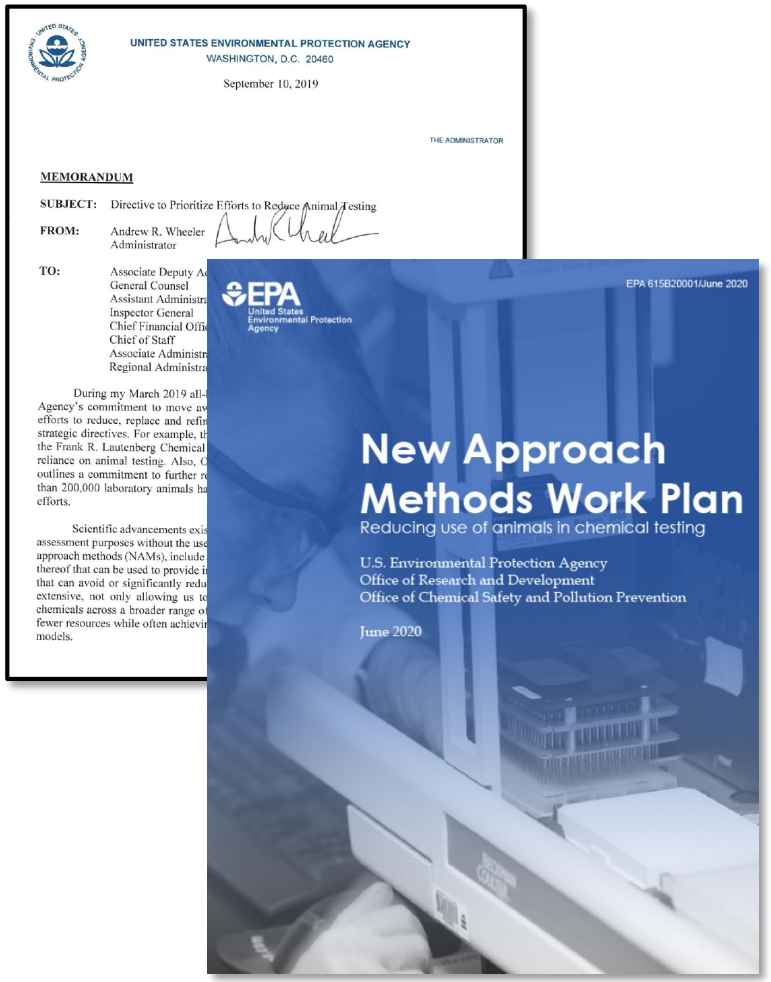
Reliability/Relevance



Economics



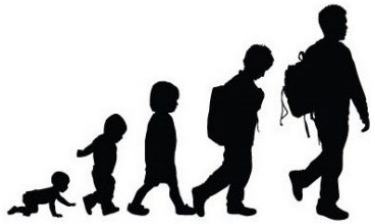
EPA Memo and Work Plan Continues to Spur Development and Shift Towards Computational Toxicology

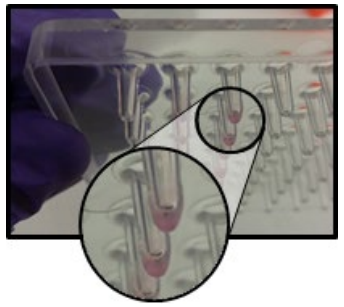


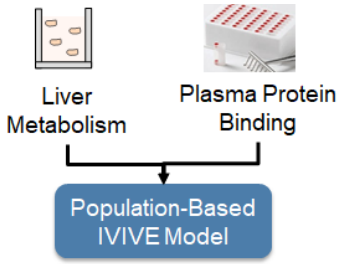
www.epa.gov/nam

- 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).
- Work Plan Objectives and Strategies:
  - Evaluate Regulatory Flexibility for Accommodating NAMs
  - Develop Baselines and Metrics for Assessing Progress
  - Establish Scientific Confidence in NAMs and Demonstrate Application to Regulatory Decisions
  - Develop NAMs to Address Scientific Challenges and Fill Important Information Gaps
  - Engage and Communicate with Stakeholders

A Few Topics for Highlighting the Emergence of Computational Toxicology from the Teenage Years…







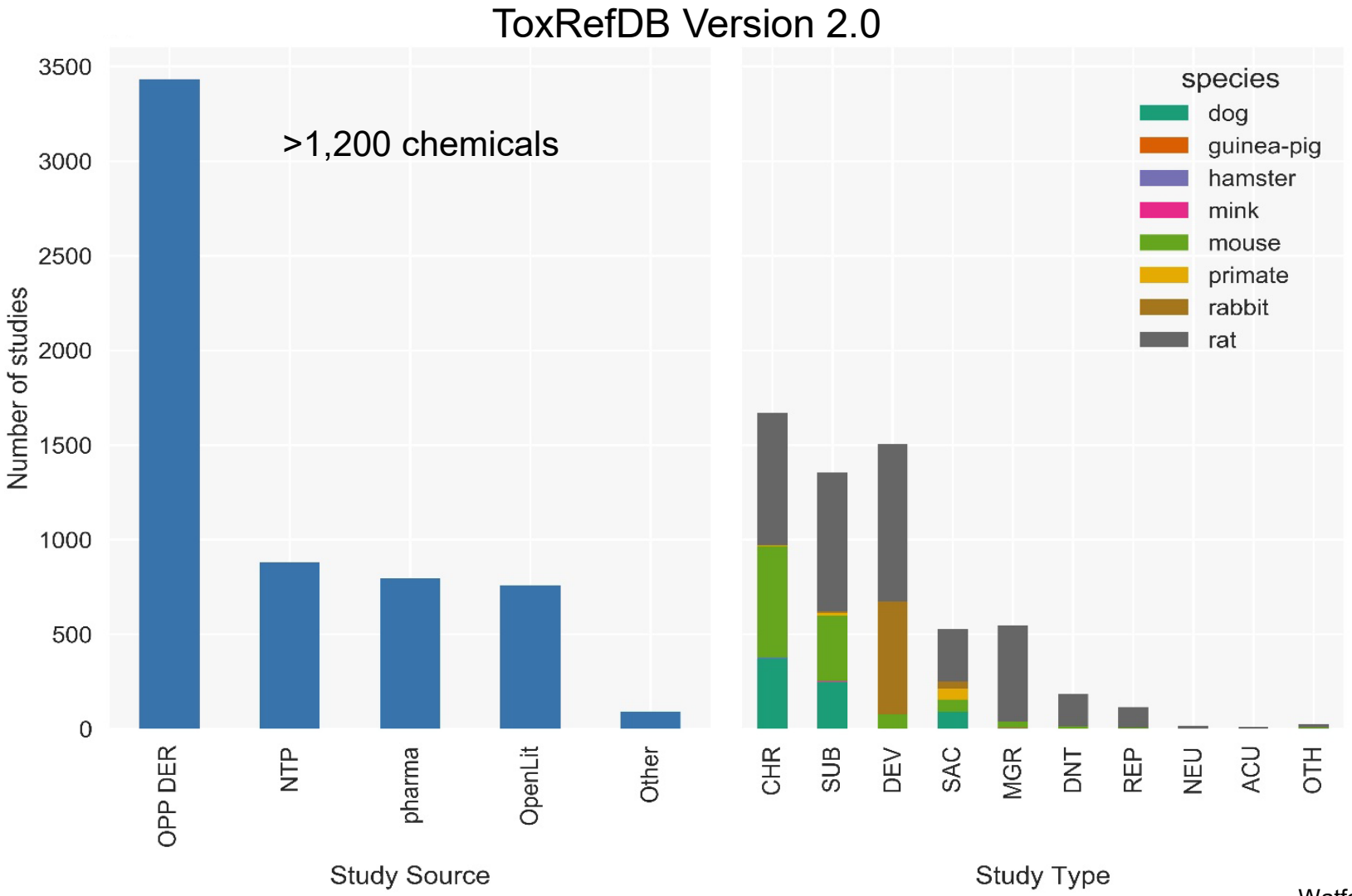
- Establishing expectations on the variability of current toxicity studies
- Technological advances to evaluate large numbers of chemicals across toxicological space
- Addressing limitations of *in vitro* test systems
- Put results in a dose context
- Building confidence through regulatory focused case studies

United States Environmental Protection Agency

Why 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
- EPA NAM Work Plan includes an objective to characterize the scientific quality and relevance of existing animal tests

Evaluating Reproducibility of Traditional Toxicity Studies By Mining Legacy Data

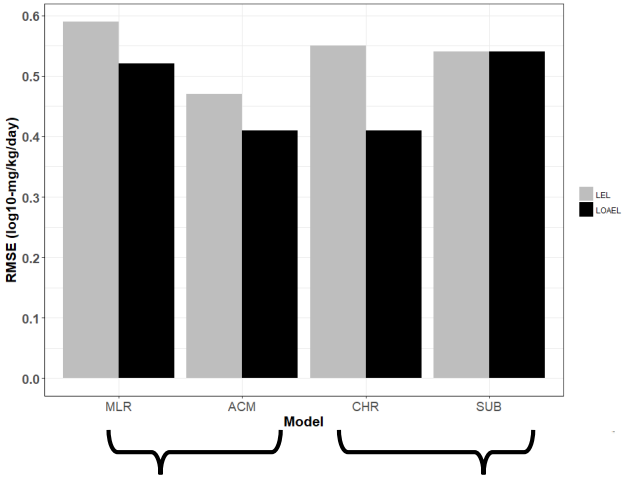


Watford et al., Reprod Toxicol. 2019

Qualitative Reproducibility of Traditional Toxicity Studies

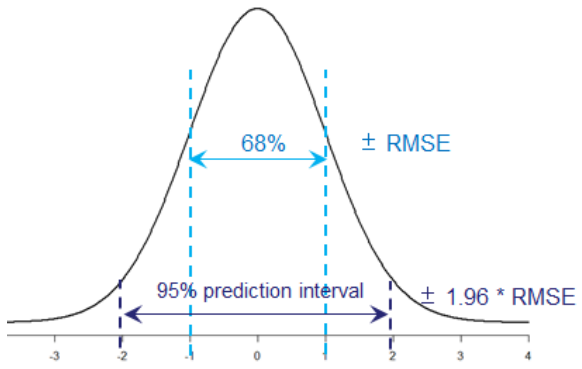
United States Environmental Protection Agency

Reproducibility in Quantitative Effect Levels from *In Vivo* Repeat Dose Toxicity Studies



Two ways to statistically model the data across multiple study types

Variability within a specific study type



Using an RMSE=0.59, the 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.

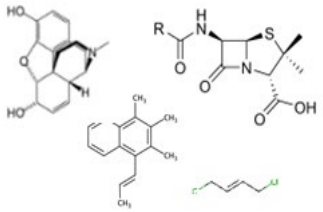
Pham et al., Comp Toxicol., 2020

Reproducibility in Qualitative Target Organ Effects in Repeat Dose Toxicity Studies

Organ	Species	Repeated negative	Mixed effects	Repeated positive	% Concordance
Liver	dog	20	26	46	71.7
	mouse	30	40	69	71.2
	rat	42	71	132	71.0
Kidney	dog	49	33	10	64.1
	mouse	61	51	27	63.3
	rat	60	105	80	57.1
Spleen	dog	64	21	7	77.2
	mouse	93	31	15	77.7
	rat	132	84	29	65.7
Testes	dog	65	20	7	78.3
	mouse	110	20	9	85.6
	rat	135	87	23	64.5
Adrenal gland	dog	76	12	4	87.0
	mouse	109	23	7	83.5
	rat	142	83	20	66.1

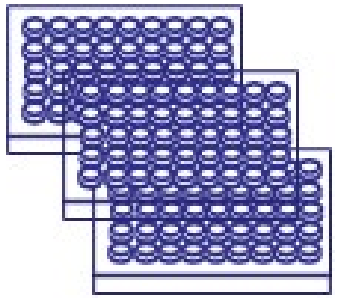
LyLy Pham and Katie Paul-Friedman, Unpublished

Application of High-Throughput Assays to Test Thousands of Chemicals



Thousands of Chemicals

Concentration Response Screening

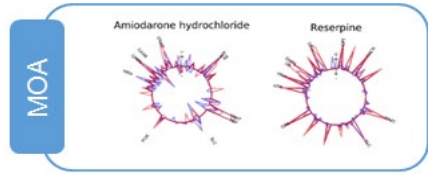


ToxCast Assays

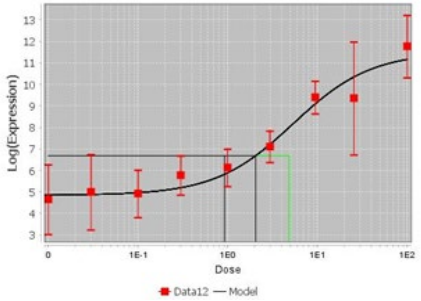
Transcription Factors
Transporter
Cytokines
Kinases
Nuclear Receptors
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion channels

~700 Assay Endpoints

Mode-of-Action Identification



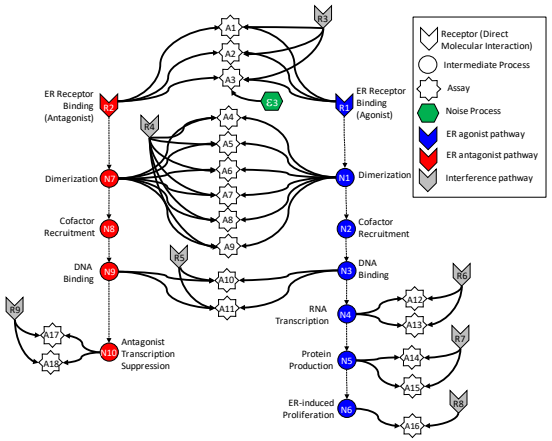
Concentration Response Modeling



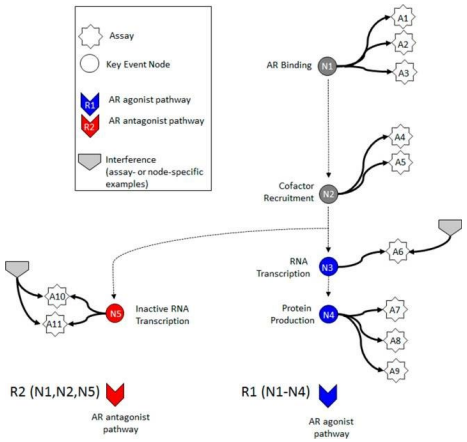
- 96, 384, and 1536-well, laboratory automation compatible
- Relatively expensive (~$20,000 - $30,000 / chemical)
- Coverage of molecular and phenotypic responses
- Multiple assay vendors/labs

Application of High-Throughput Assays to Identify Potential Endocrine Disrupting Chemicals

ToxCast *In Vitro* Assays Measure ER- and AR-Related Activity



Judson *et al., Tox Sci.* 2015
Browne *et al., ES&T.* 2015
Kleinstreuer *et al., EHP* 2016



Kleinstruer *et al., Chem Res Toxicol.* 2017

ER Reference Agonists

Accuracy	**0.93**
Sensitivity	**0.93**
Specificity	**0.92**

AR Reference Antagonists

Accuracy	**0.98**
Sensitivity	**1.00**
Specificity	**0.95**

Application to Regulatory Decisions

35350 **Federal Register** / Vol. 80, No. 118 / Friday, June 19, 2015 / Notices

may claim all or part of a response confidential. EPA will disclose information that is covered by a claim of confidentiality only to the extent permitted by, and in accordance with, the procedures in TSCA section 14 and 40 CFR part 2.

Burden statement: The annual public reporting and recordkeeping burden for this collection of information is estimated to average 31.5 hours per response. Burden is defined in 5 CFR 1320.3(b).

The ICR, which is available in the docket along with other related materials, provides a detailed explanation of the collection activities and the burden estimate that is only briefly summarized here:

Respondents/Affected Entities: Entities potentially affected by this ICR are companies that manufacture, process or import chemical substances, mixtures or categories.

Estimated total number of potential respondents: 1.

Frequency of response: On occasion.

Estimated total average number of responses for each respondent: 1.

Estimated total annual burden hours: 31.5 hours.

Estimated total annual costs: $2,388. This includes an estimated burden cost of $2,388 and an estimated cost of $0 for capital investment or maintenance and operational costs.

III. Are There Changes in the Estimates from the Last Approval?

There is a decrease of 916 hours in the total estimated respondent burden compared with that identified in the ICR currently approved by OMB. This decrease reflects additional both adjustment changes from a reduction in the assumed number of PAIR reports filed annually, and program changes resulting from mandatory electronic submissions of PAIR reports. In recent years (FY 2011–FY 2014), EPA has received no PAIR submissions and, for the purposes of this analysis, EPA assumes an annual rate of one submission per year. At the time OMB last renewed this ICR, EPA estimated an average of 33 reports from 14.8 submitters based on fiscal year 2006–2010 data. The ICR supporting statement provides a detailed analysis of the change in burden estimate. This change is both an adjustment and a program change.

IV. What is the Next Step in the Process for this ICR?

EPA will consider the comments received and amend the ICR as appropriate. The final ICR package will then be submitted to OMB for review and approval pursuant to 5 CFR 1320.12. EPA will issue another **Federal Register** document pursuant to 5 CFR 1320.5(a)(1)(iv) to announce the submission of the ICR to OMB and the opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under **FOR FURTHER INFORMATION CONTACT.**

Authority: 44 U.S.C. 3501 *et seq.*

Dated: June 10, 2015.

James Jones,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

[FR Doc. 2015–14946 Filed 6–18–15; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–OPPT–2015–0305; FRL–9928–69]

Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1400 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.

DATES: Comments must be received on or before August 18, 2015.

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA–HQ–OPPT–2015–0305, by one of the following methods:

- *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.
- *Mail:* Document Control Office (7407M), Office of Pollution Prevention and Toxics (OPPT), Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001.
- *Hand Delivery:* To make special arrangements for hand delivery or delivery of boxed information, please follow the instructions at *http://www.epa.gov/dockets/contacts.html.*

Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at *http://www.epa.gov/dockets.*

FOR FURTHER INFORMATION CONTACT: *For technical information contact:* Jane Robbins, Office of Science Coordination and Policy (OSCP), Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001; telephone number: (202) 564–6625; email address: robbins.jane@*epa.gov.*

For general information contact: The TSCA-Hotline, ABVI-Goodwill, 422 South Clinton Ave., Rochester, NY 14620; telephone number: (202) 554–1404; email address: *TSCA-Hotline@epa.gov.*

SUPPLEMENTARY INFORMATION:

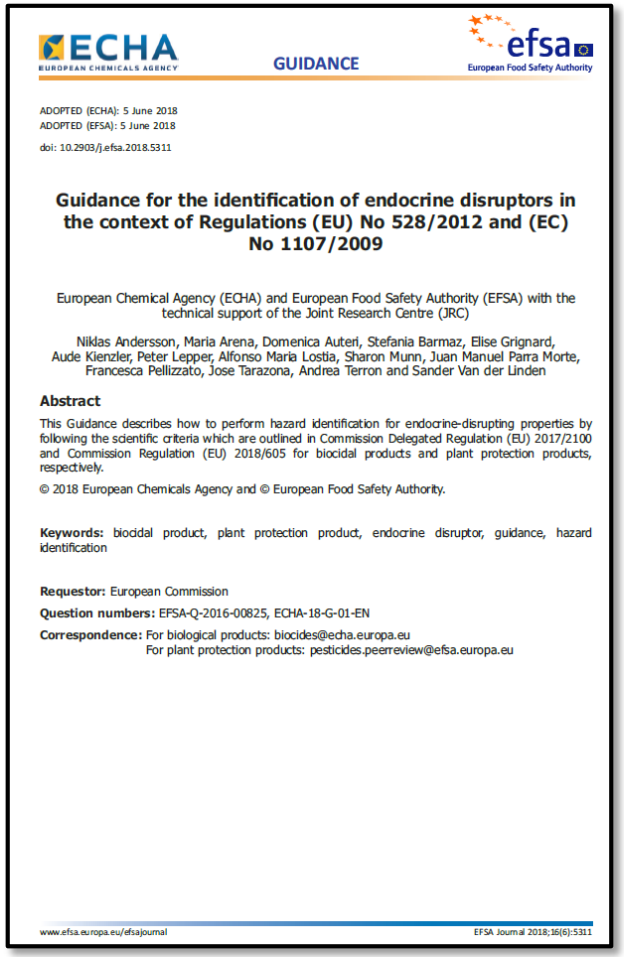
I. General Information

A. Does this action apply to me?

This action is directed to the public in general, and may be of interest to a wide range of stakeholders including those interested in endocrine testing of chemicals (including pesticides), and the EDSP in general. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What is the agency authority for taking this action?

The EDSP is established under section 408(p) of the Federal Food, Drug and



ECHA EUROPEAN CHEMICALS AGENCY — GUIDANCE — efsa European Food Safety Authority

ADOPTED (ECHA): 5 June 2018
ADOPTED (EFSA): 5 June 2018

doi: 10.2903/j.efsa.2018.5311

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC)

Niklas Andersson, Maria Arena, Domenica Auteri, Stefania Barmaz, Elise Grignard, Aude Kienzler, Peter Lepper, Alfonso Maria Lostia, Sharon Munn, Juan Manuel Parra Morte, Francesca Pellizzato, Jose Tarazona, Andrea Terron and Sander Van der Linden

Abstract

This Guidance describes how to perform hazard identification for endocrine-disrupting properties by following the scientific criteria which are outlined in Commission Delegated Regulation (EU) 2017/2100 and Commission Regulation (EU) 2018/605 for biocidal products and plant protection products, respectively.

Keywords: biocidal product, plant protection product, endocrine disruptor, guidance, hazard identification

Requestor: European Commission

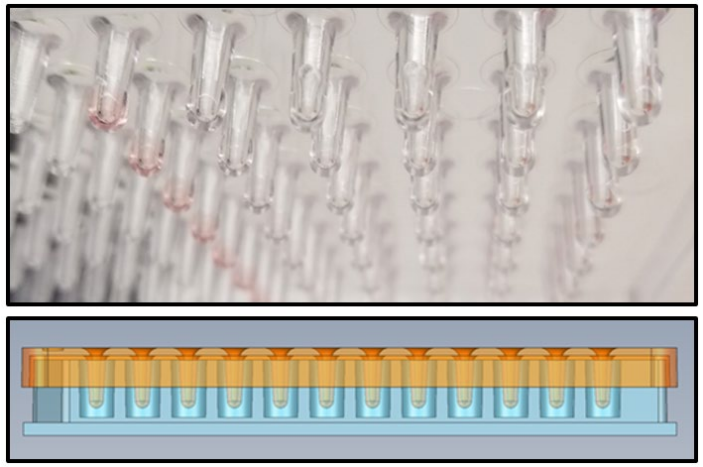
Question numbers: EFSA-Q-2016-00825, ECHA-18-G-01-EN

Correspondence: For biological products: biocides@echa.europa.eu
For plant protection products: pesticides.peerreview@efsa.europa.eu

Beginning to Incorporate Xenobiotic Metabolism Into *In Vitro* Assays

EPA United States Environmental Protection Agency

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

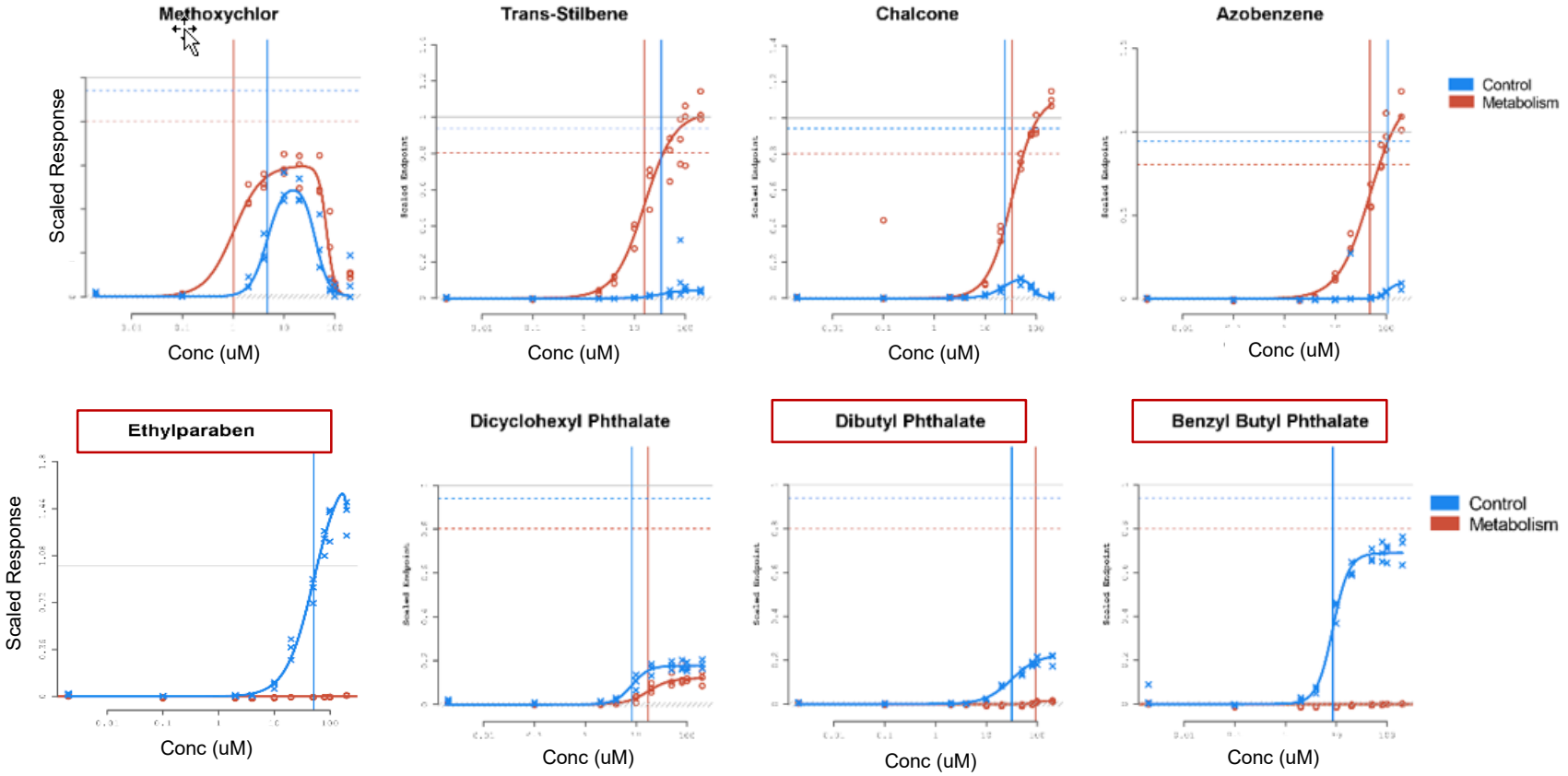


Screening Window of VM7 (formerly BG1) ER Transactivation Assay

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

Application to ER Transactivation Assay (ERTA)

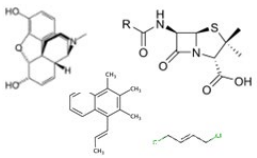
Screening Results of Pinto et al., 2016 Library



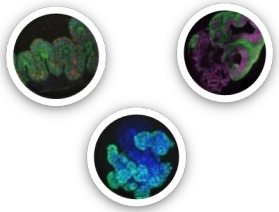
Collaboration with Unilever

C. Deisenroth et al., Toxicol. Sci., In Press

Incorporating High-Content Technologies to Increase Biological Coverage

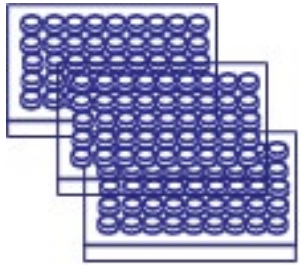


Thousands of Chemicals

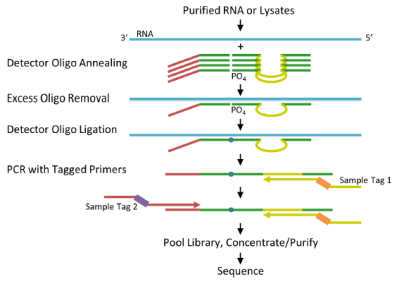


Multiple Cell Types

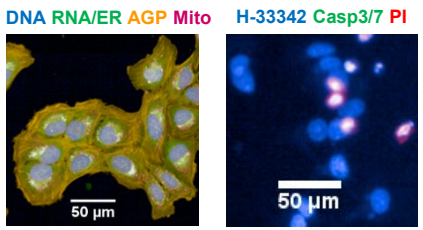
Concentration Response Screening



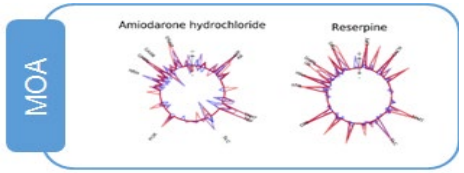
Whole Genome Transcriptomics



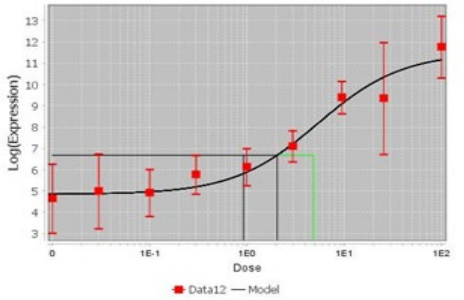
Multi-Parameter Cellular Phenotypic Profiling



Mode-of-Action Identification

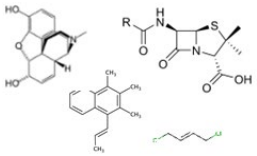


Concentration Response Modeling

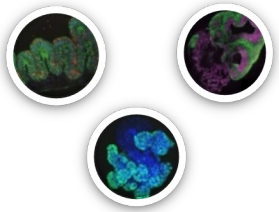


- 384-well, laboratory automation compatible
- Relatively inexpensive ($2.50 - $1,500 per chemical)
- Broad complementary coverage of molecular and phenotypic responses
- Integration of reference materials and controls for performance standards
- Increased portability

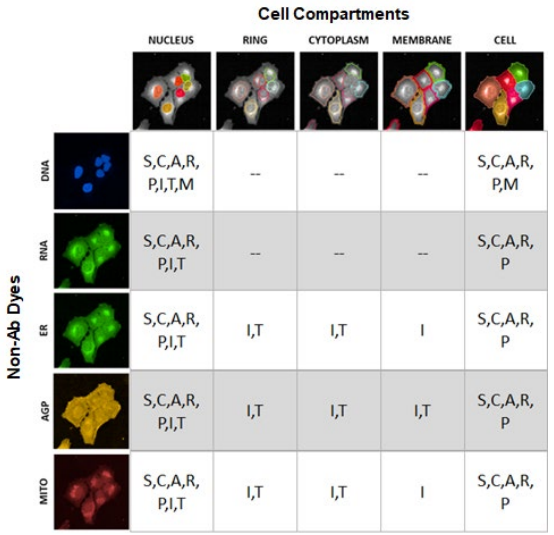
High-Throughput Phenotypic Profiling as a Measure of 'Cellular Pathology'

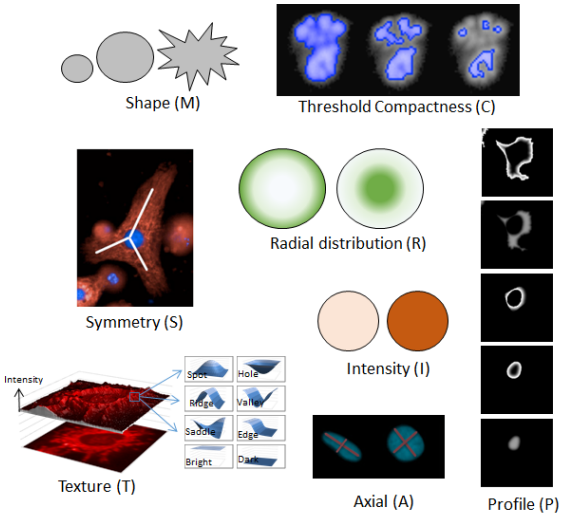


Thousands of Chemicals



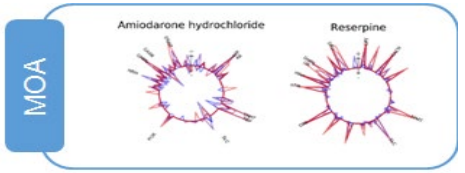
Multiple Cell Types



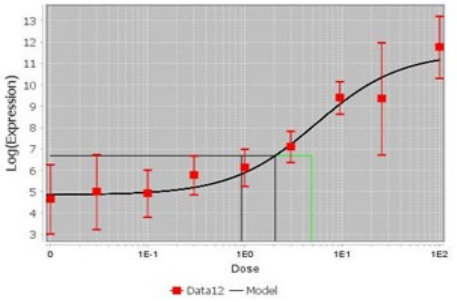


Images from PerkinElmer

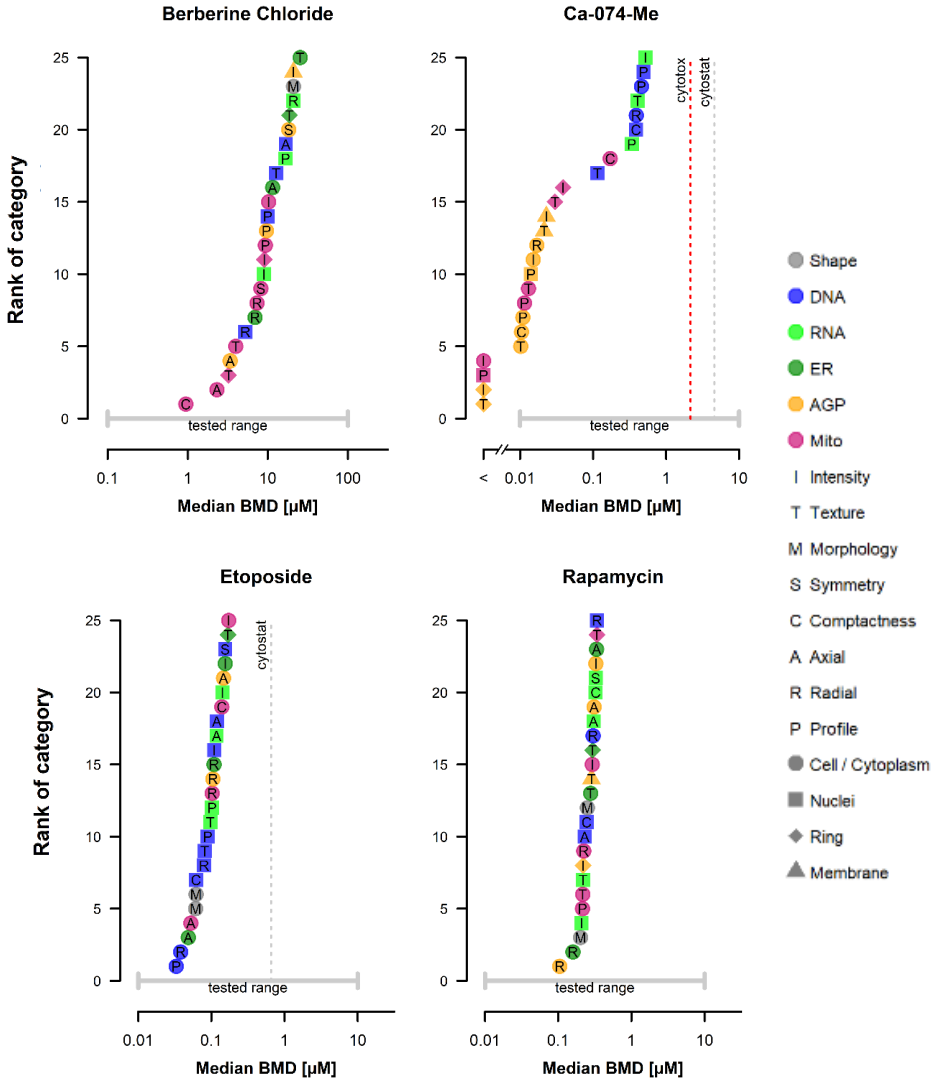
Mode-of-Action Identification

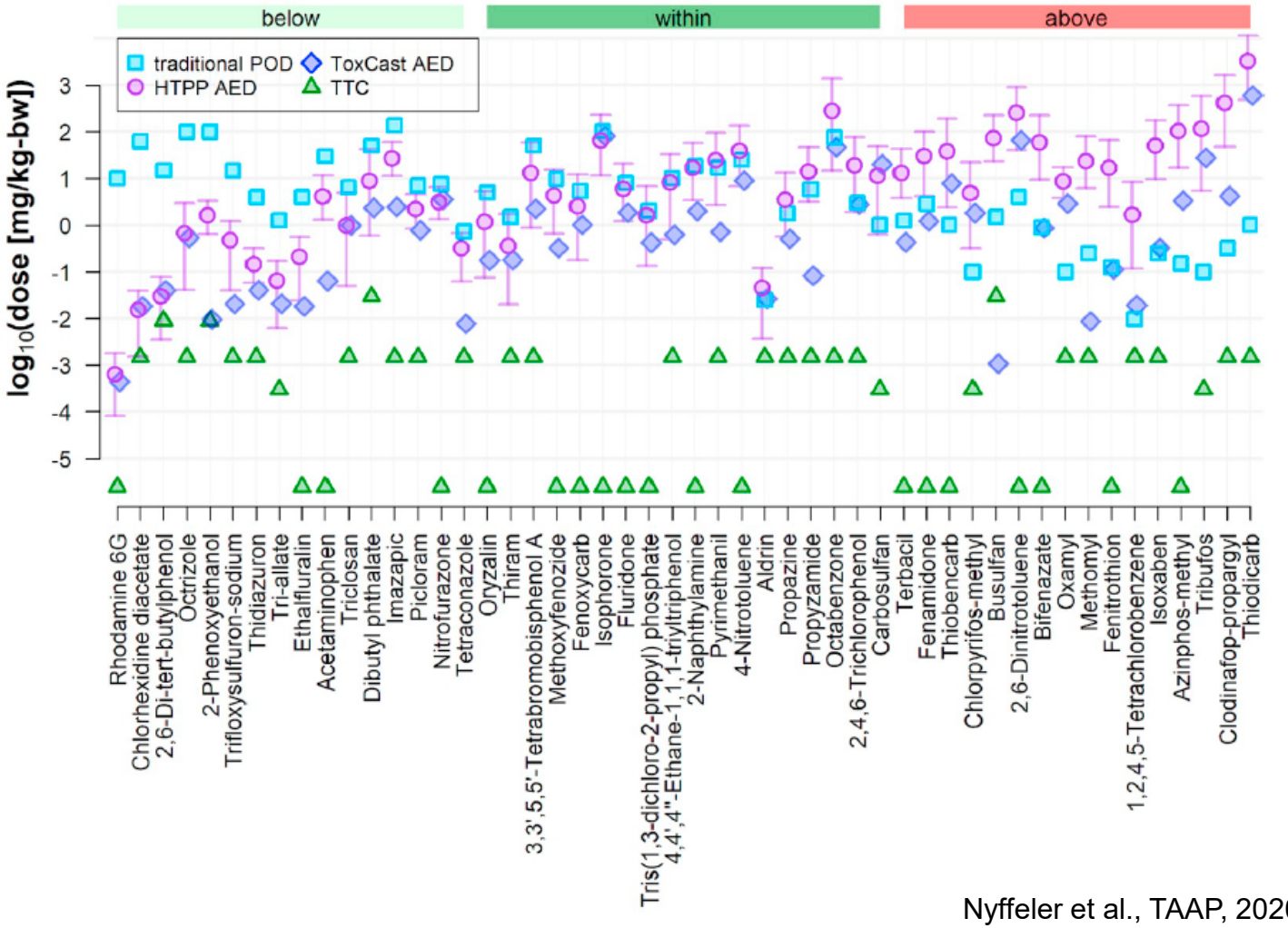


Concentration Response Modeling



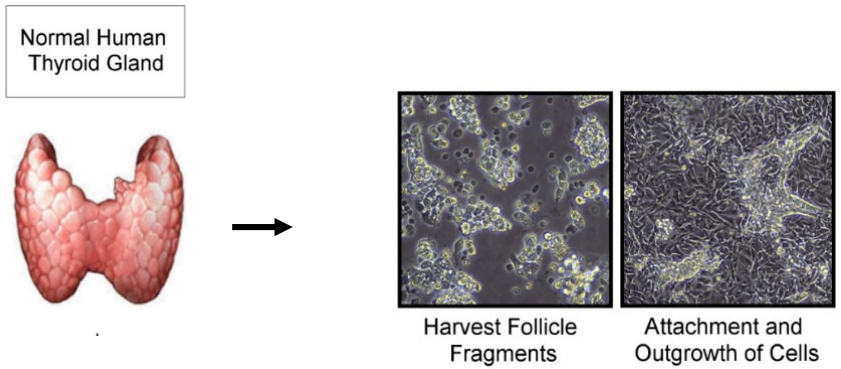
Evaluating 'Cellular Pathology' in U2OS Cells for Different MOAs and *In Vivo* Pathology Responses

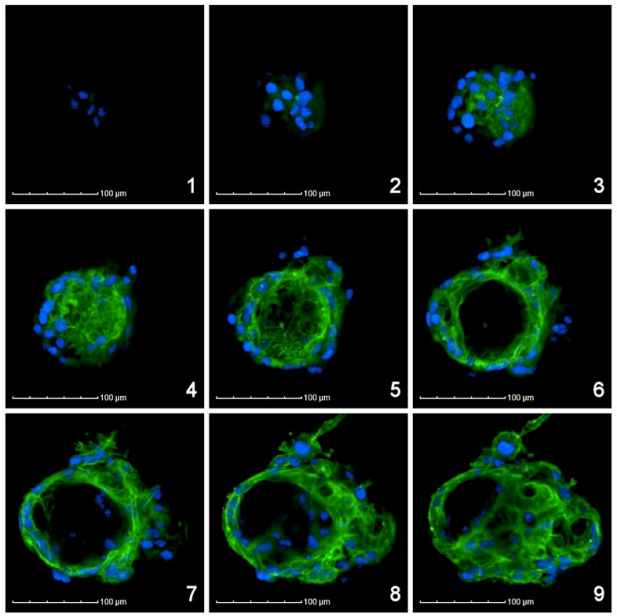




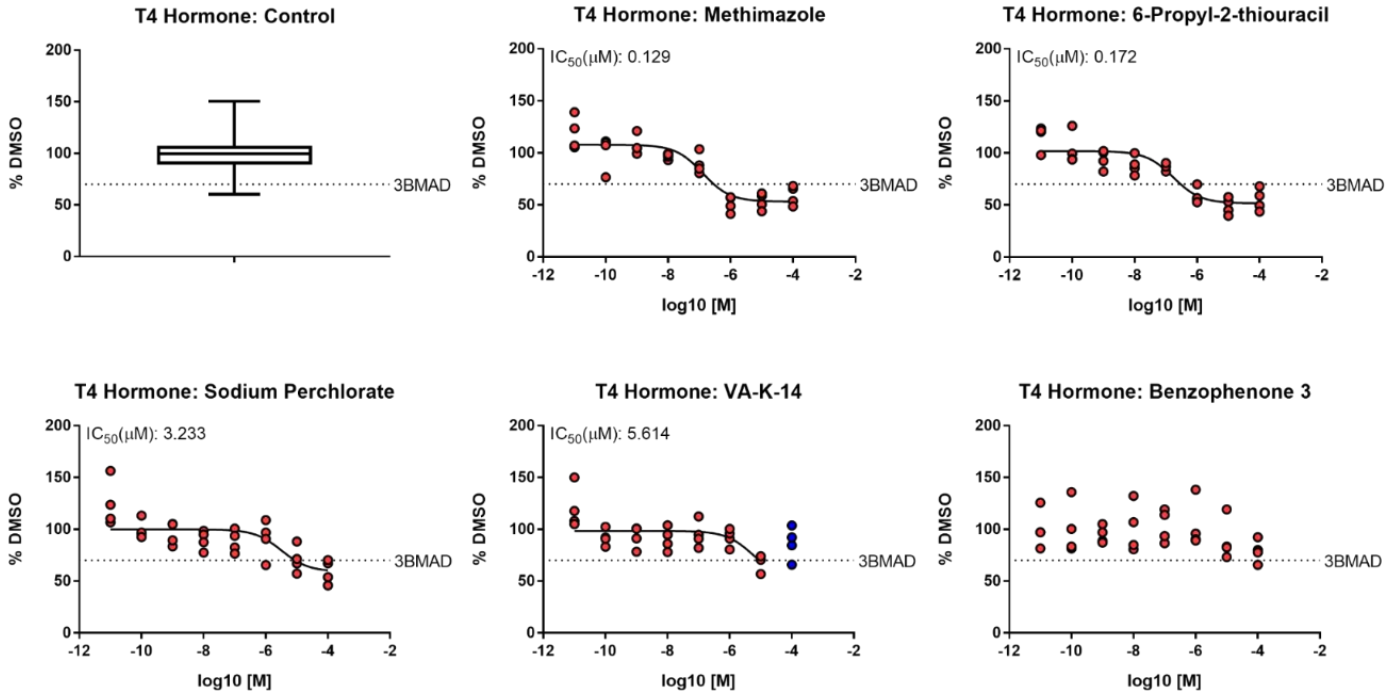
Nyffeler et al., TAAP, 2020

Developing Organotypic Culture Models to Translate Molecular Events into Tissue/Organ Effects



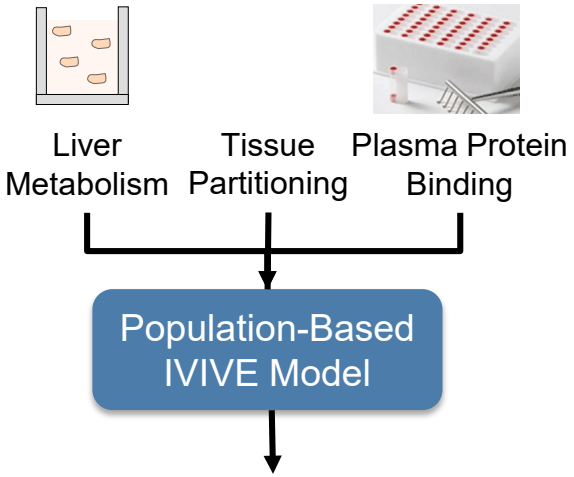


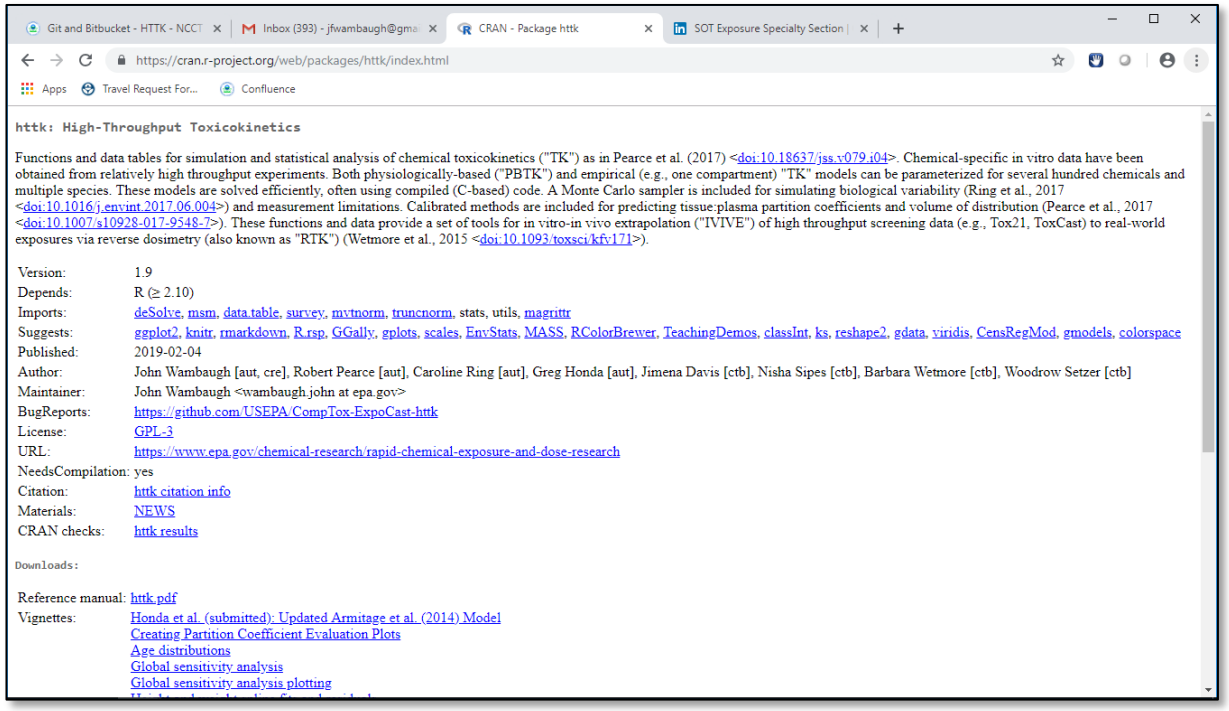
Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin



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

Putting *In Vitro* Test Results in a Dose Context





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

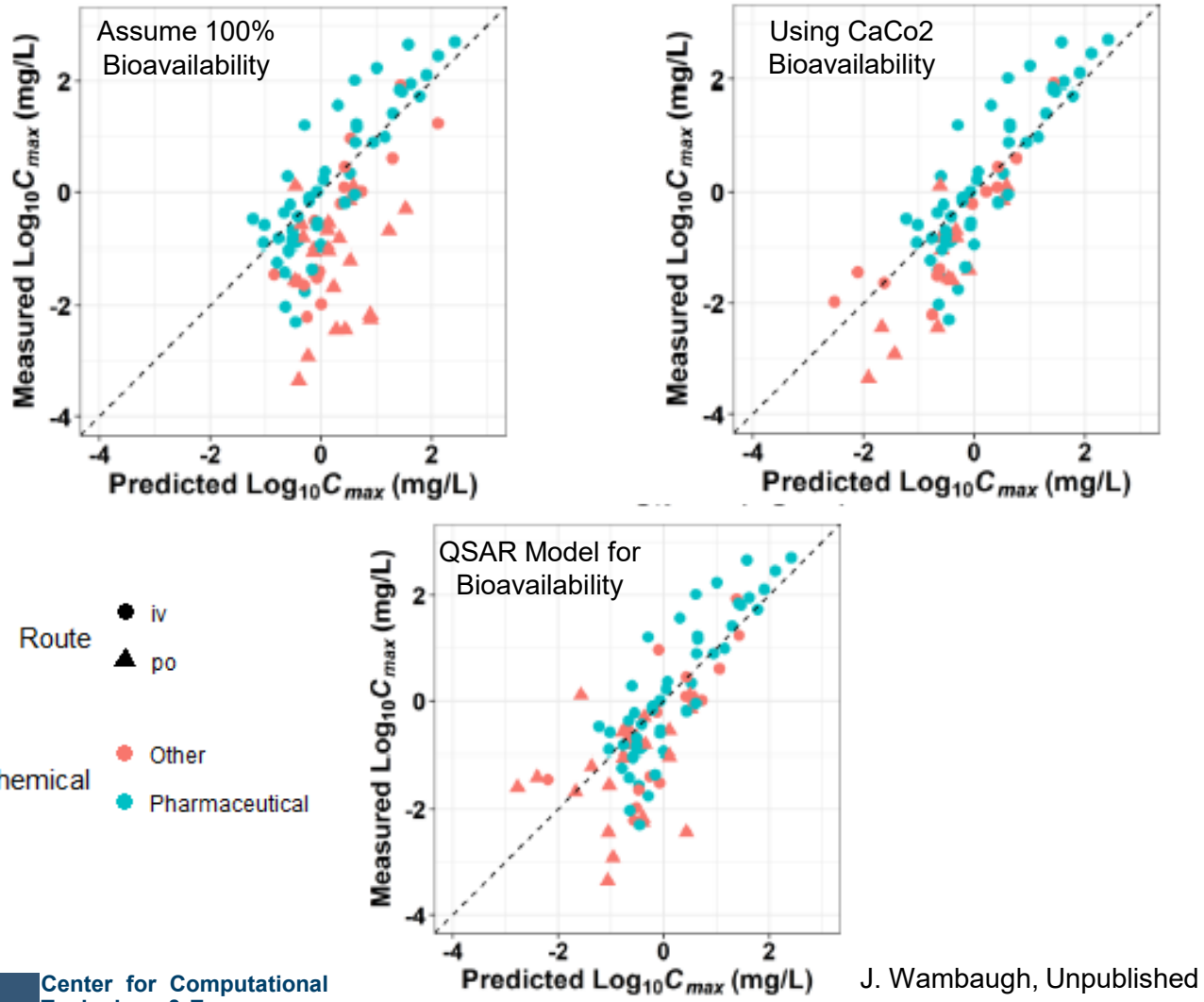
R package "httk"

- 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)
- v1.10 features **942 total chemicals**
- Now allows propagation of uncertainty

Continued Improving and Expanding Toxicokinetic Modeling Capabilities

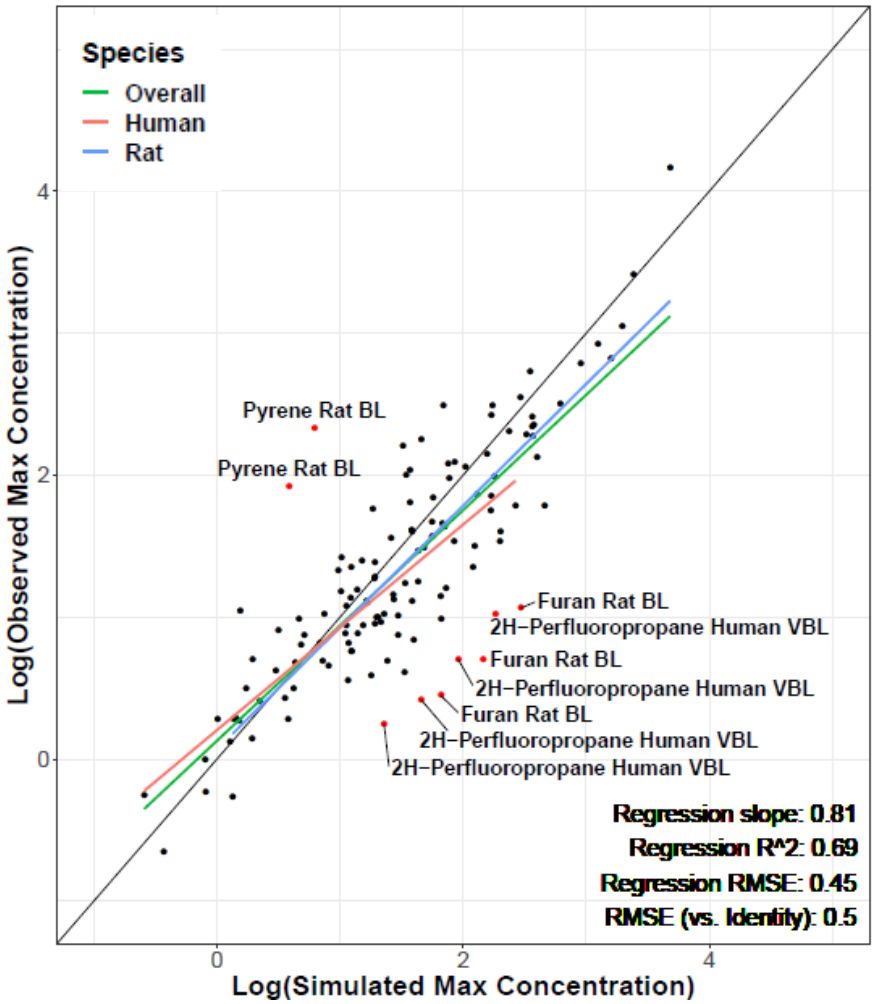
United States Environmental Protection Agency

Improving Predictivity of Oral TK and PBTK Models



J. Wambaugh, Unpublished

Expanding Exposure Routes to Inhalation

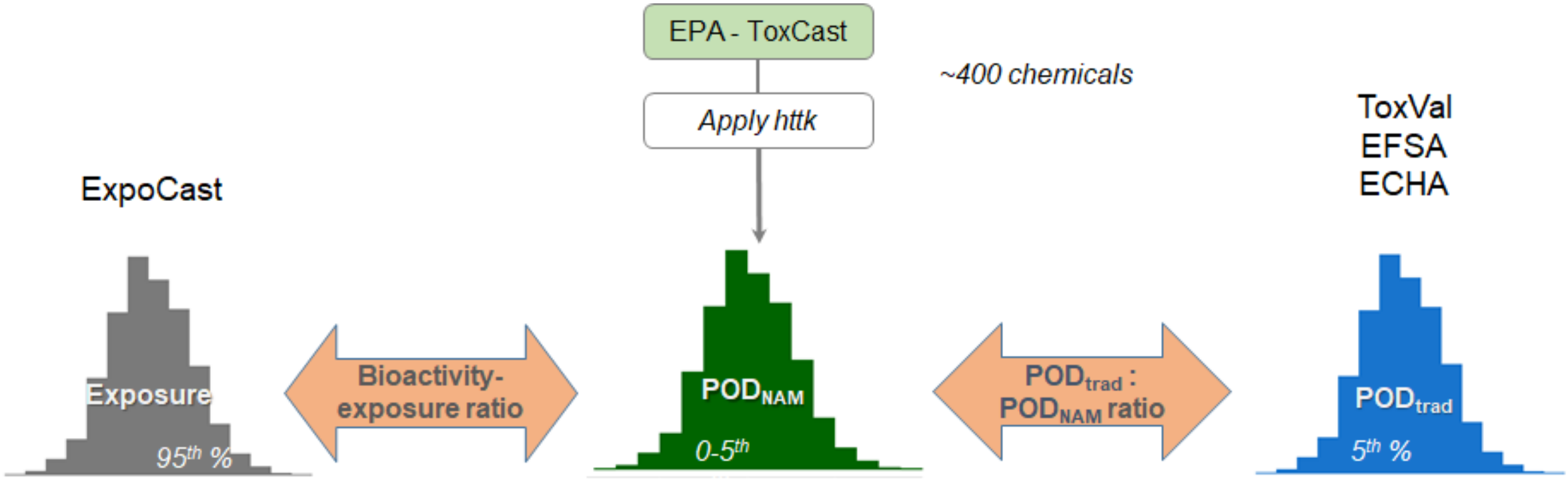


Linakis *et al.,* In Press.

Case Study on Application To Screening Level Assessments

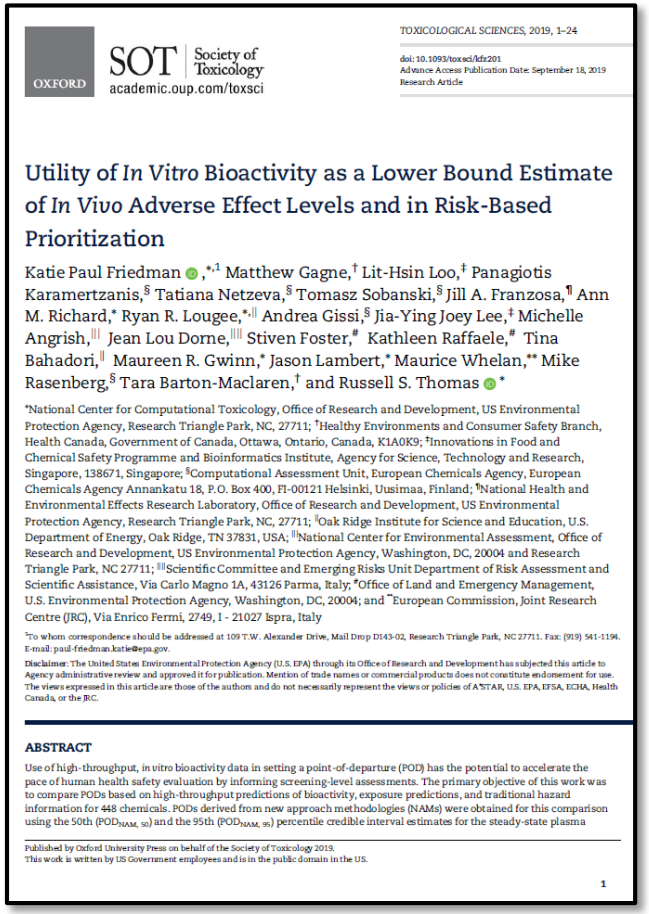
- Multiple international case studies stemming from 2016 inter-governmental workshop
- Example: *In Vitro* Bioactivity as a Conservative Point of Departure
- Participants include EPA, Health Canada, ECHA, EFSA, JRC, and A*STAR
- Goal: Determine whether *in vitro* bioactivity from broad high-throughput screening studies (e.g., ToxCast) can be used as a conservative point-of-departure and when compared with exposure estimates serve to prioritize chemicals for future study or as lower tier risk assessment.

Case Study on Application To Screening Level Assessments



- *NOEL, LOEL, NOAEL, or LOAEL*
- *Oral exposures*
- *Mg/kg-bw/day units*

Regulatory Focused Case Study on Bioactivity as a Point-of-Departure



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

TOXICOLOGICAL SCIENCES, 2019, 1–24
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Research Article

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

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Franzosa,¶ Ann M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Ying Joey Lee,‡ Michelle Angrish,|| Jean Lou Dorne,|| Stiven Foster,# Kathleen Raffaele,# Tina Bahadori,|| Maureen R. Gwinn,* Jason Lambert,* Maurice Whelan,** Mike Rasenberg,§ Tara Barton-Maclaren,† and Russell S. Thomas *

*National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, 27711; †Healthy Environments and Consumer Safety Branch, Health Canada, Government of Canada, Ottawa, Ontario, Canada, K1A0K9; ‡Innovations in Food and Chemical Safety Programme and Bioinformatics Institute, Agency for Science, Technology and Research, Singapore, 138671, Singapore; §Computational Assessment Unit, European Chemicals Agency, European Chemicals Agency Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Uusimaa, Finland; ¶National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, 27711; ||Oak Ridge Institute for Science and Education, U.S. Department of Energy, Oak Ridge, TN 37831, USA; ||National Center for Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Washington, DC, 20004 and Research Triangle Park, NC 27711; ||Scientific Committee and Emerging Risks Unit Department of Risk Assessment and Scientific Assistance, Via Carlo Magno 1A, 43126 Parma, Italy; #Office of Land and Emergency Management, U.S. Environmental Protection Agency, Washington, DC, 20004; and ~European Commission, Joint Research Centre (JRC), Via Enrico Fermi, 2749, I - 21027 Ispra, Italy

*To whom correspondence should be addressed at 109 T.W. Alexander Drive, Mail Drop D143-02, Research Triangle Park, NC 27711. Fax: (919) 541-1194. E-mail: paul-friedman.katie@epa.gov.

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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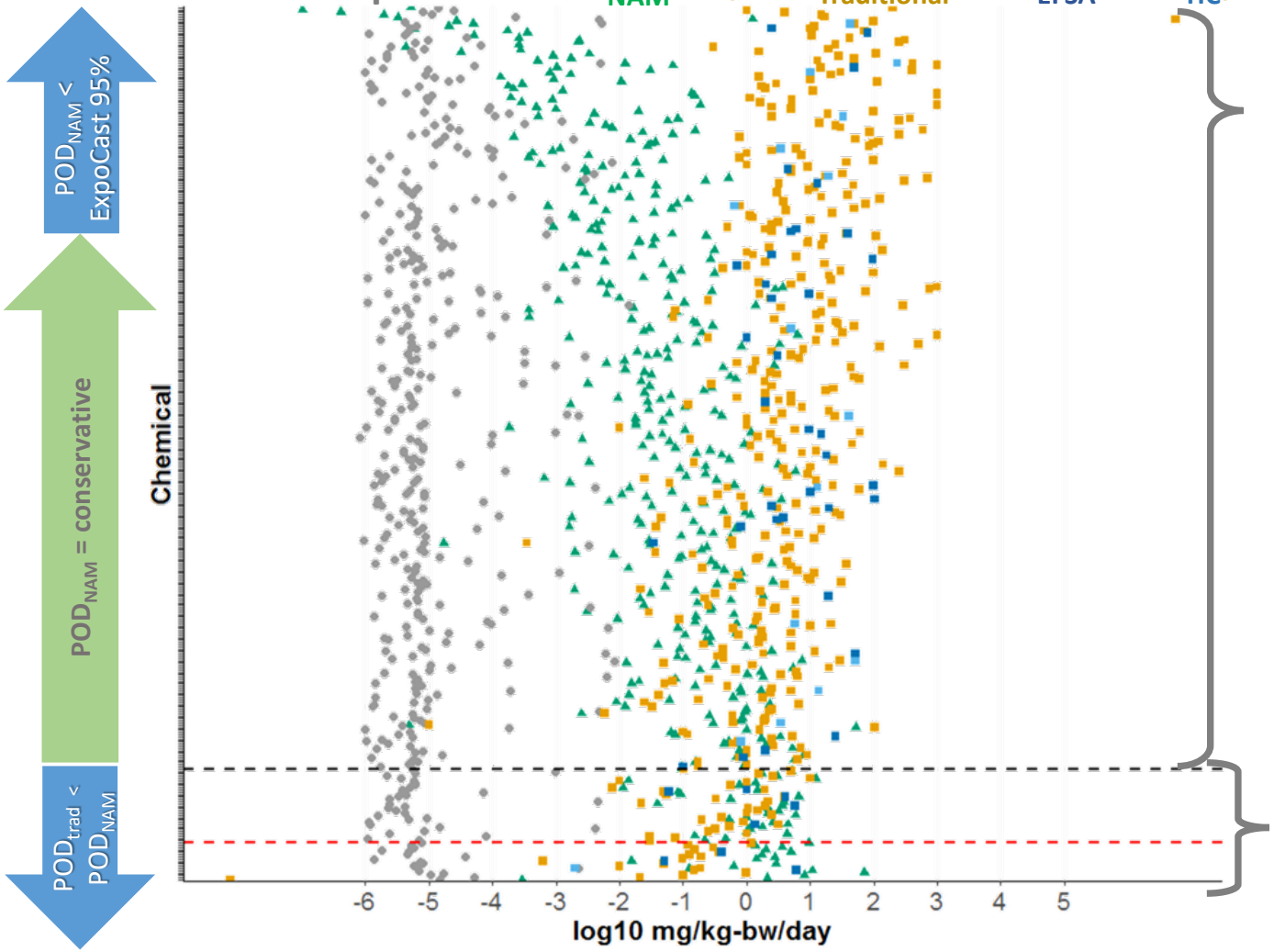
This work is written by US Government employees and is in the public domain in the US.



For ~89% of the chemicals, POD_{NAM} was conservative.

(~100-fold on average), but less conservative than a TTC

Chemicals where POD_{NAM} was not conservative enriched in OPs/carbamates

Others Are Applying the Concept to Screening-Level Regulatory Decisions

Computational Toxicology 7 (2018) 20–26

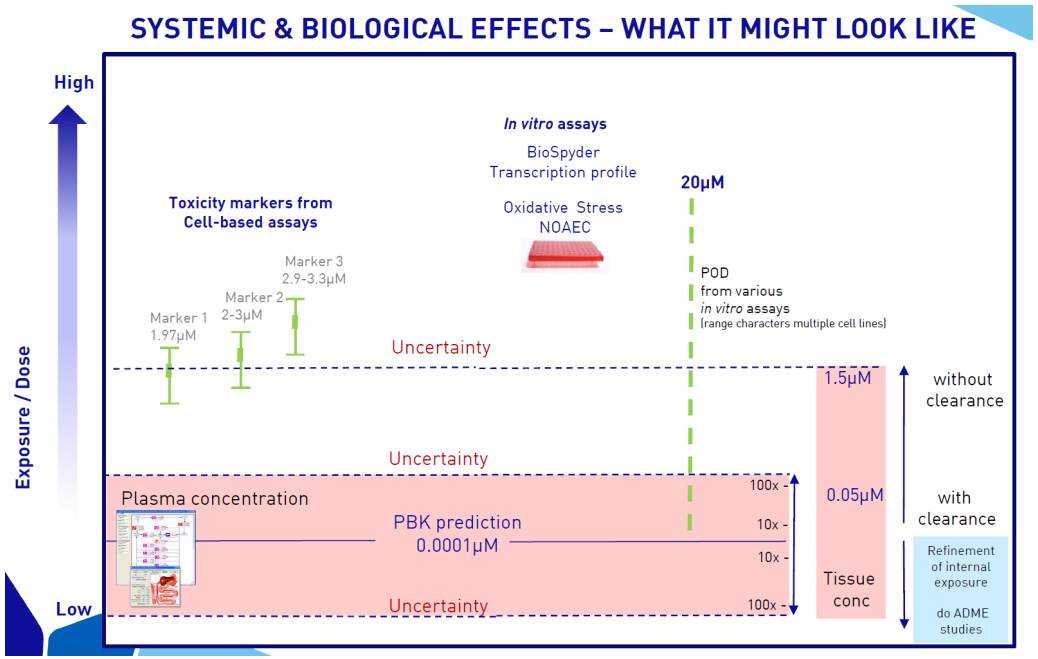
Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.elsevier.com/locate/comtox

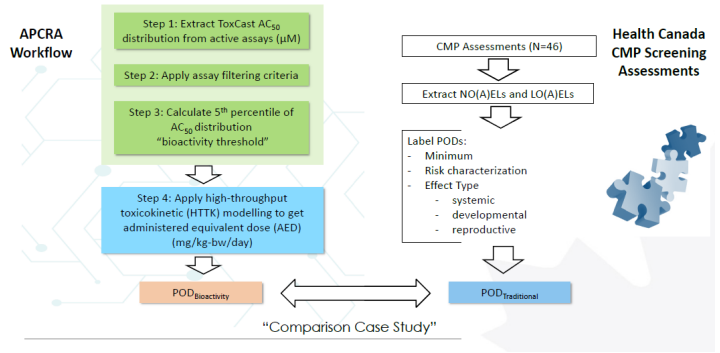
Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o], Rajeshwar Verma[l], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

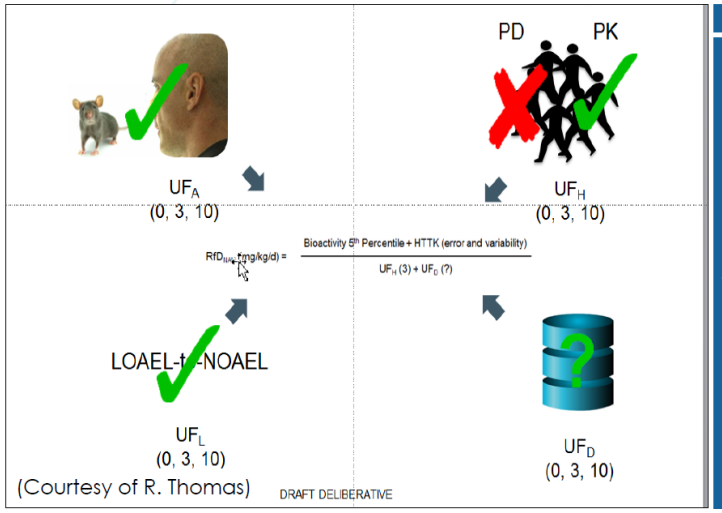


https://www.nc3rs.org.uk/sites/default/files/A.%20Scott%20-%20Unilever%20funders%20perspective.pdf

Overview of key elements in Health Canada SciAD



Uncertainties and Variabilities Characterized



Health Canada Santé Canada (Under Consideration)

Type	Factor	Rationale
Deriving $POD_{Bioactivity}$ ($UF_{Bioactivity}$)	3	Incomplete biological space covered by assays in ToxCast as well as limited metabolic competence. Uncertainties associated with the three compartment model to estimate C_{ss} using in vitro toxicokinetic parameters.
Immortalized Monocultures and Culture Conditions (UF_{Cells})	3	Considers effects of using monocultures and immortalized cell lines, as well as culture conditions, on endpoint measurements. Limitations of single cell type as a surrogate for systemic effects.
Inter-individual Human Variability (UF_{Human})	10	Inter-individual variability related to toxicodynamics and toxicokinetics. Note this is likely conservative as HTTK model partially accounts for this.
TOTAL	~100	

https://www.epa.gov/sites/production/files/2020-01/documents/6_508_tara_barton-maclaren_nams_2019.pdf

EPA United States Environmental Protection Agency

Take Home Messages…

- Computational toxicology is emerging from the teenage years and new Agency initiatives are accelerating the maturation of the field
- Statutory language and the EPA NAM Work Plan require establishing expectations for the performance of computational toxicology methods by better characterizing the variability and relevance of existing models
- New technologies exist for rapidly and comprehensively covering toxicological space at significantly less cost
- Addressing previous technical limitations such as a lack of metabolism and higher-level tissue effects are within reach
- Toxicokinetic modeling and in vitro-to-in vivo extrapolation methods continue to be improved and expanded for broader application
- Continuing to partner with regulators on case studies will increase confidence and acceleration application to chemical risk assessment

Acknowledgements

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- FDA
- NCATS

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- CPHEA
- CESER

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- A*STAR
- ECHA
- EFSA
- Health Canada

Center for Computational Toxicology and Exposure (CCTE) Staff

Research Triangle Park, NC

Duluth, MN

Washington, DC

Gulf Breeze, FL

Cincinnati, OH

Athens, GA