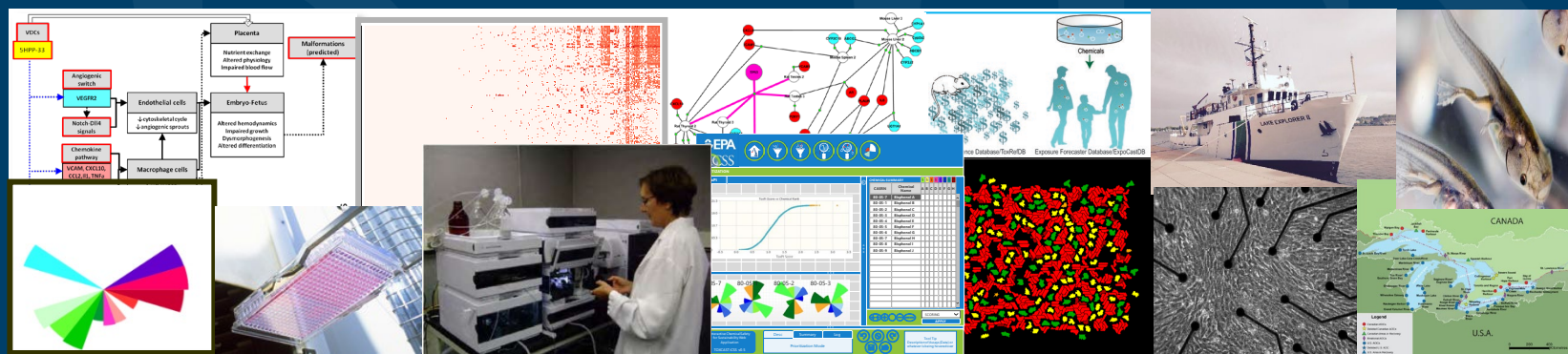


EPA Office of Research and Development: Update on Alternatives Research Activities



ICCVAM Public Forum

May 26, 2021

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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 NAM Work Plan provided clear objectives, strategies and deliverables



- Five objectives for achieving the reduction goals while ensuring that Agency decisions remain fully protective of human health and the environment
 - Evaluate regulatory flexibility
 - Develop baselines and metrics
 - Establish scientific confidence and demonstrate application
 - Develop NAMs to address information gaps
 - Engage and communicate with stakeholders
- Changes in 2021 updated work plan:
 - Modified timelines & deliverables through 2024; two case studies
 - Covered species now includes all vertebrate animals, consistent with TSCA
 - Pilot study to develop NAMs training courses for a broad range of stakeholders

EPA NAMs Confidence Framework

- 2021 NAMs Work Plan: Requested NAS report - scope now includes review of validation and scientific confidence frameworks
- EPA's Confidence Framework will be informed by internal and external case studies, variability analysis, NASEM committee recommendations, and stakeholder feedback
- Workshop being planned for ~Fall 2022 to engage the public/stakeholders in developing the framework, with delivery date of 2024



Building confidence: Progress toward NAM Work Plan deliverable to set expectations for alternative models

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Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment

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Animal testing is often used to evaluate the potential risks, uses, and environmental impacts of chemicals. New Approach Methodologies (NAMs) are technologies and approaches that can potentially provide the same hazard and risk assessment information without the use of animal testing. To further establish scientific confidence in these approaches, this study will review the variability and relevance of existing mammalian toxicity tests, specifically when it comes to human health risk assessment. The goal of this study is to set data-driven and science-based expectations for NAMs based on the variability and relevance of the traditional toxicity testing models.

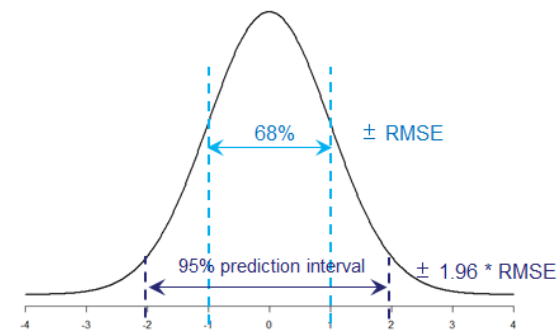
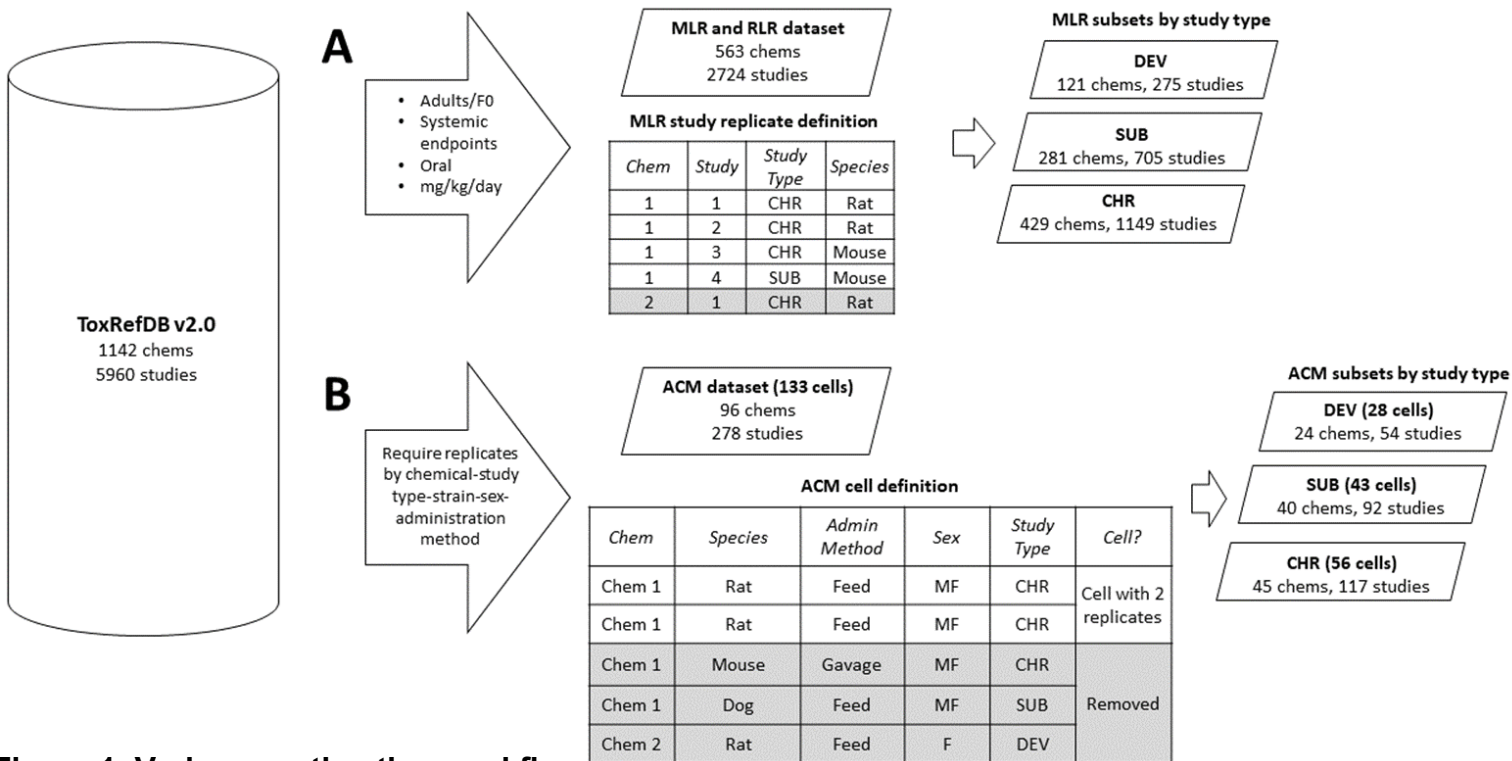
 [Provide feedback on this project](#)

- **Two Workshops:**
 - December 2021: Workshop report released 3/2022
 - May 2022
- Committee **report** will be informed by workshops and comprehensive literature review that addresses the variability and human relevance of current laboratory mammalian toxicity tests and approaches to validation and establishing scientific confidence in using NAMs.

Case Study: Evaluating reproducibility of traditional repeat dose toxicity studies in adult animals

Katie Paul-Friedman and team built 28 different statistical models to approximate total variance, unexplained variance, and the spread of the residuals from statistical models of study-level points-of-departure in adult animals.

The variance, as approximated by RMSE, approaches 0.4-0.6 log10-mg/kg-bw/day regardless of the dataset or approach used. This helps us estimate a minimum prediction interval for a new estimation of study-level point-of-departure and to set a benchmark for NAMs to predict these values.



Using an RMSE=0.59, the minimum 95% PI of an LEL/LOAEL is:

1 mg/kg/day → 0.07 – 14 mg/kg/day.

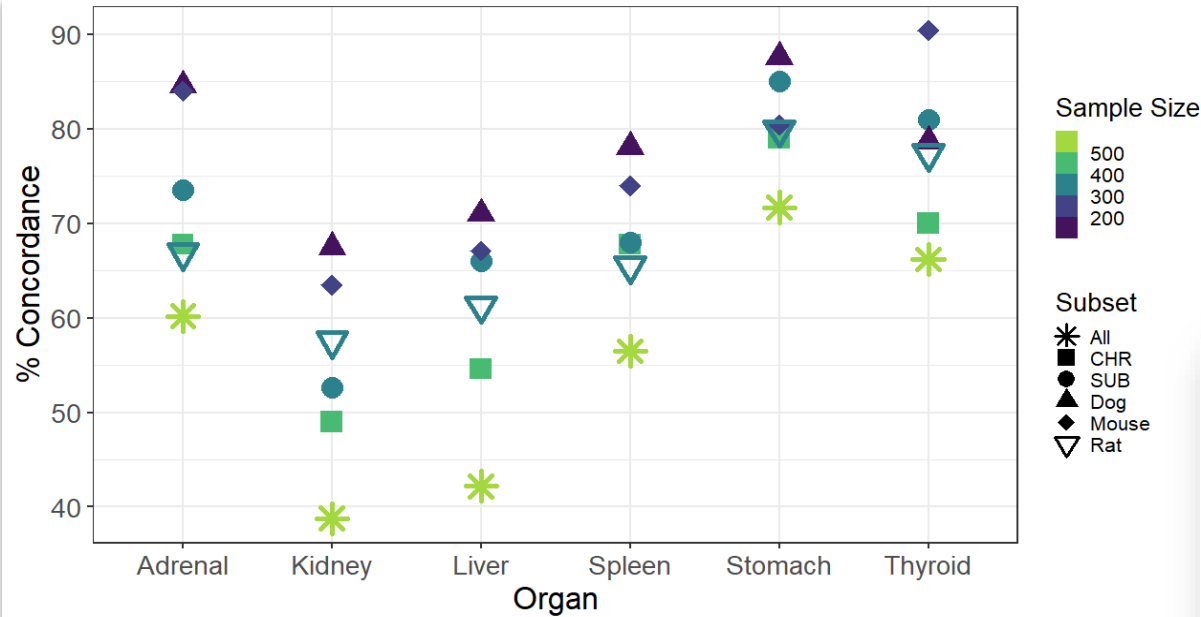
10 mg/kg/day → 0.7 – 143 mg/kg/day.

Figure 1. Variance estimation workflow.

CHR = chronic; DEV = developmental (adults only); SUB = subchronic; cells are defined by the factor of all categorical variables; MF = males and females; F = females; MLR = multilinear regression; POD = point of departure; RLR = robust linear regression; ACM = augmented cell means.

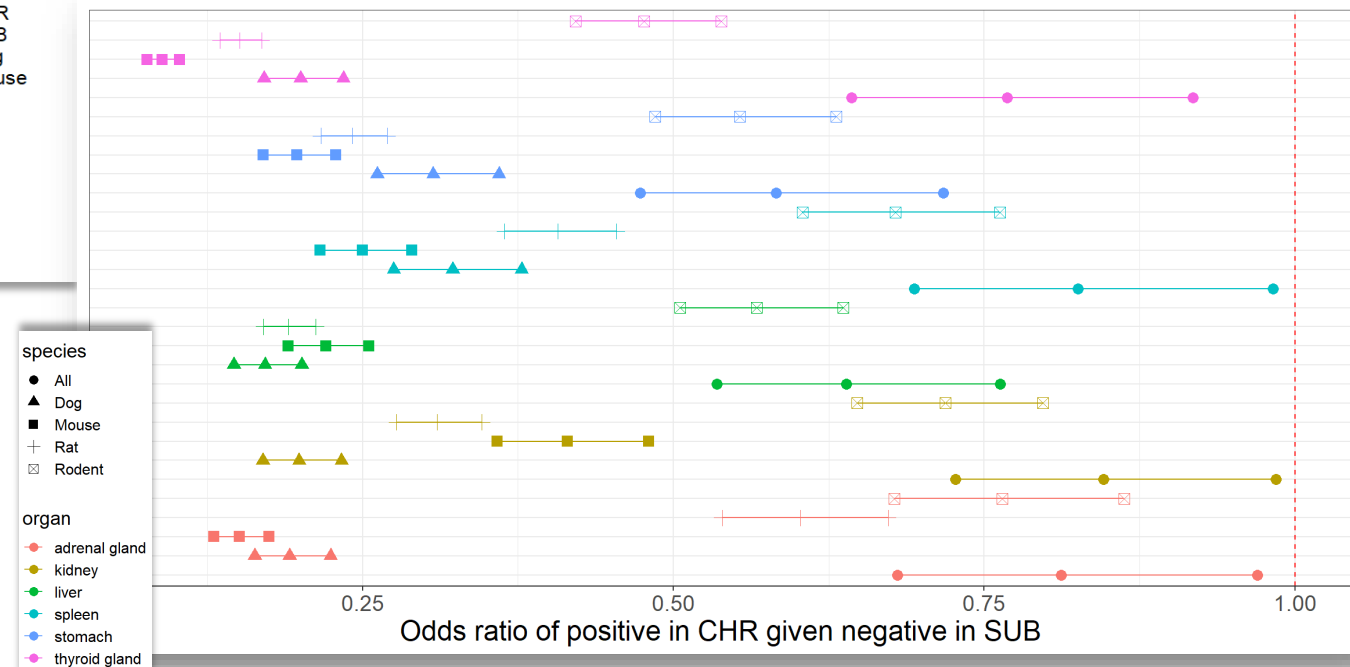
Reproducibility of organ-level findings across replicate studies ranged from 33-88%

$$\% \text{ Concordance} = \frac{\text{chemical with positive finding in all studies} + \text{chemicals with negative finding in all studies}}{\text{total chemicals tested}}$$



- Qualitative reproducibility of organ-level effect observations in repeat dose studies of adult animals was 33-88%, depending on grouping
- Organs associated with more negative chemicals (stomach, thyroid, adrenal) had higher rates of concordance
- Within-species concordance tended to be greater than within-study concordance

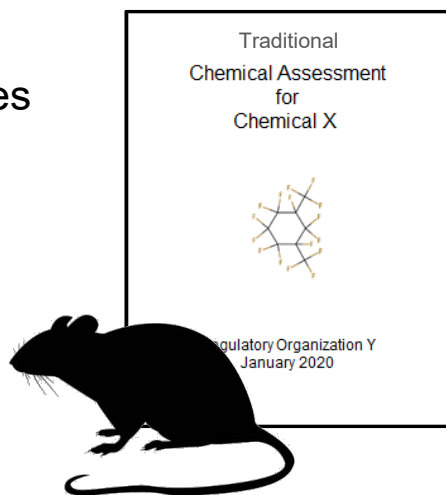
- A negative in a subchronic (SUB) study indicates a greater likelihood of a negative in a chronic (CHR) study, as all odds ratios for a positive finding by organ were < 1 in this case
- A SUB target organ POD, particularly for liver and kidney where have larger datasets, is likely protective for a CHR target organ finding



Quantifying trade-offs of uncertainty, cost, and time in toxicity testing methods

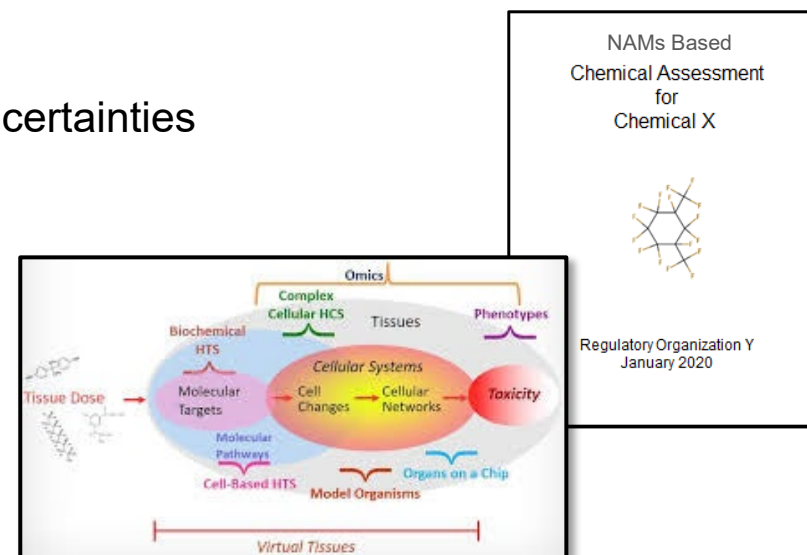
Option 1

- 6 – 20 years
- “Smaller” uncertainties
- \$Ks - \$Ms



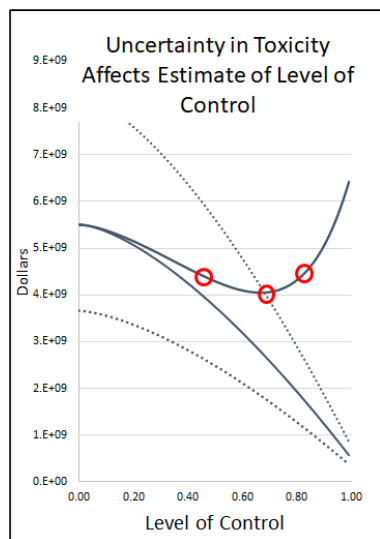
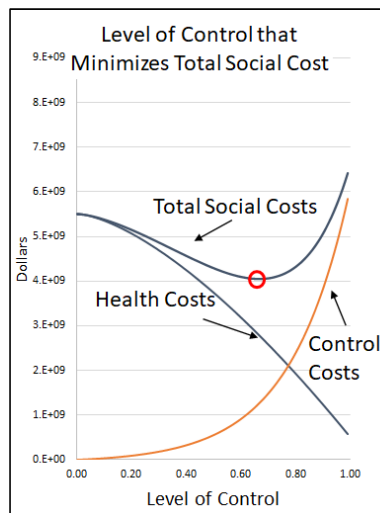
Option 2

- <1 year
- “Bigger” uncertainties
- \$Ks



What are the trade-offs between the approaches?

Development of a Value of Information Framework to evaluate the trade-offs in toxicity testing



- Value of information (VOI) analysis is a decision analytic method that quantifies the expected value of additional testing/data in reducing decision uncertainty (Tuffaha, 2021).
- VOI requires a method to determine the cost of uncertainty
 - $Total\ Social\ Cost = Total\ Control\ Cost + Total\ Health\ Cost$
- Lots of work in VOI evaluating different tests (e.g., medical tests), but few studies evaluating the impact of time.
- The impact of time can be incorporated by discounting the costs on an annual basis.
- Multiple metrics can be used to compare the value of different toxicity tests adjusted for time and cost of the test
 - **Expected Value of Delayed Sample Information (EVDSI)**
 - Expected Net Benefit of Sampling (ENBS)
 - Return on Investment (ROI)

General conclusions from the Value of Information studies

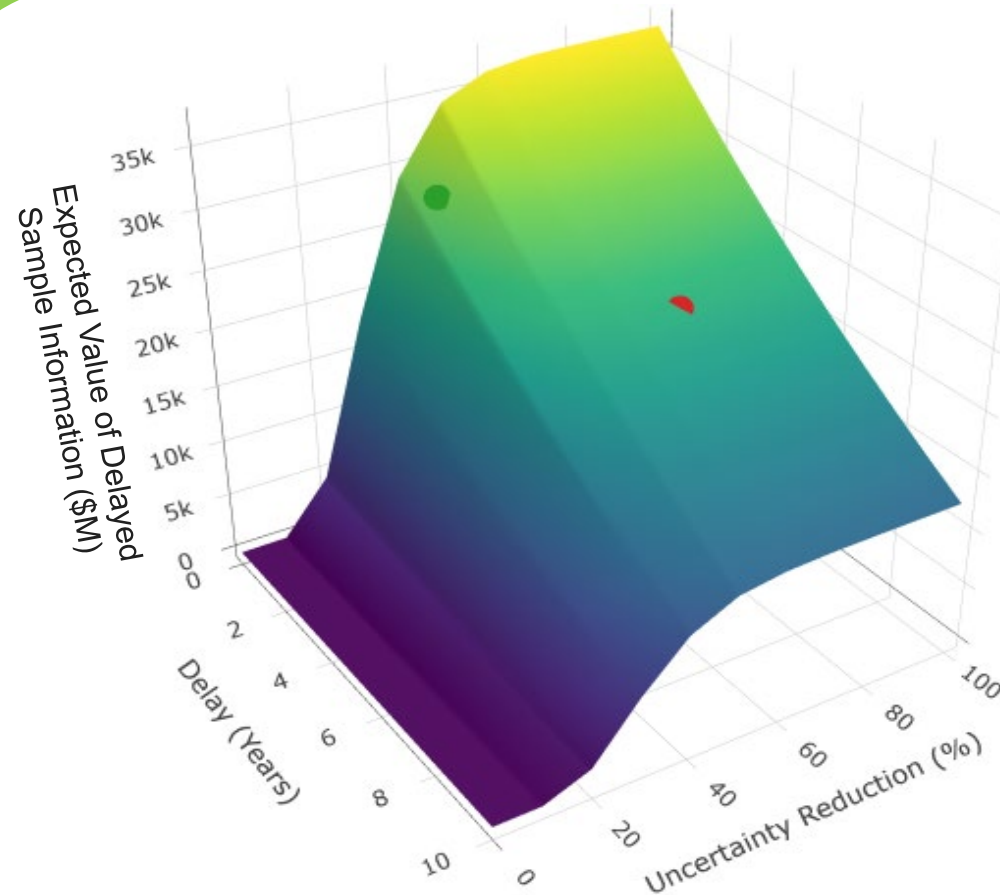
Example Scenarios

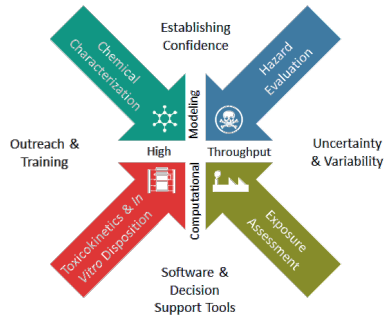
- Two hypothetical toxicity tests
 - Option 1 – lower cost (\$5K), shorter duration (1 yr), higher uncertainty (4 orders of magnitude)
 - Option 2– higher cost (\$5M), longer duration (5 yr), lower uncertainty (2 orders of magnitude)
- Different health endpoints and decision types
 - Chronic and acute effects
 - Chemicals regulated based on benefit-cost analysis and target risk levels

Overall Conclusions

- ***Timeliness has a significant positive impact on the VOI of toxicity tests, even in the presence of smaller reductions in uncertainty.***
- The positive impact of the shorter tests may be multiplicatively amplified by the ability to test more chemicals.

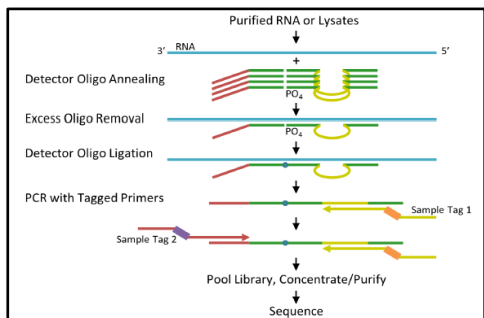
Trade-Offs of Uncertainty and Time of Hypothetical Toxicity Testing Methods
(Chronic Effect, Target Risk Decision Maker)



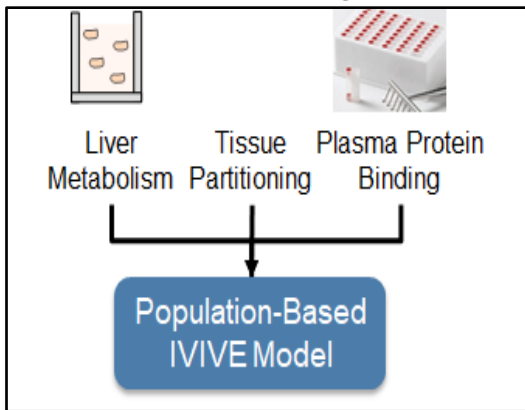


EPA continues to innovate and address limitations in NAMs

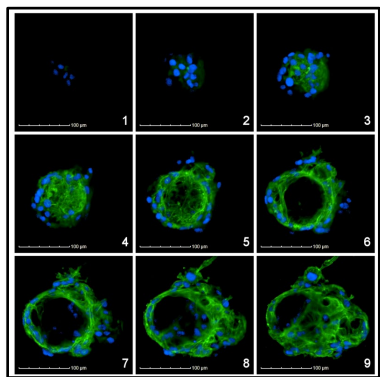
Whole Genome Transcriptomics



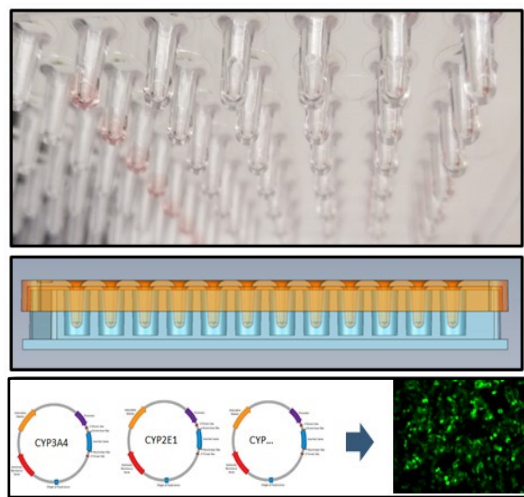
Toxicokinetic Measurements and Modeling



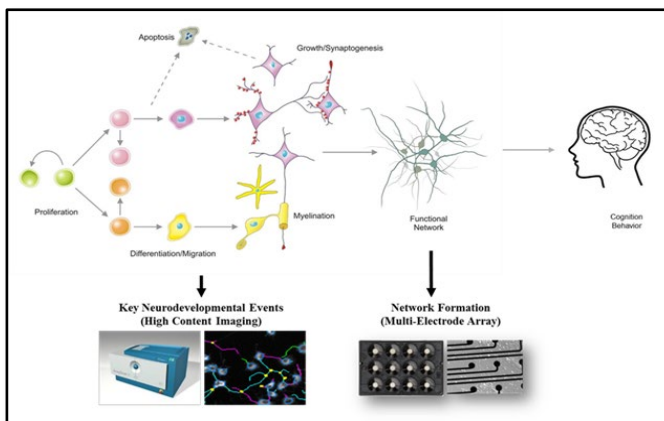
Organotypic Culture Models



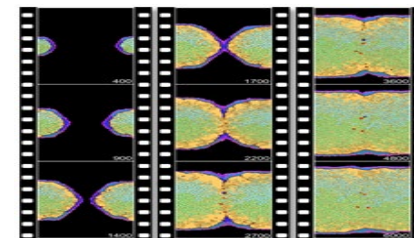
Metabolic Retrofitting



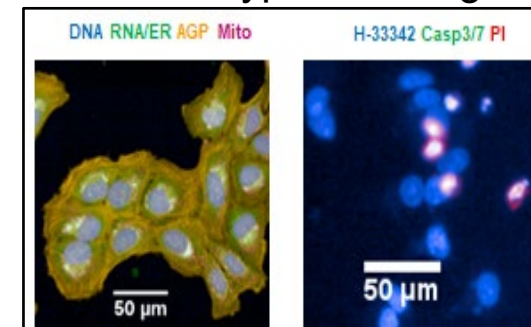
Integrated Approach to Testing and Assessment for DNT



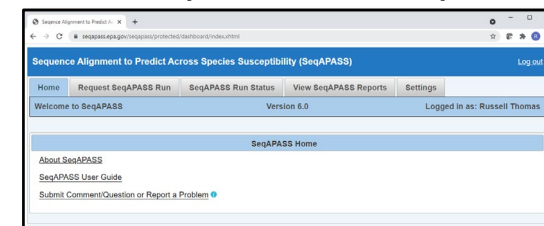
Virtual Tissue Models



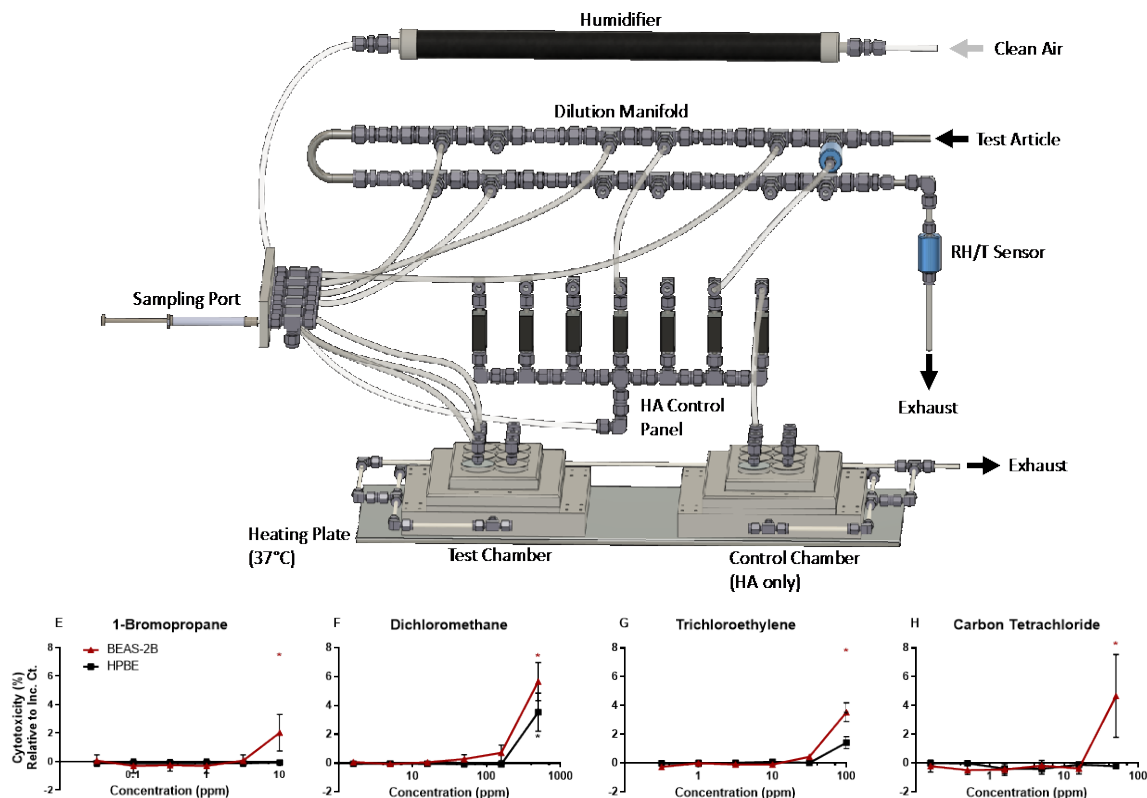
Multi-Parameter Cellular Phenotypic Profiling



Sequence Alignment to Predict Across Species Susceptibility



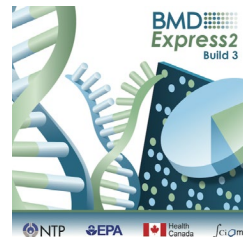
Progress: *in vitro* systems for volatile chemicals



ITFB's CCES achieves higher-throughput ALI exposures:

- 6 doses with 4 technical replicates/dose, maintained at physiological RH/T
- Real-time analytical quantification of VOCs
- Sub-cytotoxic doses included in study design

Chemical Name	BEAS-2B Median BMD (ppm)	HPBE MedianBMD (ppm)	Representative LOAEL (ppm)	Representative NOAEL (ppm)	TLV (ppm)
Acrolein	0.586	--	0.25	NR	0.1
1-Bromopropane	2.246	N/A	62.5	250	0.1
Formaldehyde	N/A	--	2	1	0.3
1,3-Butadiene	13.979	--	625	200	10
Carbon Tetrachloride	9.563	N/A	20	5	10
Acetaldehyde	N/A	--	400	150	25
Trichloroethylene	44.842	28.148	50	25	50
Dichloromethane	142.127	226.73	8400	4200	100



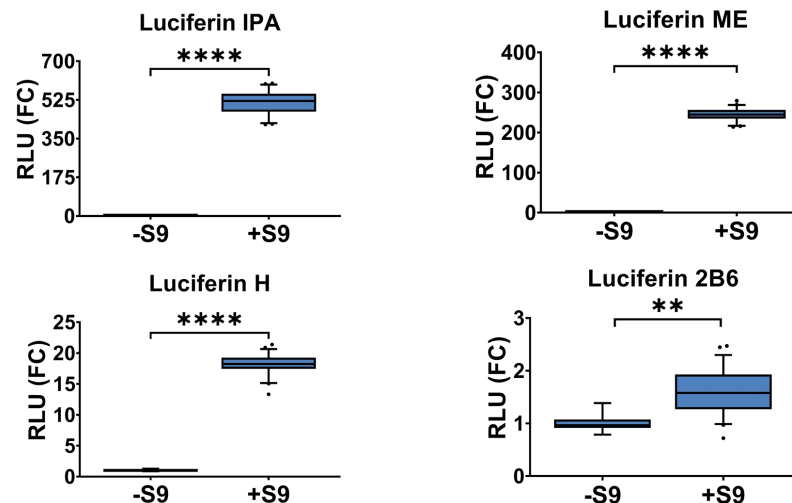
Benchmark Dose Analysis:

- HTTr TempO-Seq analysis at sub-cytotoxic concentrations
- Comparative to representative *in vivo* LOAEL/NOAEL values
- Within a magnitude of ACGIH occupational exposure TLVs

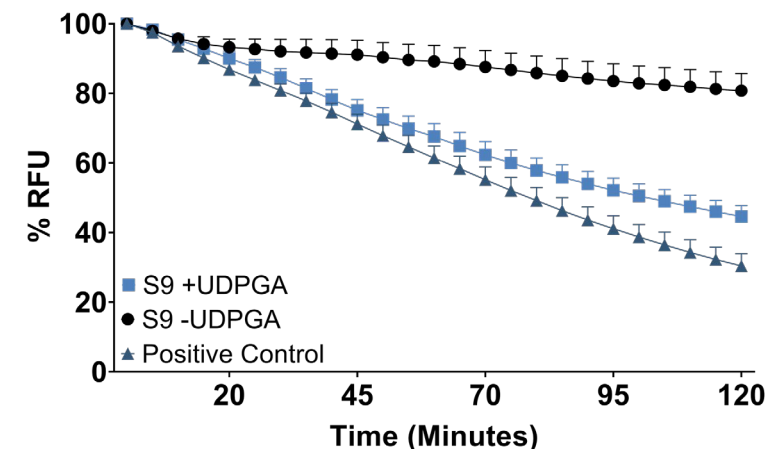
Metabolic competence: Development of a bioprinting approach to adapt the AIME method for high-throughput screening applications



Phase I



Phase II

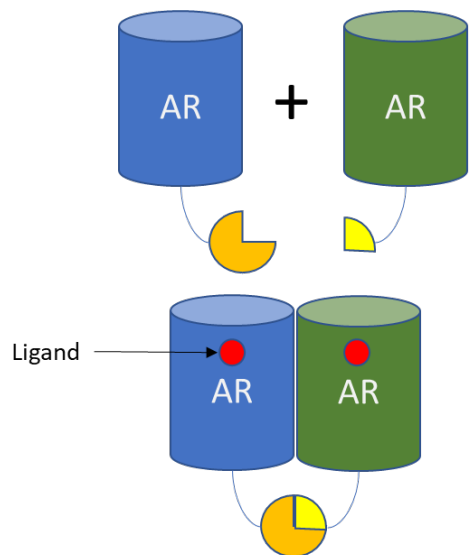


Luminogenic CYP Substrates

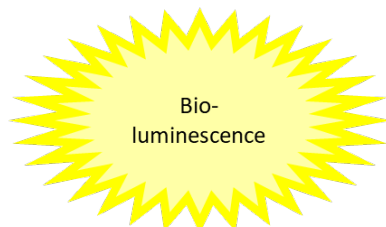
CYP Substrate	Human CYP	Rat CYP
IPA	CYP3A4	CYP3A1
ME	CYP1A2	CYP1A2
H	CYP2C9	CYP2C6
2B6	CYP2B6	CYP2B1

- Goal: Adapt AIME* method to an automated approach using bioprinting for routine application to high-throughput screening.
- The bioprinter method expands the functional capacity for hepatic phase I (CYPs) and phase II (UGTs, SULTs, GSTs) metabolic enzymes.

HepG2-AR2 Assay and Metabolic Retrofit



+ NanoLuc substrate



Journal of Pharmacological and Toxicological Methods 92 (2018) 77-94



Research article

mRNA transfection retrofits cell-based assays with xenobiotic metabolism

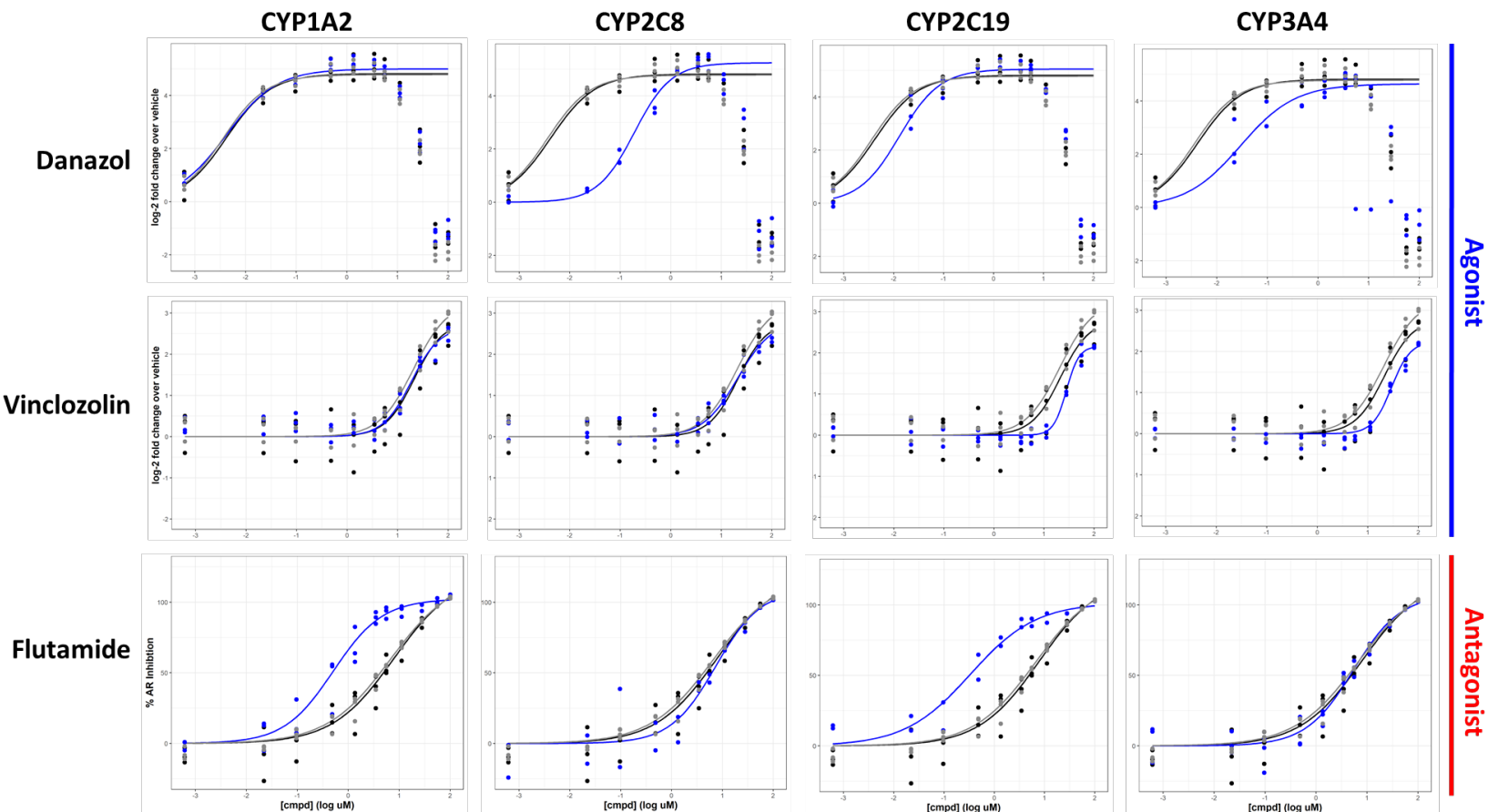
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^dUnilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire, UK





Take Home Messages...

- The EPA NAM Work Plan and CompTox Blueprint provide strategic and operational direction for research and translation of NAMs
- ORD is working on a diverse portfolio of research activities to address information gaps and build scientific confidence in NAMs
- Continued development and refinement of new technologies and analysis approaches will help comprehensively evaluate potential toxicological effects for both humans and ecological species
- Systematically addressing technical limitations such as a lack of metabolism, testing challenging chemicals, and filling important information gaps
- Partnering with regulators and national and international partners on proof-of-concepts and case studies will increase confidence in alternatives and accelerate application for a range of decision contexts

Acknowledgements

Center for Computational Toxicology and Exposure (CCTE) Staff

EPA Colleagues:

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OCSP
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Collaborative Partners:

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ECHA
JRC
EFSA
A*STAR



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Athens, GA



Gulf Breeze, FL