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

ICCVAM Public Forum

May 26, 2021

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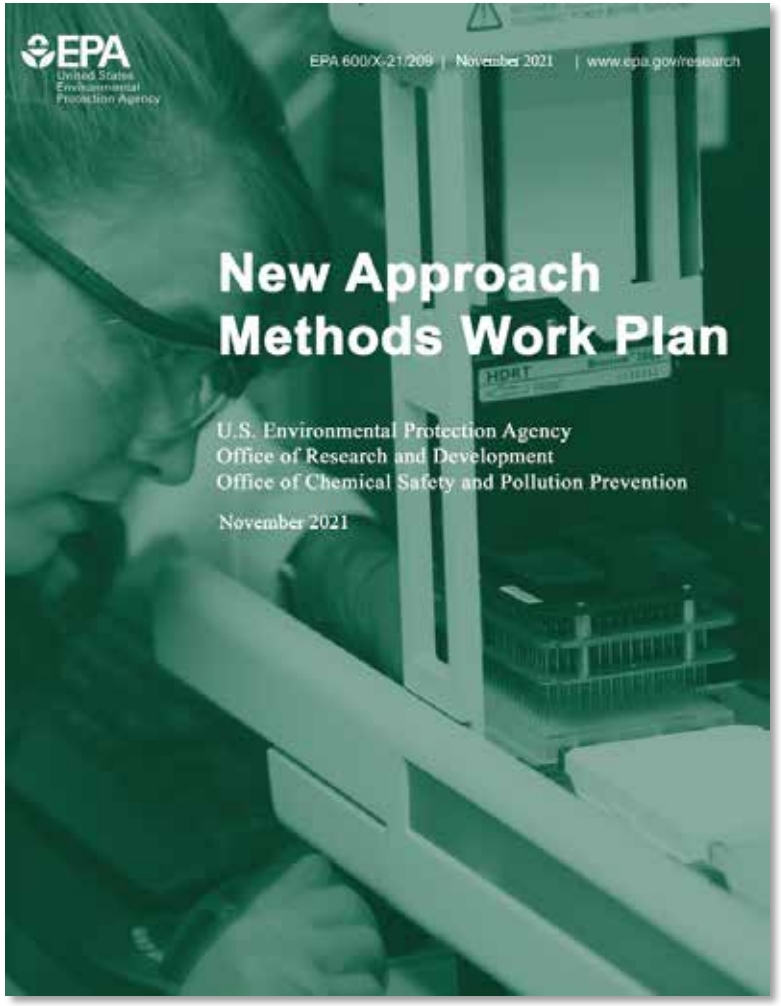
Center for Computational Toxicology and Exposure

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

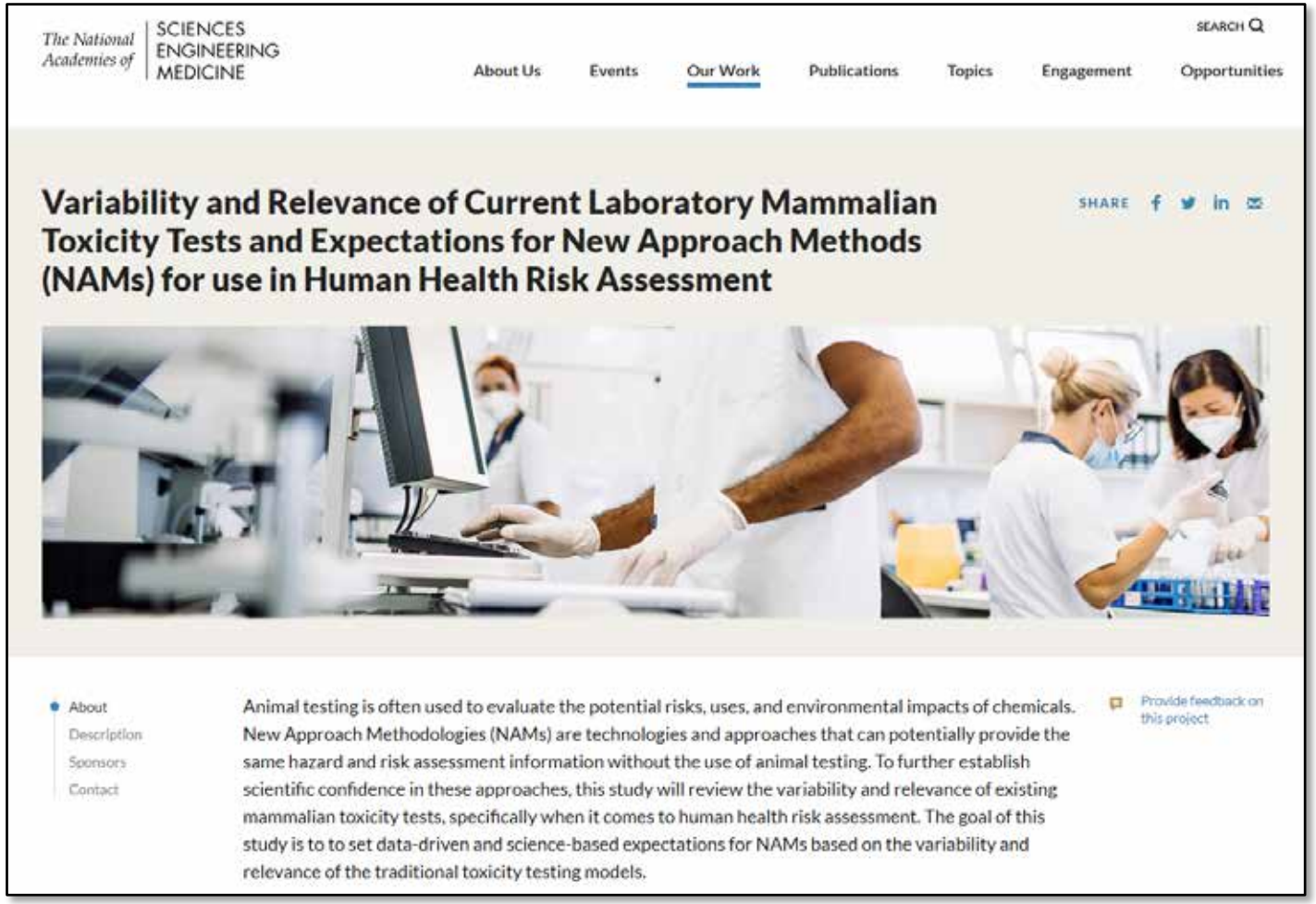
EPA NAMs Confidence Framework

- 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

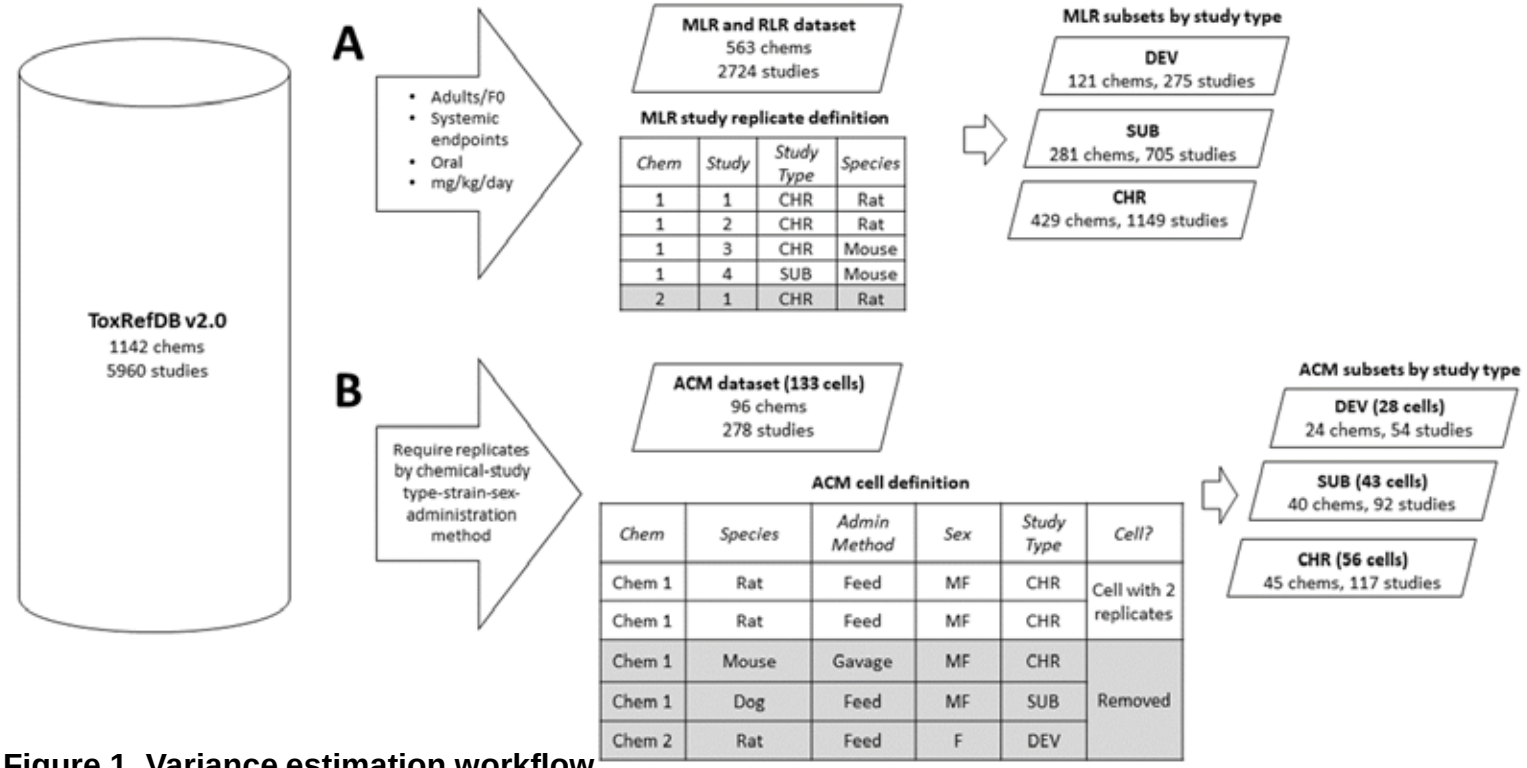
Building confidence: Progress toward NAM Work Plan deliverable to set expectations for alternative models



- **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.



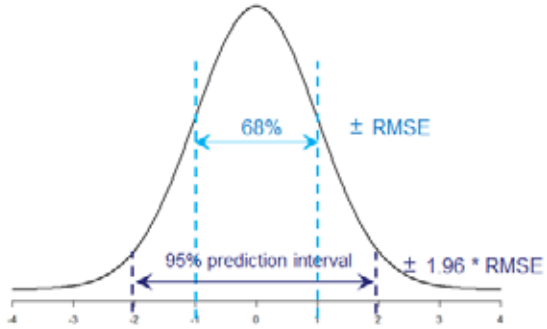
Chem	Study	Study Type	Species
1	1	CHR	Rat
1	2	CHR	Rat
1	3	CHR	Mouse
1	4	SUB	Mouse
2	1	CHR	Rat

Chem	Species	Admin Method	Sex	Study Type	Cell?
Chem 1	Rat	Feed	MF	CHR	Cell with 2 replicates
Chem 1	Rat	Feed	MF	CHR	
Chem 1	Mouse	Gavage	MF	CHR	Removed
Chem 1	Dog	Feed	MF	SUB	
Chem 2	Rat	Feed	F	DEV	

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.

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.

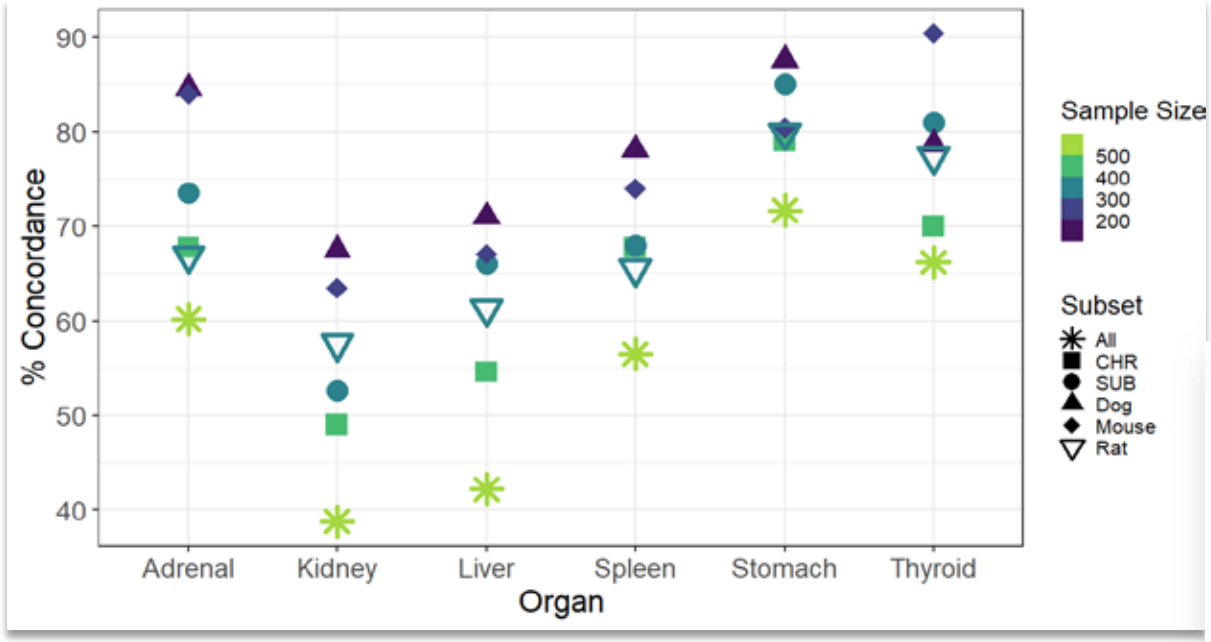


Using an RMSE=0.59, the minimum 95% PI of an LEL/LOAEL is:
For 1 mg/kg/day: 0.07 – 14 mg/kg/day.
For 10 mg/kg/day: 0.7 – 143 mg/kg/day.

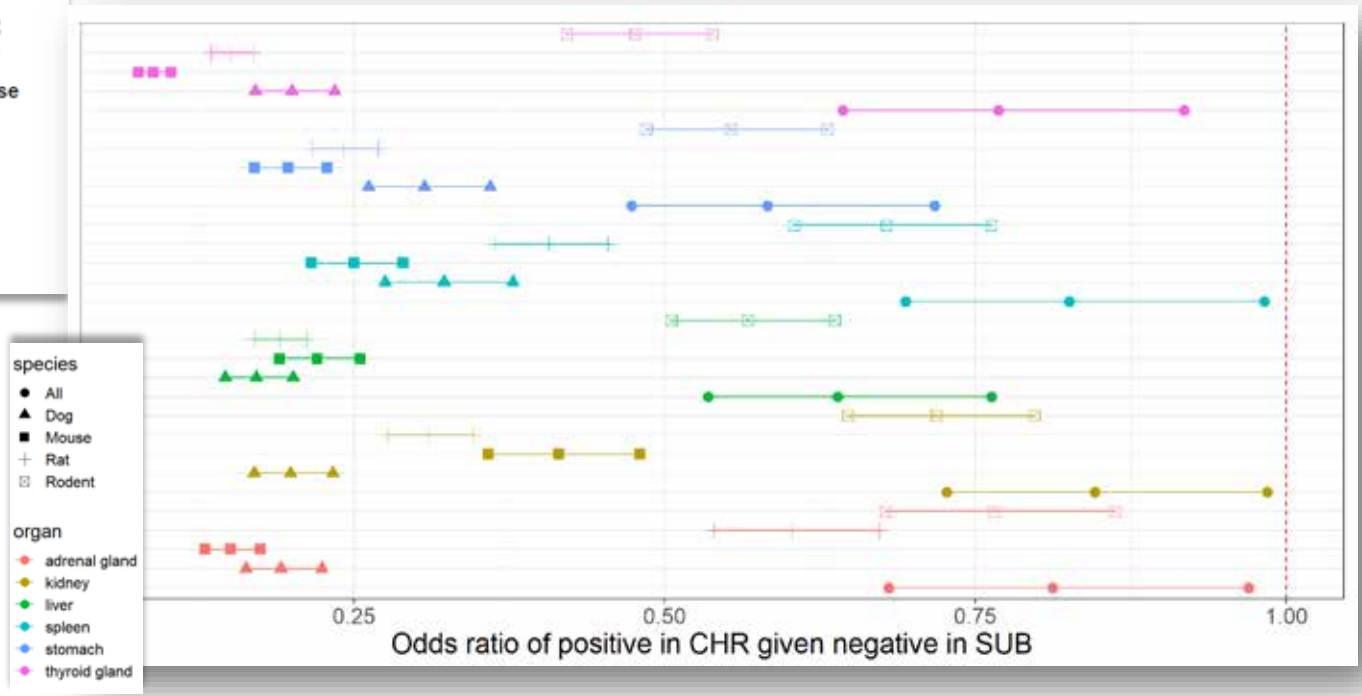
Pham *et al., Comp Toxicol., 2020*

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

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



- 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



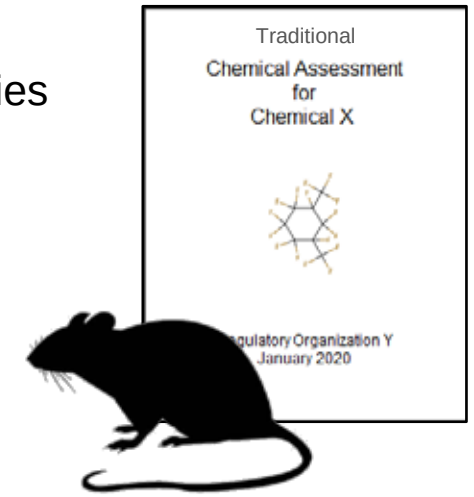
- A negative in a subchronic (SUB) study indicates a greater likelihood of a negative in a chronic (CHR) study, as all odds ratios for a positive finding by organ were < 1 in this case
- A SUB target organ POD, particularly for liver and kidney where have larger datasets, is likely protective for a CHR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

Paul Friedman K, Foster MJ, Setzer RW, Judson RS. *In prep.* Reproducibility of organ-level effects in repeat dose animal studies.

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

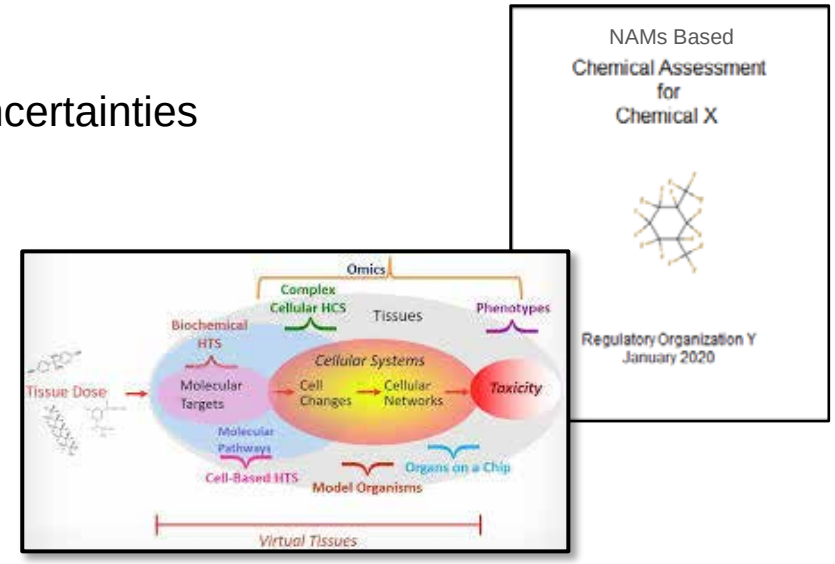
Option 1

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



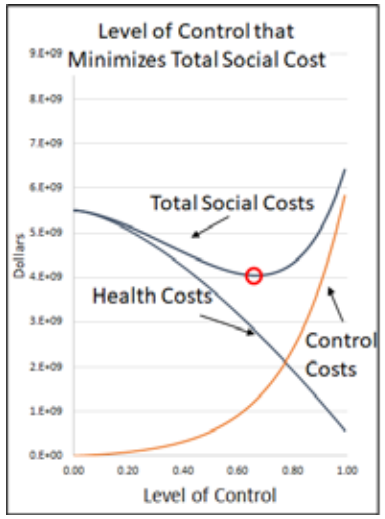
Option 2

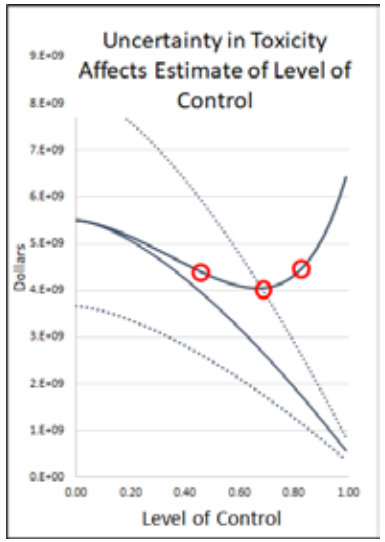
- <1 year
- “Bigger” uncertainties
- $Ks



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)

S. Hagiwara, G. Paoli, D. Krewski (RSI)
P. Price, A. Guiseppi-Elie, M. Gwinn, B. Hubbell, R. Thomas (EPA)

General conclusions from the Value of Information studies

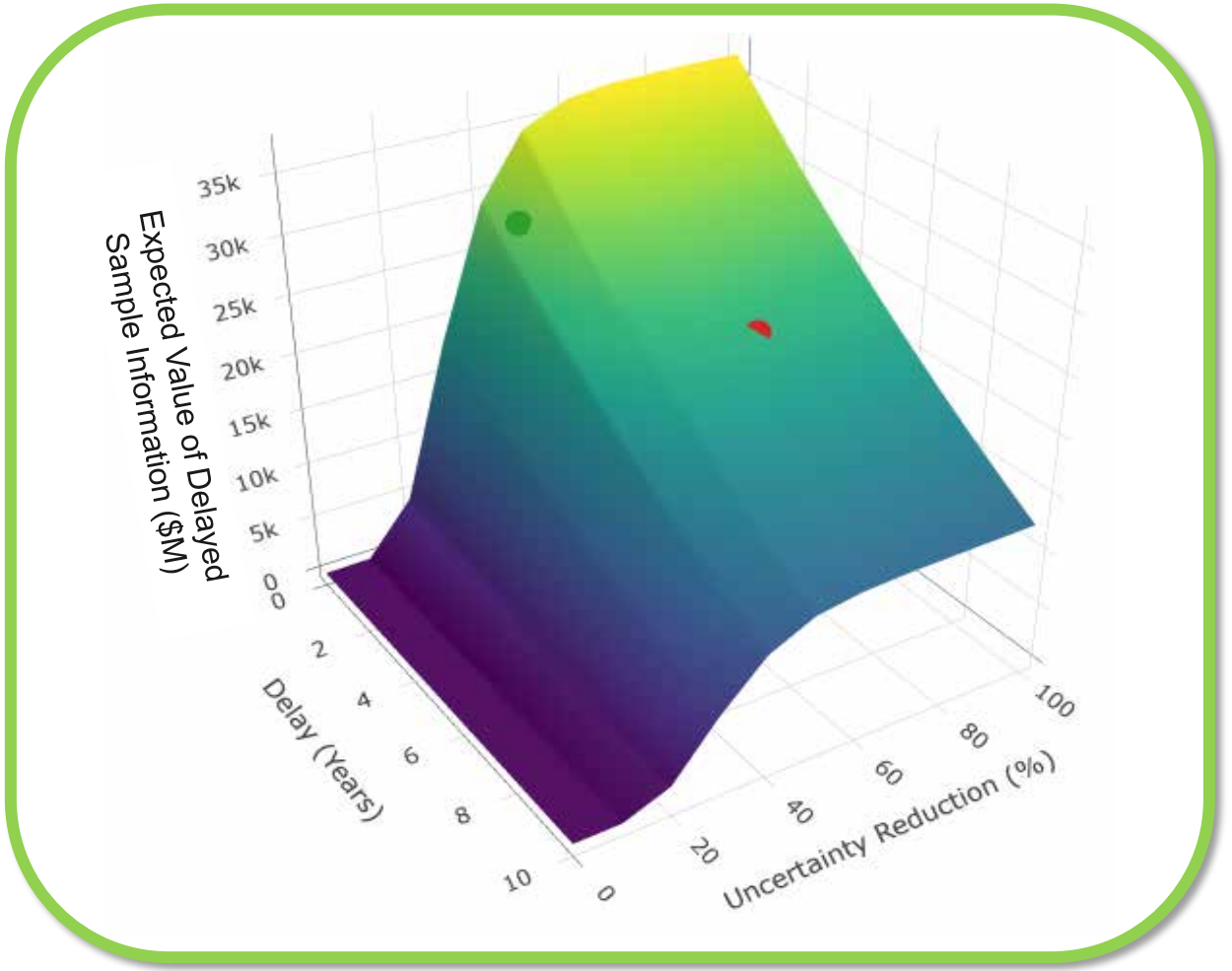
Example Scenarios

- 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.

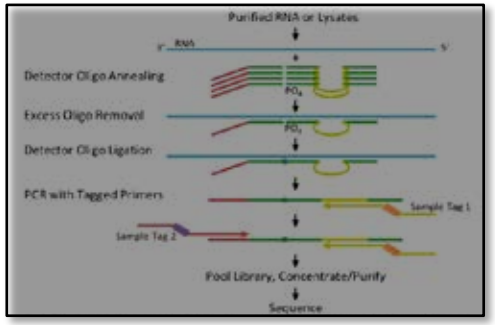
Trade-Offs of Uncertainty and Time of Hypothetical Toxicity Testing Methods
(Chronic Effect, Target Risk Decision Maker)



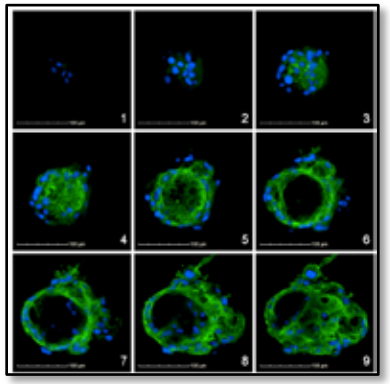
S. Hagiwara, G. Paoli, D. Krewski (RSI)
P. Price, A. Guiseppi-Elie, M. Gwinn, B. Hubbell, R. Thomas (EPA)

EPA continues to innovate and address limitations in NAMs

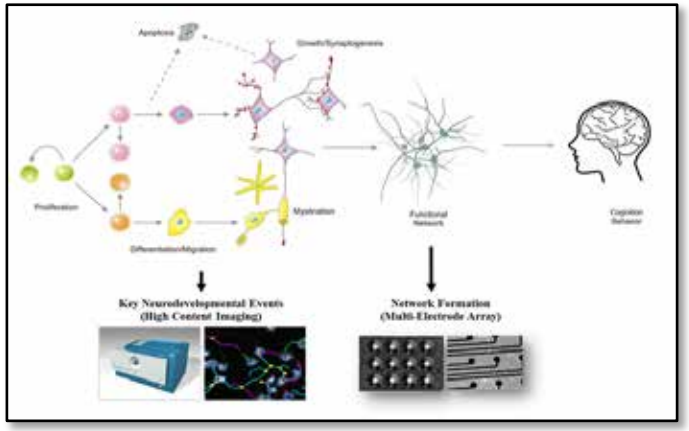
Whole Genome Transcriptomics



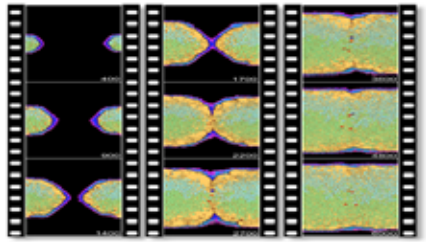
Organotypic Culture Models



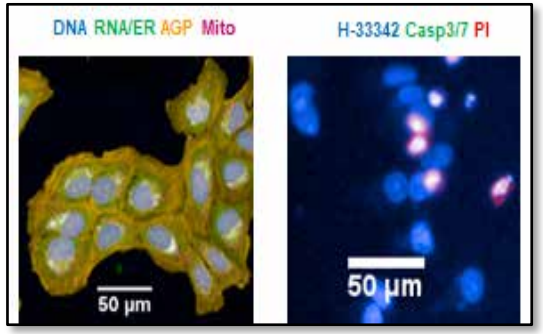
Integrated Approach to Testing and Assessment for DNT



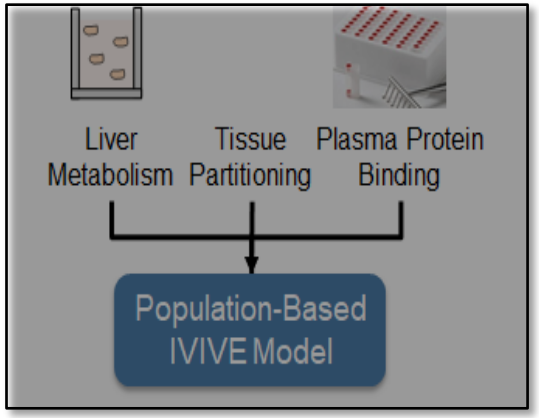
Virtual Tissue Models



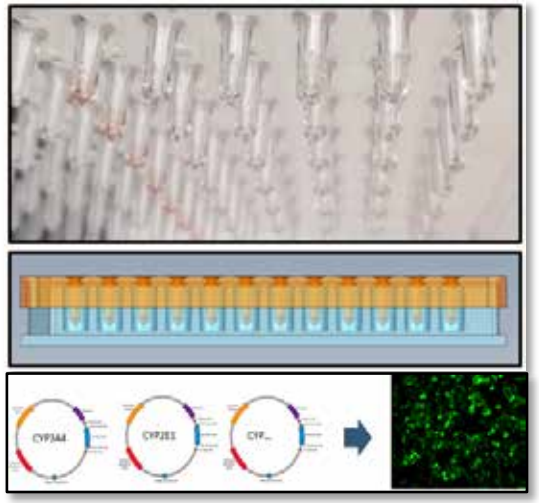
Multi-Parameter Cellular Phenotypic Profiling



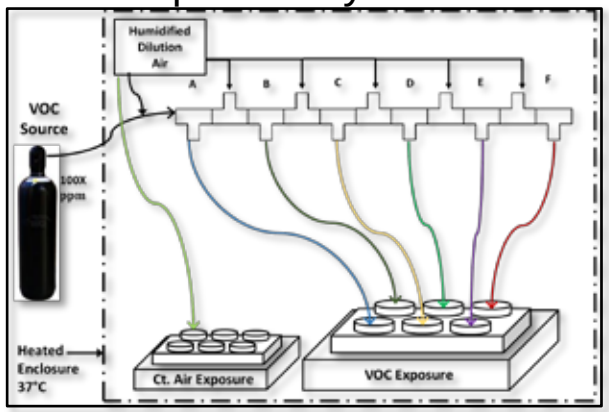
Toxicokinetic Measurements and Modeling



Metabolic Retrofitting

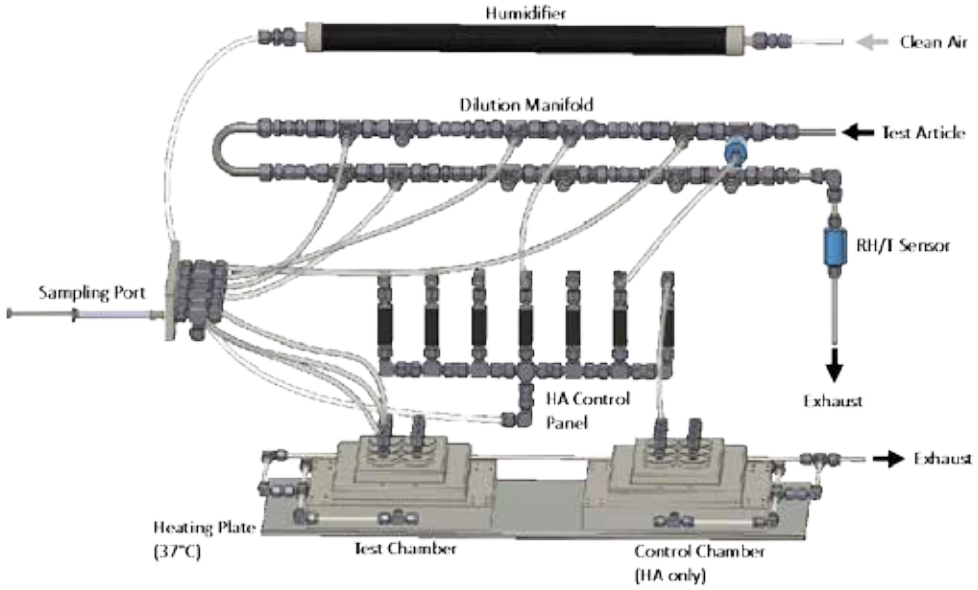


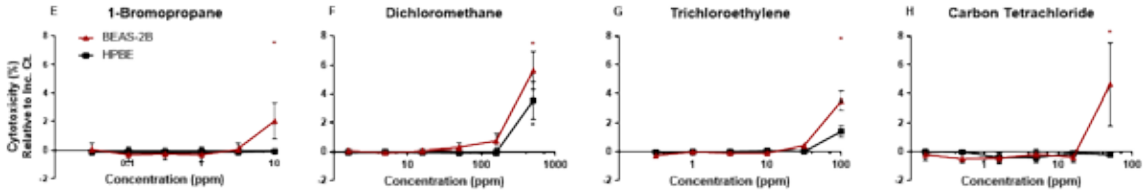
Volatile/Aerosol *In Vitro* Exposure Systems



Sequence Alignment to Predict Across Species Susceptibility

Progress: *in vitro* systems for volatile chemicals





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

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

Benchmark Dose Analysis:

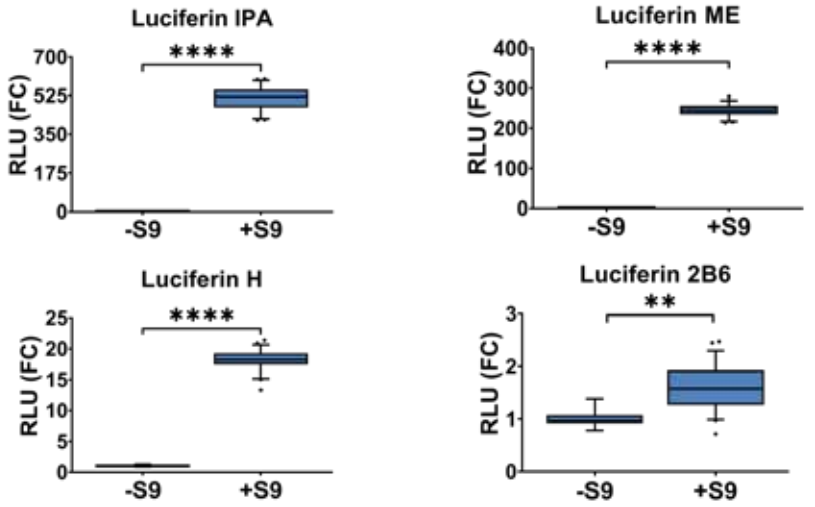
- HTTr TempO-Seq analysis at sub-cytotoxic concentrations
- Comparative to representative in vivo LOAEL/NOAEL values
- Within a magnitude of ACGIH occupational exposure TLVs

Mark Higuchi and colleagues

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

Initiate print | Crosslink on liquid handler | Ready for assay

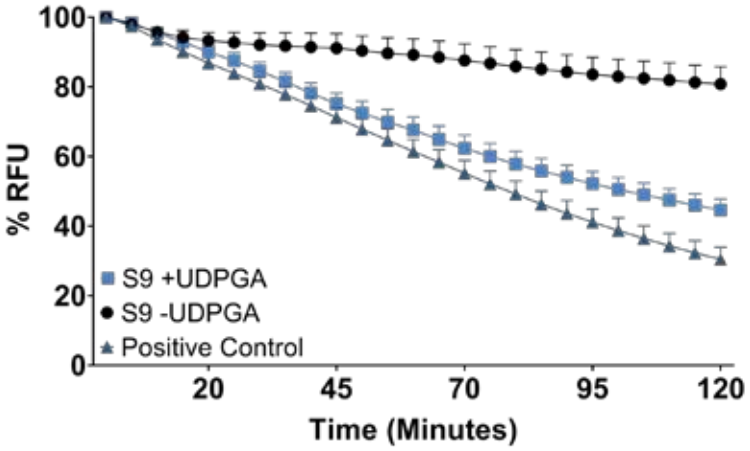
Phase I



Luminogenic CYP Substrates

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

Phase II

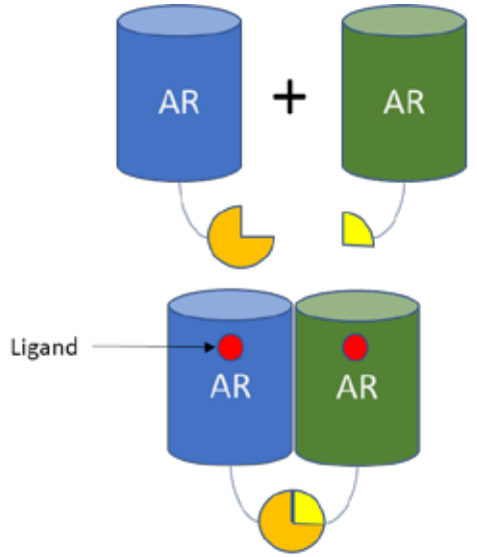


- Goal: Adapt AIME* method to an automated approach using bioprinting for routine application to high-throughput screening.
- The bioprinter method expands the functional capacity for hepatic phase I (CYPs) and phase II (UGTs, SULTs, GSTs) metabolic enzymes.

Kristen Hopperstad, Deisenroth Lab

*AIME: Alginate immobilization of metabolic enzymes

HepG2-AR2 Assay and Metabolic Retrofit

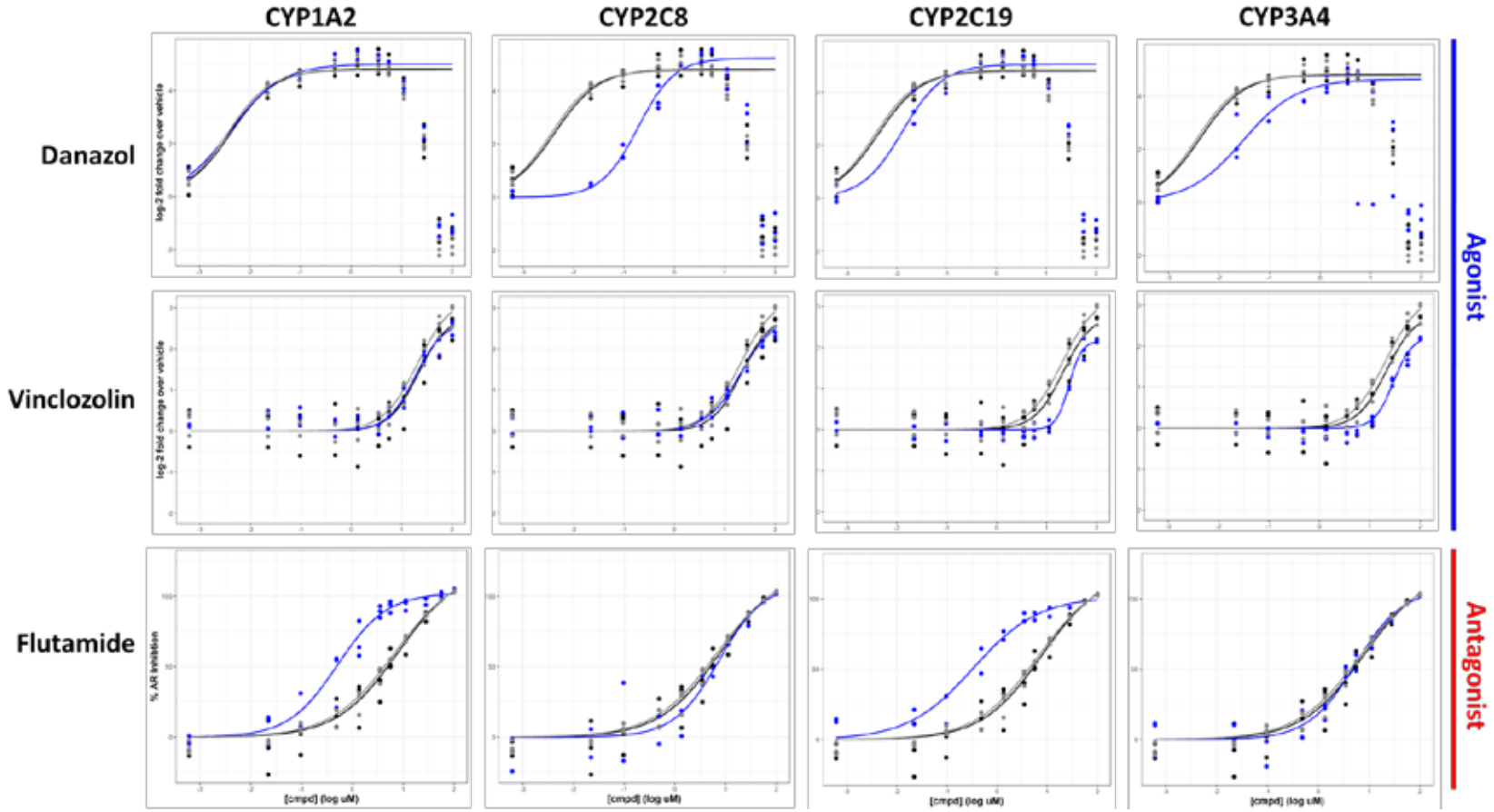



Journal of Pharmacological and Toxicological Methods

Research article

mRNA transfection retrofits cell-based assays with xenobiotic metabolism

Danica E. DeGroot, Adam Swank, Russell S. Thomas, Mark Strynar, Mi-Young Lee, Paul L. Carmichael, Steven O. Simmons



Building test batteries for organ toxicity (DNT)

United States Environmental Protection Agency

Data Integration

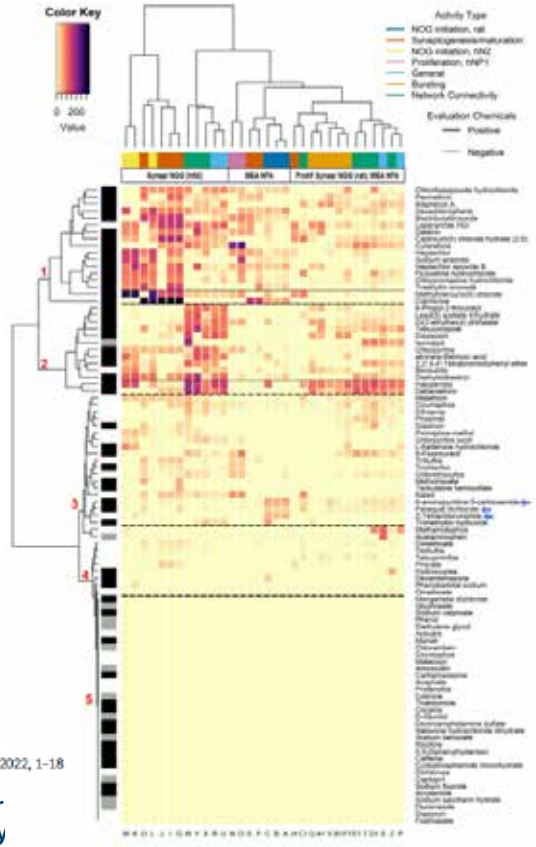
Developmental Neurotoxicity

Genetic Susceptibility

Calculating a selectivity metric at sub-cytotoxic doses is informative for identifying patterns of biological activity.

Integrating Data From *In Vitro* New Approach Methodologies for Developmental Neurotoxicity

Kelly E. Carstens,*,† Amy F. Carpenter,*,† Melissa M. Martin,* Joshua A. Harrill,* Timothy J. Shafer,* and Katie Paul Friedman*,1



TOXICOLOGICAL SCIENCES, 2022, 1–18

Establishing Methods

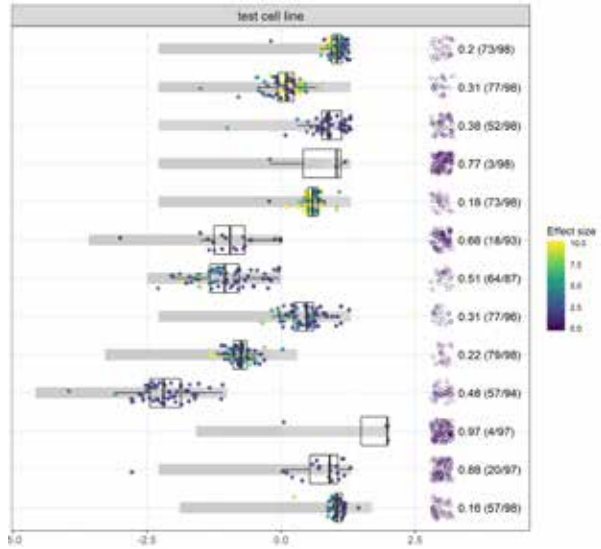
Establishing methods for the community to facilitate assay standardization and adoption.

"Assessment of Larval Zebrafish Locomotor Activity for Developmental Neurotoxicity Screening" in Experimental Neurotoxicology Methods (Stephanie Padilla; July 2021).

"Using Zebrafish to Assess Developmental Neurotoxicity" in Reproductive and Developmental Toxicology (Stephanie Padilla; June 2022).

OECD DNT Expert Group
Guidance on evaluation of data from the developmental neurotoxicity in vitro testing battery (Target 2022 publication)

Genetic diversity across cell lines enables determination of inter-individual variability in biological potency.



Tox21 Cross-Partner Project lead by EPA, NTP, FDA
Cell painting / high content imaging in 98 Diversity Outbred neural progenitor cell lines
[A. Harrill & J. Harrill unpublished data]

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 meet the 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:
- CPHEA
- CEMM
- OCSPP
- OLEM
- Regions

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- NCATS
- Health Canada
- ECHA
- JRC
- EFSA
- A*STAR

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