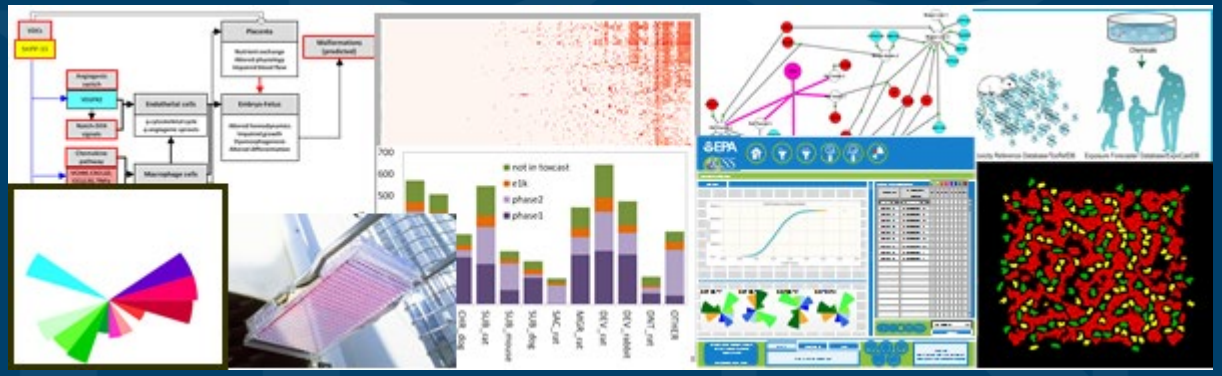
EPA
United States
Environmental Protection
Agency

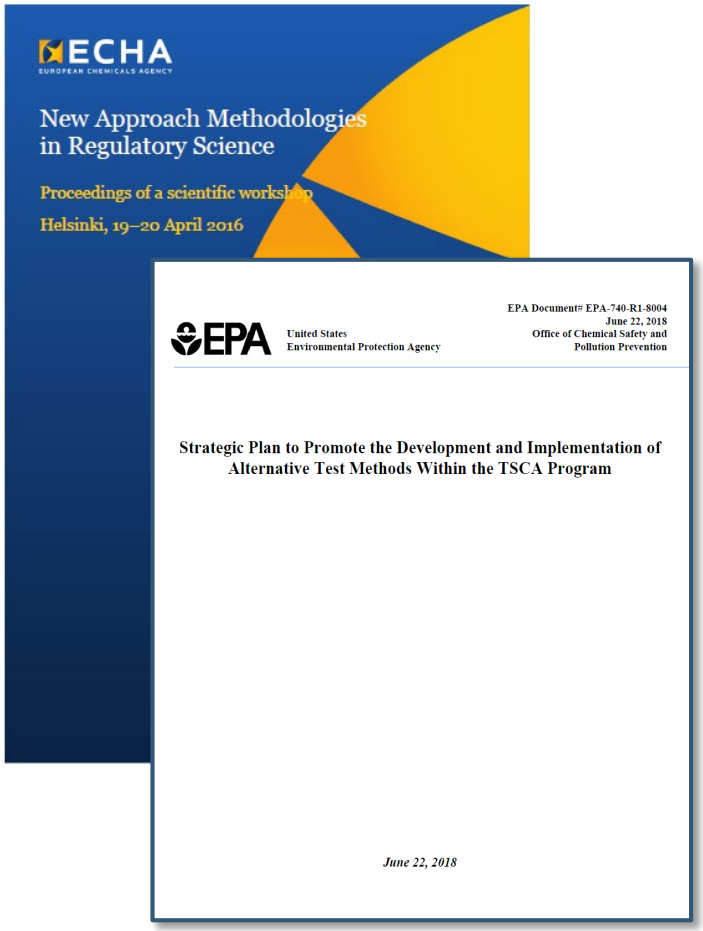
Current Status of New Approach Methodologies



WORKSHOP ON THE DEVELOPMENT OF AN EVIDENCE BASED RISK ASSESSMENT FRAMEWORK
December 17 – 18, 2018

Dr. Maureen R. Gwinn
National Center for Computational Toxicology
Office of Research and Development
US Environmental Protection Agency

New Approach Methodologies (NAMs)



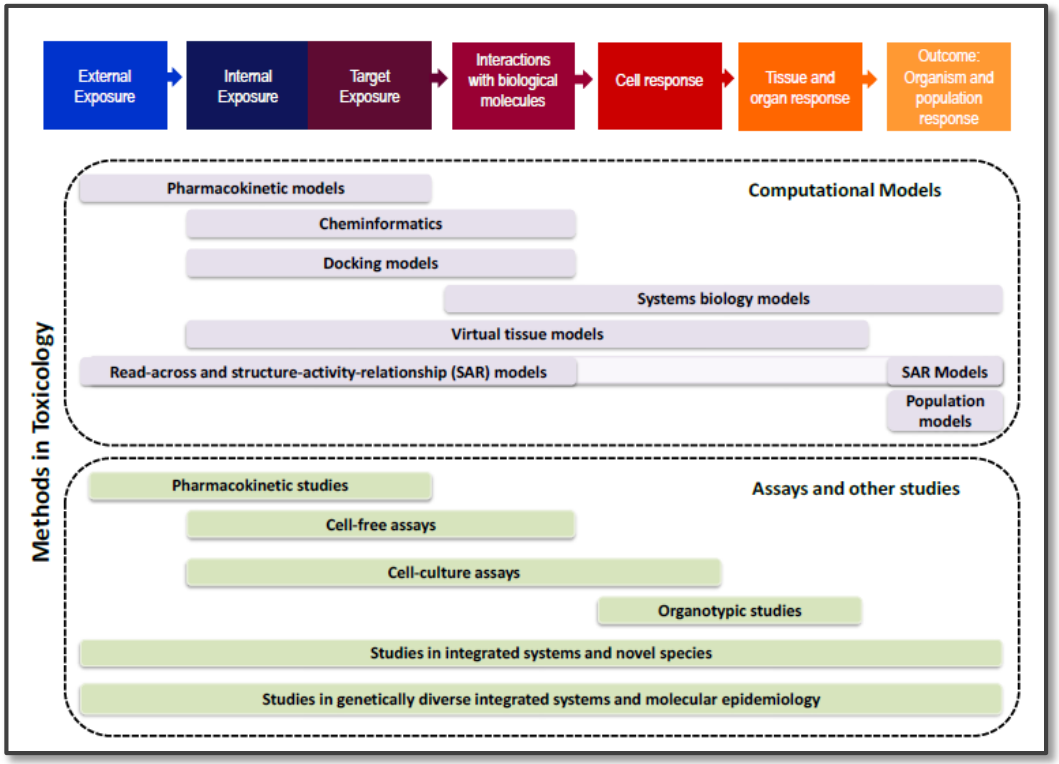
https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf

https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

- Commonly broadly defined to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment.

- Recently defined in the TSCA Alternative Toxicity Strategy as:
  - a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals.

Toxicology Moving to Embrace 21st Century Methods

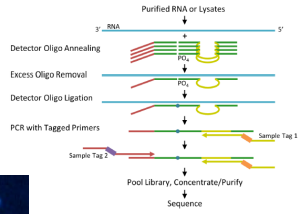


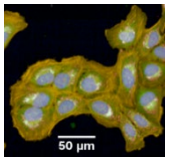
https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations

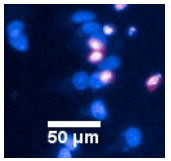
Use of NAMs in Filling Gaps in Hazard and Exposure Information

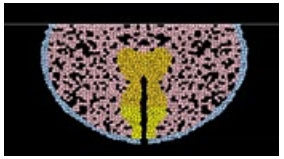
Interactions with biological molecules → Cell response

- Use of comprehensive screening to inform hazard characterization
  - High-Throughput Transcriptomics
  - High-Throughput Phenotypic Profiling
  - High-Throughput Metabolism



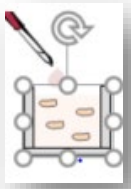


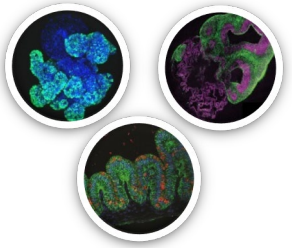




Tissue and organ response → Outcome: Organism and population response

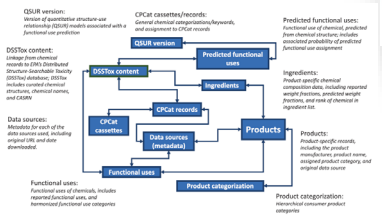
- Higher Tier Adversity
  - Organotypic Cellular Models
  - Virtual Tissue Models
- High-throughput toxicokinetics
  - *In-vitro* studies
  - *In-silico* models and tools

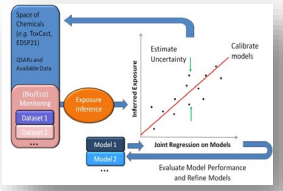




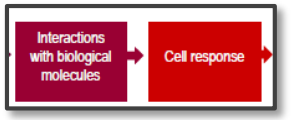
External Exposure → Internal Exposure → Target Exposure

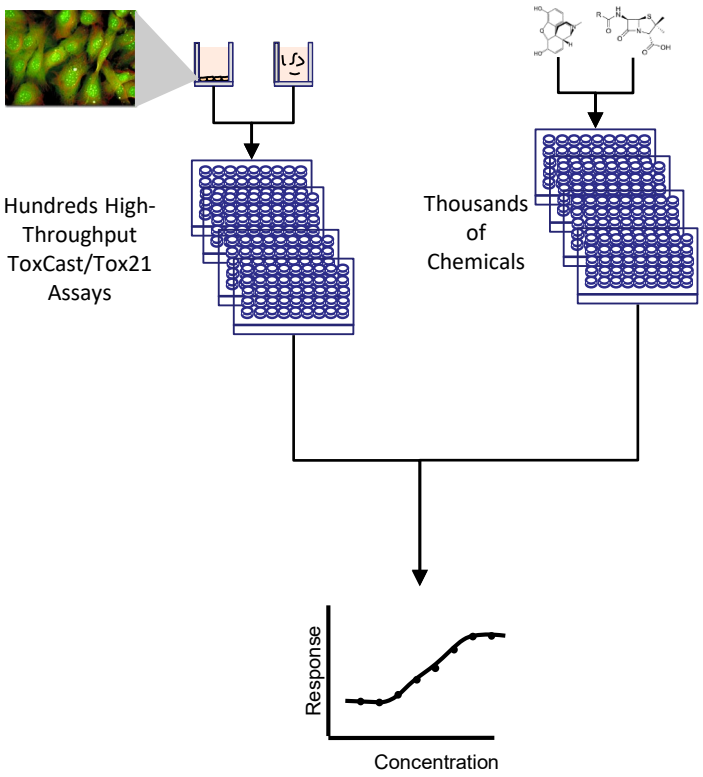
- Use of structure-based machine-learning QSAR models to predict exposure information
  - Functional use
  - Exposure pathways
- Consensus multi-pathway modeling approaches (e.g., ExpoCast SEEM)

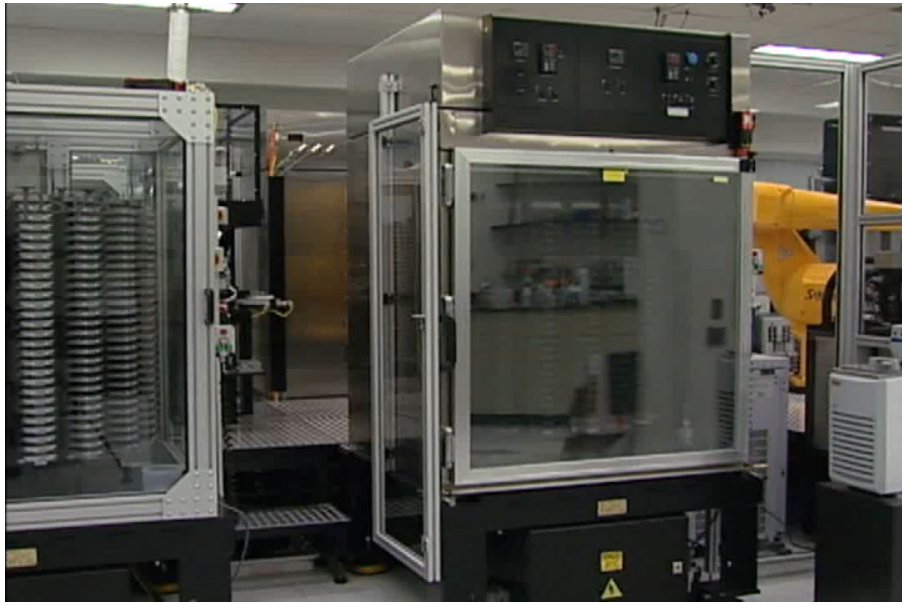




High-Throughput Assays Used to Screen Chemicals for Potential Toxicity

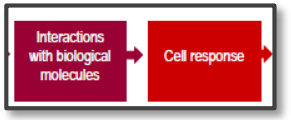




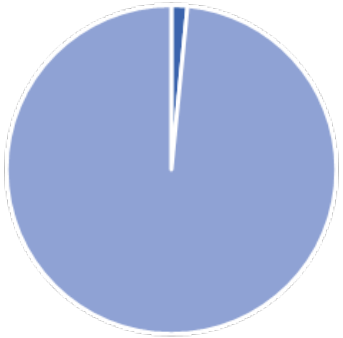


- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

High-Throughput Transcriptomics



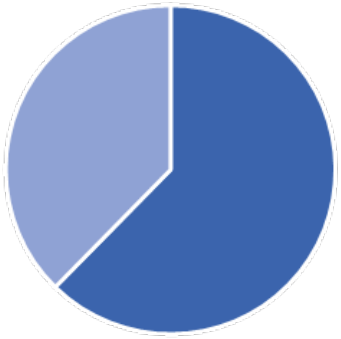
Gene Coverage



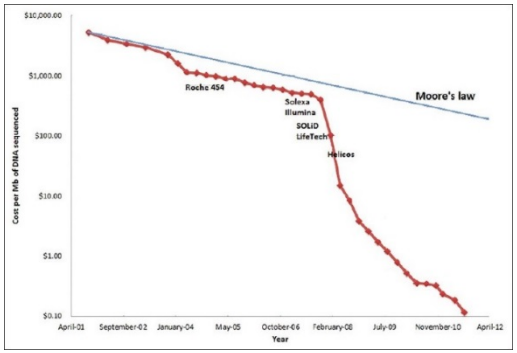
ToxCast

Not in ToxCast

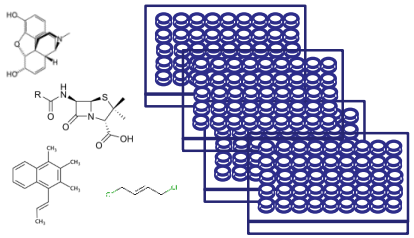
Pathway Coverage*



*At least one gene from pathway represented

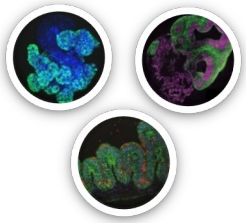


Thousands of chemicals



X

Multiple Cell Types

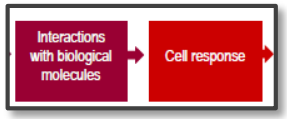


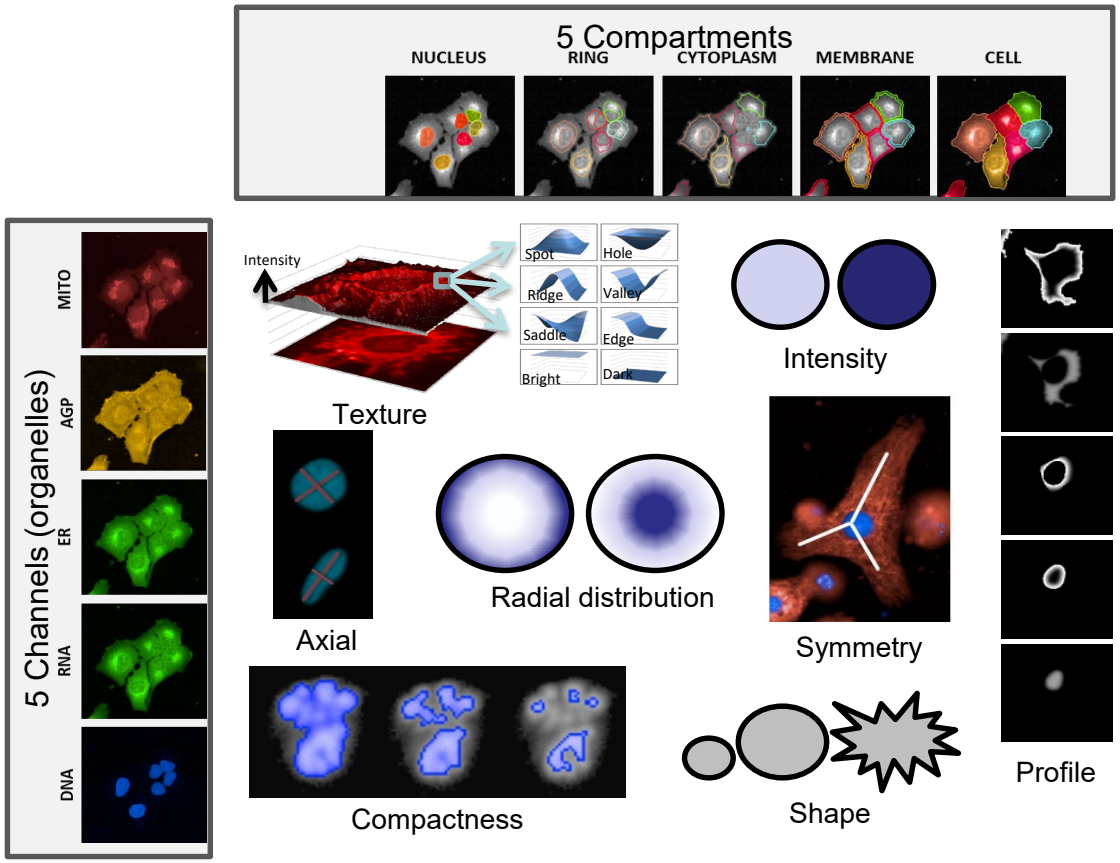
Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

High Throughput Phenotypic Profiling (HTPP)

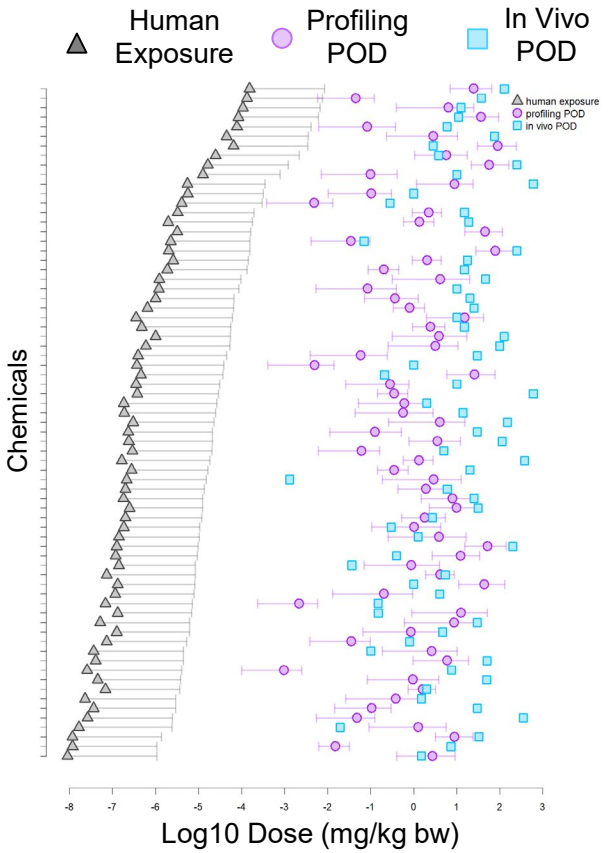
United States Environmental Protection Agency



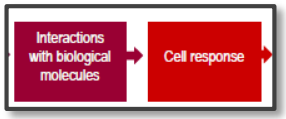


Illustrations from Perkin Elmer

~ 1300 endpoints
(tcpl: "components")

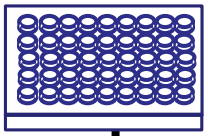


Innovations in Incorporating Xenobiotic Metabolism

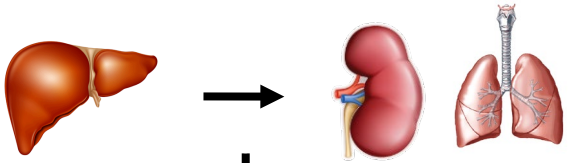


"Extracellular" Approach

Chemical metabolism in the media or buffer of cell-based and cell-free assays

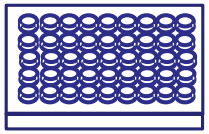


More closely models effects of hepatic metabolism and generation of circulating metabolites

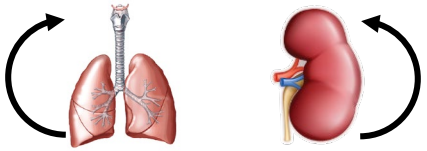


"Intracellular" Approach

Chemical metabolism inside the cell in cell-based assays



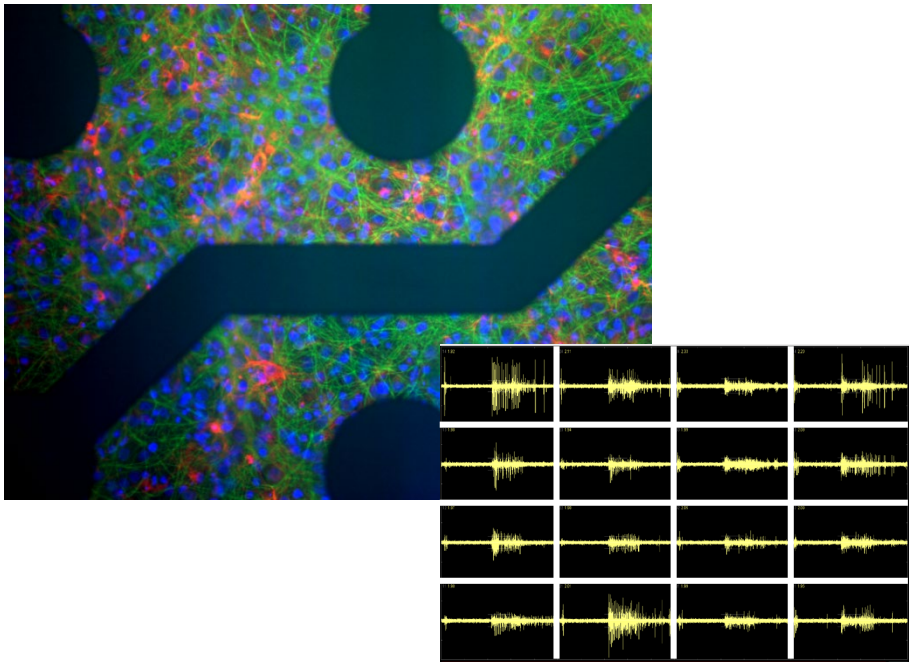
More closely models effects of target tissue metabolism



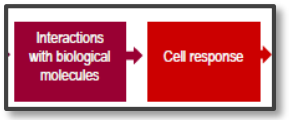
Integrated strategy to model *in vivo* metabolic bioactivation and detoxification

Microelectrode Array (MEA) Assays

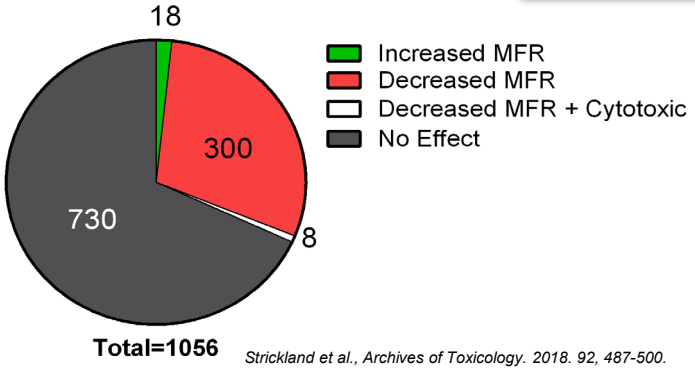
16 microelectrodes/well; Cortical Neurons.



- **Spontaneous** Activity in networks of interconnected neurons
- **Acute** exposure or exposure during **Network Formation** (developmental neurotoxicology (DNT))
- **Medium Throughput** (48-well plates)



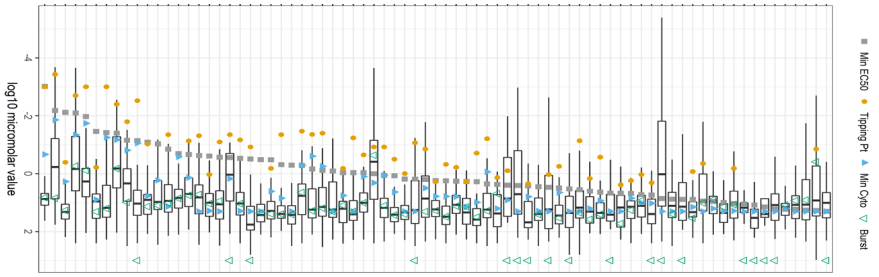
Acute



Strickland et al., Archives of Toxicology. 2018. 92, 487-500.

- Screened 1056 ToxCast Compounds
- Screening APCRA Compounds
- Plans to screen TSCA Compounds

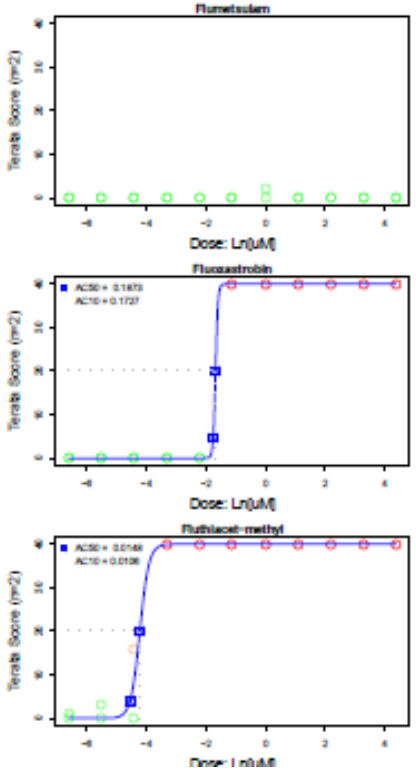
Network Formation

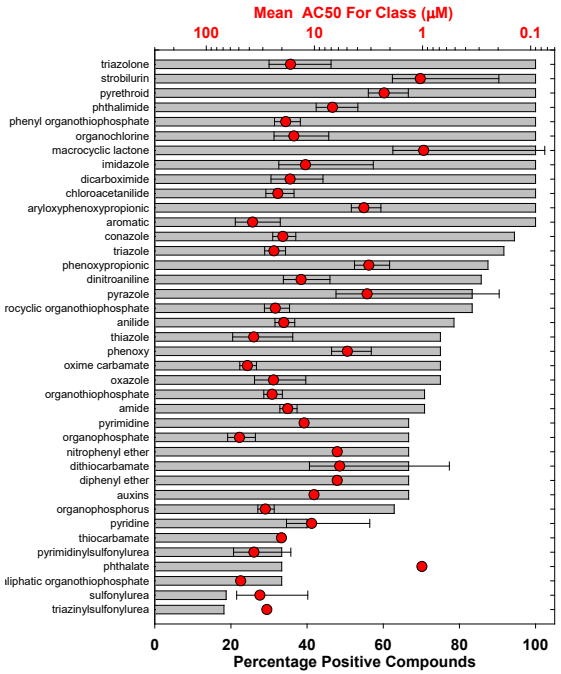


- Screened ~300 Compounds
- Identifies >74% of DNT compounds
- Network Formation is a very sensitive endpoint for some compounds compared to other ToxCast Assays.
- Frank et al., ToxSci, 2017; Toxicol Appl Pharm, 2018.

Zebrafish Model for Developmental and Neural Toxicity

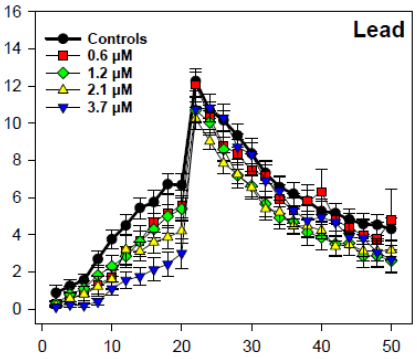
Tissue and organ response





Screening hundreds of chemicals for developmental toxicity

FIG. 41 Comparisons of embryos in three different stages of evolution. Ernst Haeckel, *The Evolution of Man: A Popular Exposition of the Principal Points of Human Ontogeny and Phylogeny*



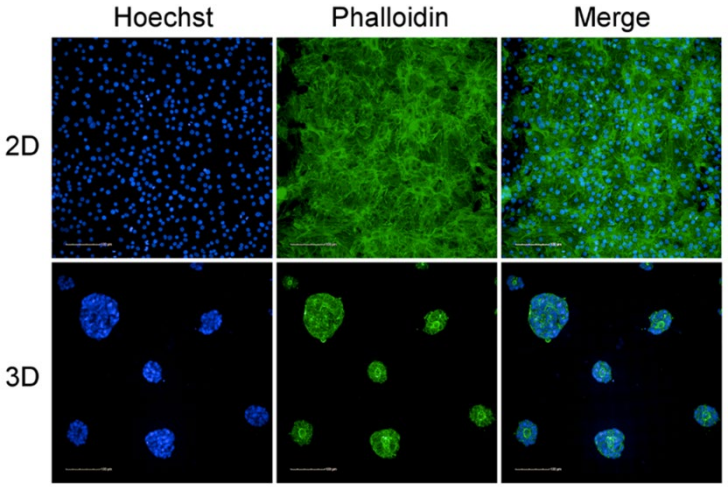
Screening chemicals for NEUROtoxicity

Innovating in Organotypic Culture Models to Predict Tissue Effects

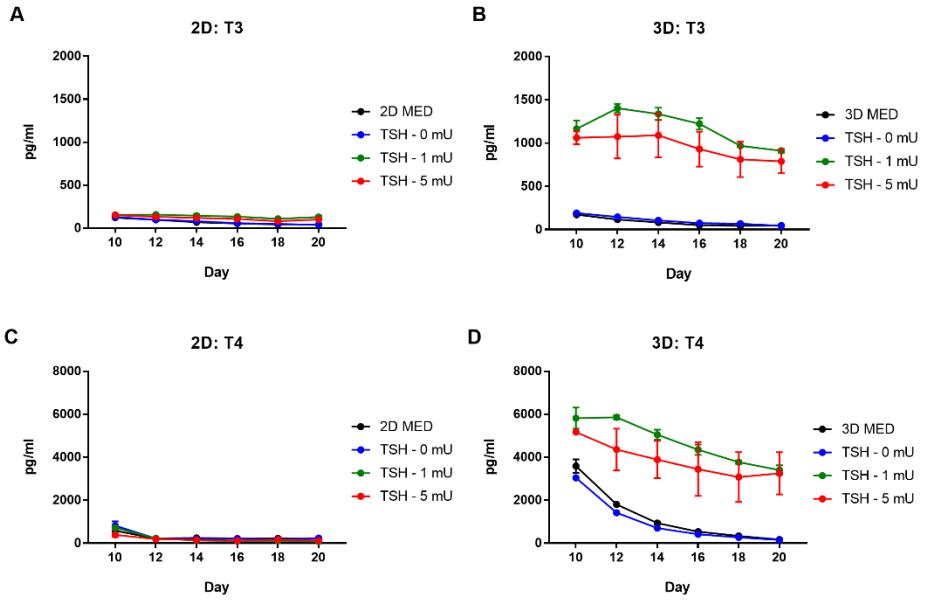
United States Environmental Protection Agency

Tissue and organ response

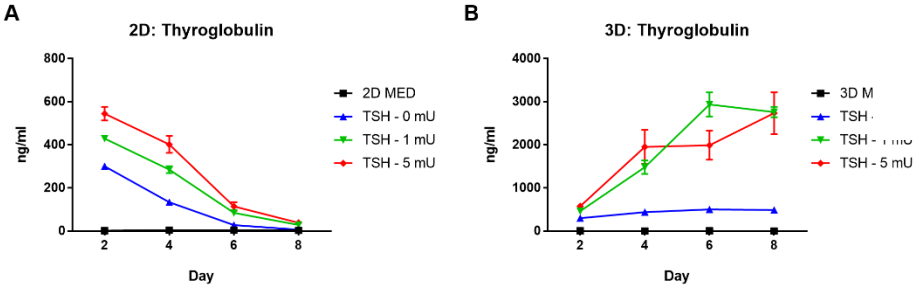
3D Microtissue Model of Primary Human Thyrocytes



Thyroid hormone is synthesized and secreted over time in a 3D culture model

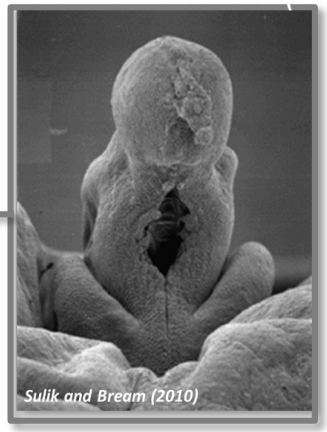


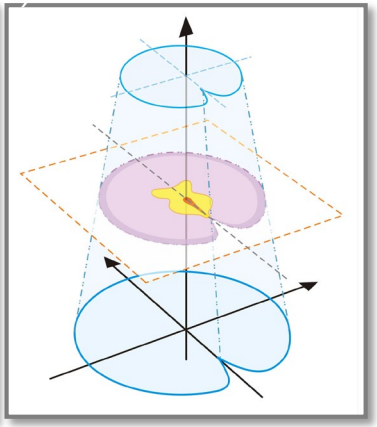
Thyroglobulin secretion is enhanced over time in a 3D culture model

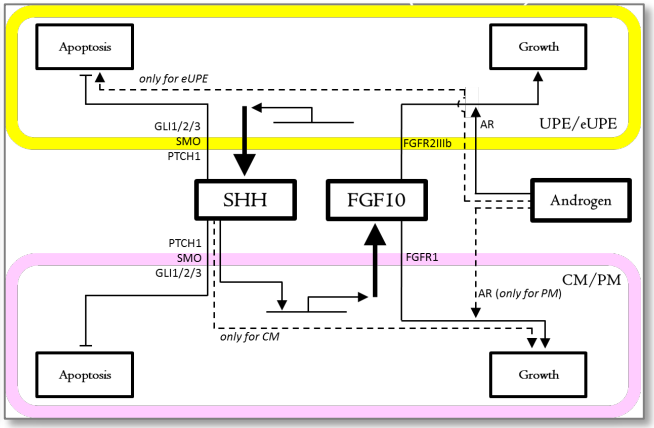


C. Deisenroth, Unpublished

Developing Virtual Tissue Models to Simulate Tissue and Organ Development and Function

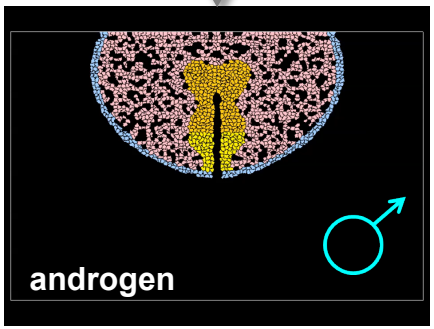


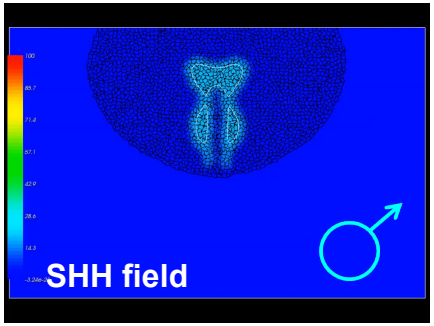


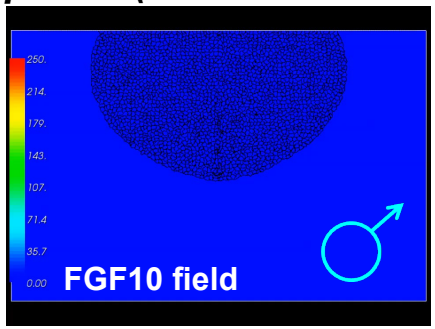


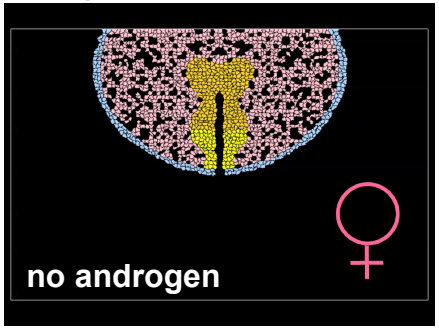
Tissue and organ response

Simulation for sexual dimorphism (mouse GD13.5 – 17.5)





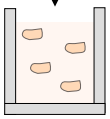




Leung et al., *Repro Toxicol*, 2016

High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals



Human Liver Metabolism

Human Plasma Protein Binding

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through "'httk" R package (https://cran.r-project.org/web/packages/httk/)

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

In Vitro Potency Value

Plasma Concentration

Exposure Route

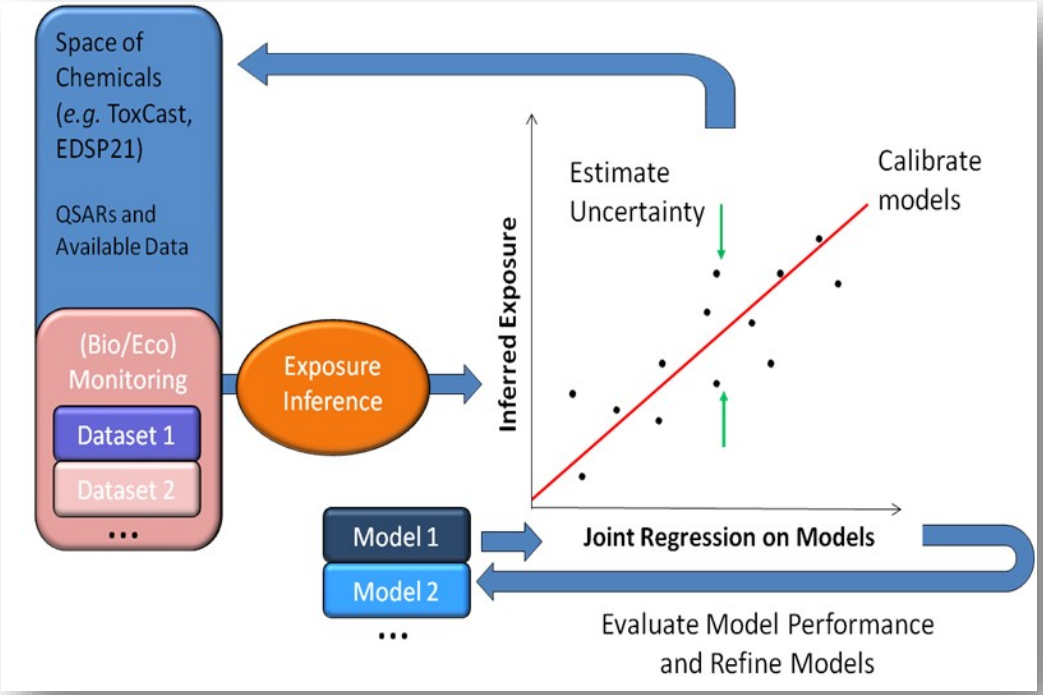
Reverse Dosimetry

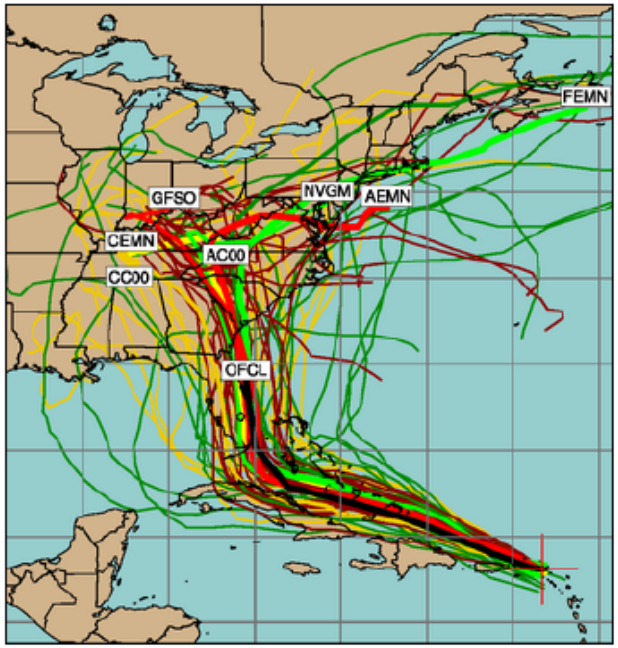
Administered Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

Consensus Exposure Predictions with SEEM Framework

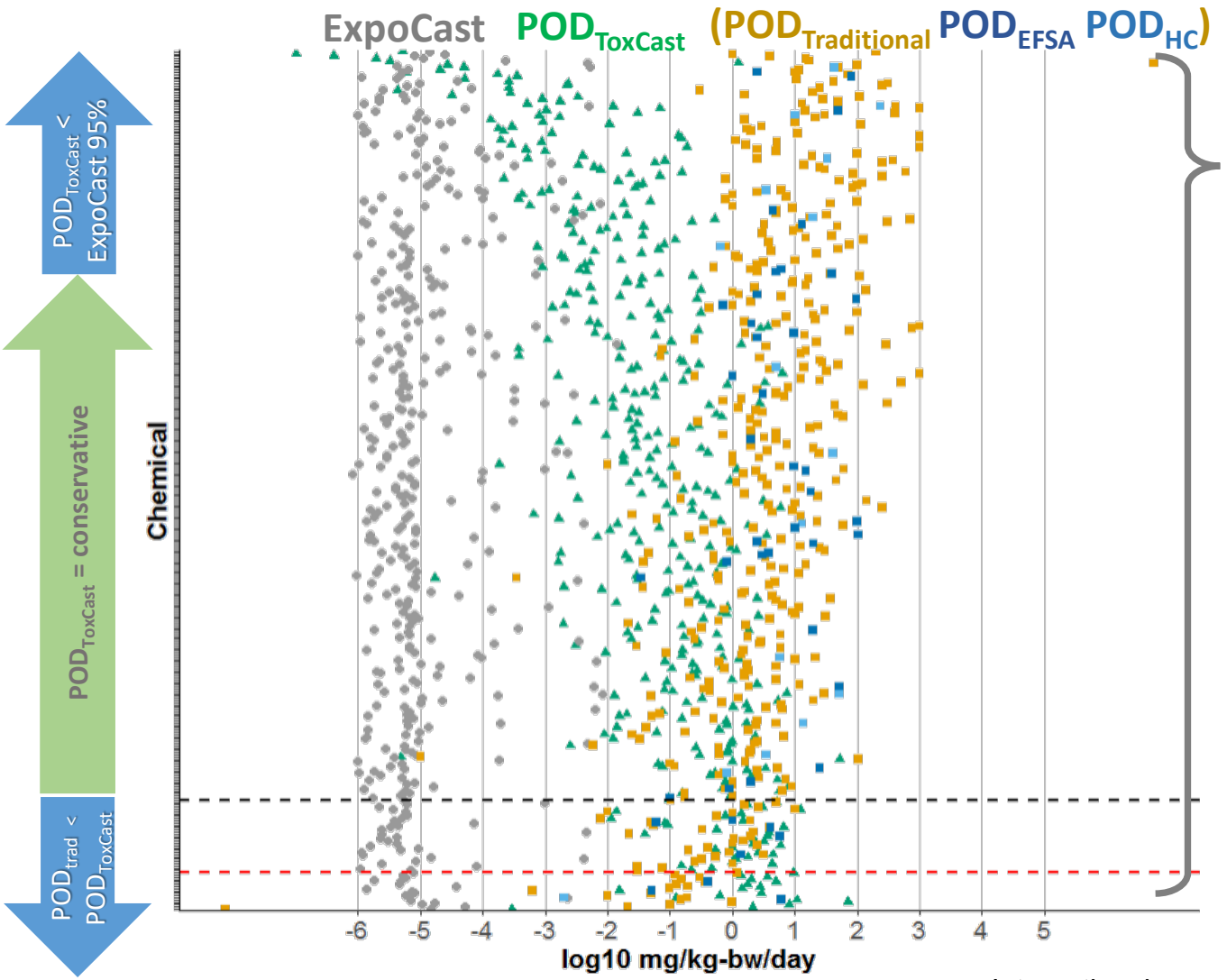
- Different exposure models incorporate knowledge, assumptions, and data (Macleod, et al., 2010)
- We incorporate multiple models into consensus predictions for 1000s of chemicals within the Systematic Empirical Evaluation of Models (SEEM) framework (Wambaugh et al., 2013, 2014; Ring et al., 2018).





Integrating Multiple Models

Results from High Throughput Assays Provide a Conservative Estimate of Adverse Effects



Total =

448 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~92% of the chemicals, $POD_{ToxCast}$ was conservative.

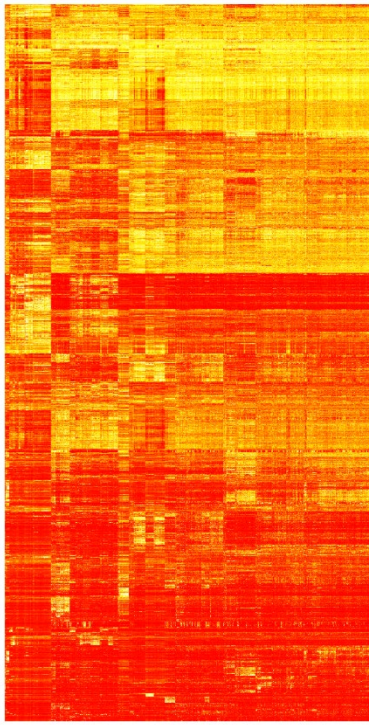
(~100-fold with human HTTK

~50-fold with rat HTTK)

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA

Broad Success Derived from High-Throughput Screening Approaches

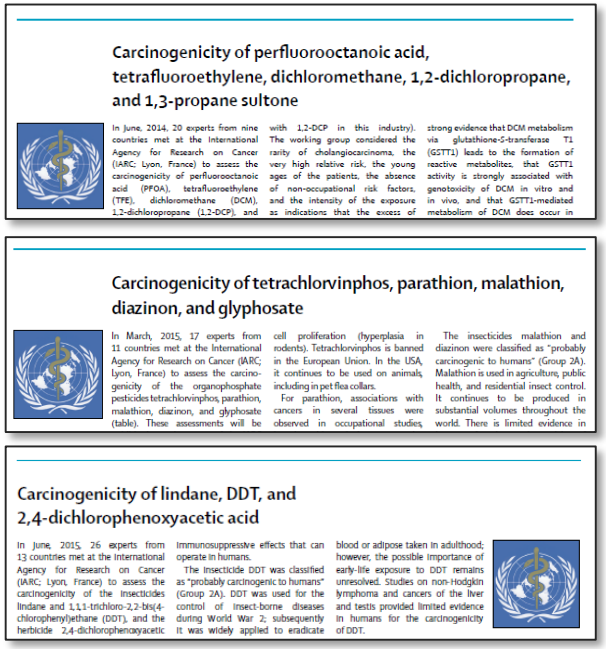
Group Chemicals by Similar Bioactivity and Predictive Modeling



Chemicals

Assays/Pathways

Provide Mechanistic Support for Hazard ID



Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone

In June, 2014, 20 experts from nine countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of perfluorooctanoic acid (PFOA), tetrafluoroethylene (TFE), dichloromethane (DCM), 1,2-dichloropropane (1,2-DCP), and with 1,2-DCP in this industry). The working group considered the rarity of cholangiocarcinoma, the very high relative risk, the young ages of the patients, the absence of non-occupational risk factors, and the intensity of the exposure as indications that the excess of strong evidence that DCM metabolism via glutathione-S-transferase T1 (GSTT1) leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity of DCM in vitro and in vivo, and that GSTT1-mediated metabolism of DCM does occur in

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be cell proliferation (hyperplasia in rodents). Tetrachlorvinphos is banned in the European Union. In the USA, it continues to be used on animals, including in pet flea collars.

For parathion, associations with cancers in several tissues were observed in occupational studies, The insecticides malathion and diazinon were classified as "probably carcinogenic to humans" (Group 2A). Malathion is used in agriculture, public health, and residential insect control. It continues to be produced in substantial volumes throughout the world. There is limited evidence in

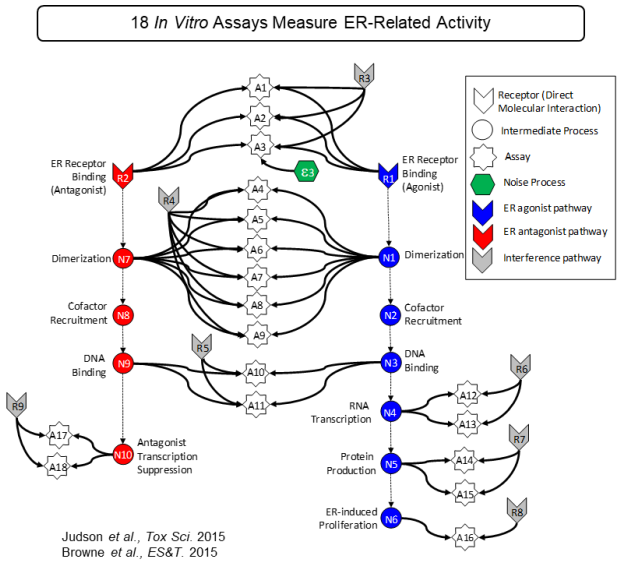
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

In June, 2015, 26 experts from 13 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the insecticides lindane and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), and the herbicide 2,4-dichlorophenoxyacetic Immunosuppressive effects that can operate in humans.

The insecticide DDT was classified as "probably carcinogenic to humans" (Group 2A). DDT was used for the control of insect-borne diseases during World War 2; subsequently it was widely applied to eradicate blood or adipose taken in adulthood; however, the possible importance of early-life exposure to DDT remains unresolved. Studies on non-Hodgkin lymphoma and cancers of the liver and testis provided limited evidence in humans for the carcinogenicity of DDT.

IARC Monographs 110, 112, 113

Prioritization of Chemicals for Further Testing



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

EPA United States Environmental Protection Agency

Conclusions

- Incorporating new technologies and innovations in toxicology can more rapidly and inexpensively screen chemicals for potential adverse biological effects
- Incorporating dosimetry and exposure provides an important dose and exposure context
- Comparisons of high-throughput *in vitro* results with traditional animal tests suggests that the *in vitro* results generally provide a conservative estimate of *in vivo* effect levels for general toxicity
- Uncertainty analysis of NAMs is an ongoing part of research and development of these new technologies
- Data management systems and decision support tools will be increasingly important for interpreting and integrating the expanding and diverse landscape of chemical safety information for use in weigh-of-evidence decisions

EPA United States Environmental Protection Agency

Thank You for Your Attention!

Tox21 Colleagues:
- NTP Crew
- FDA Collaborators
- NCATS Collaborators

EPA Colleagues:
- NERL
- NHEERL
- NCEA

EPA's National Center for Computational Toxicology