Government of Canada | Gouvernement du Canada

Regulatory Use of Bioactivity-Exposure Ratios for Priority-Setting and Chemical Risk Assessment

Humane Society International
Webinar on Risk Assessment
July 13, 2022

Source: Al-Koshi Cleaning Chemicals

Tara Barton-Maclaren, *PhD, Health Canada*

Canada

LEGISLATIVE & REGULATORY CONTEXT

CANADIAN ENVIRONMENTAL PROTECTION ACT, 1999 (CEPA)

- CEPA provides the framework for the identification, prioritization, and assessment of new and existing substances and for the control or management of those considered to pose a risk.
- This framework is broad, open, transparent and evidence-based, taking into account aspects (i.e., exposure and effects) of a substance related to the potential risk it may pose, and it builds upon work done in other jurisdictions.
- CEPA defines substances very broadly, including:
  - chemicals, polymers, biochemicals, biopolymers, nanomaterials, products of biotechnology, air pollutants and GHGs.

CEPA Review:

- Section 343 of CEPA provides for a parliamentary review of the administration of the Act every five years
- June, 2017: ENVI Committee Report: "Healthy Environment, Healthy Canadians, Healthy Economy: Strengthening the Canadian Environmental Protection Act, 1999"
- June, 2018: Follow-up report to the Standing Committee on the Canadian Environmental Protection Act
- **2021-ongoing: Bill S-5, Act to amend CEPA** (Strengthening Environmental Protection for a Healthier Canada Act)

Canada | Prime Minister of Canada Justin Trudeau

Minister of Health Mandate Letter

December 16, 2021

- ……
- To protect Canadians from harmful chemicals, strengthen the *Canadian Environmental Protection Act*, introduce mandatory labelling of chemicals in consumer products, *introduce legislation to end testing on animals*, increase testing of products for compliance with Canadian standards, and implement an action plan to protect Canadians, including firefighters, from exposure to toxic flame retardants found in household products.
- ……

[Minister of Health Mandate Letter (pm.gc.ca)]

February 9, 2022

KEY THEMES:

- A Right to a Healthy Environment
- Protecting Vulnerable Populations
- Assessing Real Life Exposures
- Supporting the Shift to Safer Chemicals
- Increased Transparency in Decision-Making
- Reducing Reliance on Animal Testing
- Informing Canadians of Risks (e.g. Labelling)

Full summary available [here]

Legislative & Regulatory Context

Opportunities for New Approach Methods (NAM) to effectively support risk assessment is dependent on requirements

Toxicology Studies	Pesticides – PCPA (Possible requirements depending on use category)	New Substances CEPA / New Substances Notification Regulations	Existing Substances CEPA - Industrial Chemicals
Acute Toxicity (oral, dermal, inhalation)	X	X	-
Eye / Dermal Irritation, Dermal Sensitization	X	X	-
Repeated Dose Toxicity 28-day (oral, dermal)	X	X (~90% OECD GD 407) *	-
Subacute Inhalation Toxicity 28-day	X	X	-
Repeated Dose Toxicity 90-day (oral, dermal, inhalation and/or 12-month dog for oral)	X	X (6-7% OECD GD 408) *	-
Combined Repeated Dose Toxicity with Reproduction/Developmental Toxicity Screening Test	X	X (3-4% OECD 422) *	-
Subchronic Toxicity 90-day (dermal, inhalation)	X		-
Chronic Toxicity (rodent)	X		-
Oncogenicity (two rodent species)	X		-
Combined Chronic Toxicity / Oncogenicity (rodent)	X		-
Multigeneration Reproductive Toxicity (rodent)	X		-
Prenatal Developmental Toxicity (rodent and non-rodent)	X		-
Genotoxicity (various in vitro, in vivo studies)	X	X	-
Metabolism/Toxicokinetcs in mammals	X		-
Acute delayed Neurotoxicity (hen); 28-day Delayed Neurotoxicity (hen)	X		-
Acute Neurotoxicity (rat)	X		-
90-day Neurotoxicity (rat)	X		-
Developmental Neurotoxicity	X		-

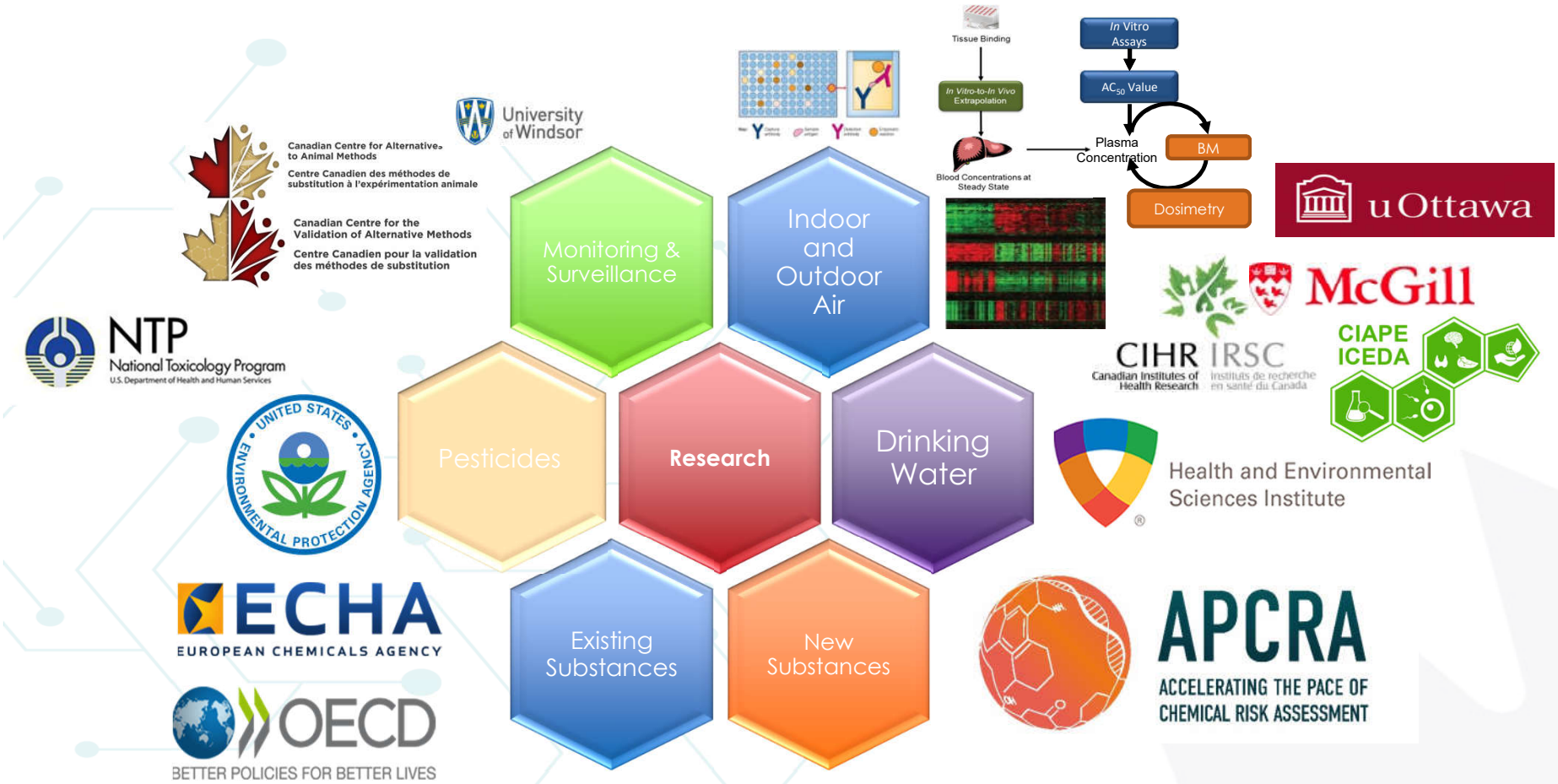
* Schedule 6 & 11 (>10 000 kg)

Common Goals

- Protect human health & the environment
- Reduce · Replace ·Refine approaches to animal testing
- Promote acceptance of NAM in fit-for-purpose uses
- Partnerships and collaborations to increase alignment
- Case examples to build confidence
- Gain experience through evidence integration, IATA, DAs, etc.
- Establish best practices and guidance (e.g. OECD)

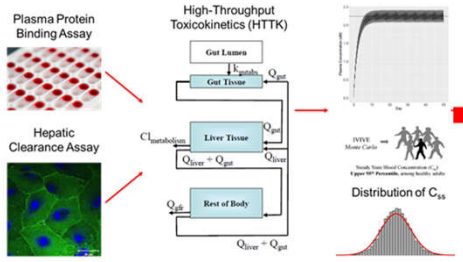
Optimize pace, accuracy and efficiency of risk assessment

PARTNERSHIPS & ENGAGEMENT ARE CRITICAL FOR MODERNIZATION



INNOVATE & ACCELERATE THE USE OF NAM: DEVELOPING FIT-FOR-PURPOSE APPROACHES

CONVERTING IN VITRO CONCENTRATIONS TO HUMAN RELEVANT DOSES



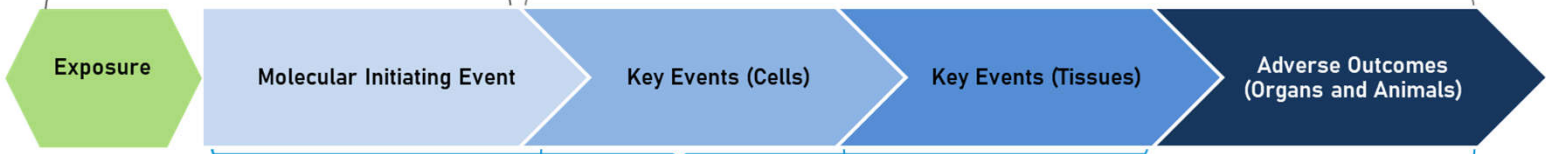
TOXICOKINETICS

TOXICODYNAMICS

ADMINISTERED EQUIVALENT DOSE (AED) MG/KG-BW/DAY

Compare to Human Exposure Estimates to Assess Potential Risk (i.e. Bioactivity Exposure Ratio [BER])

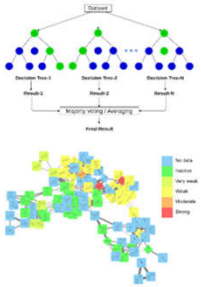
ADVERSE OUTCOME PATHWAY (AOP)



in silico
in chemico

in vitro

in vivo



MACHINE LEARNING TECHNIQUES

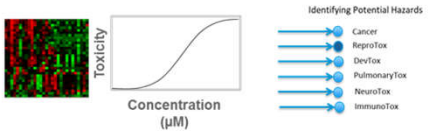
- Predictive models, Artificial Intelligence (AI)
- Natural Language Processing

NETWORK SIMILARITY MODELS

Identify clusters of substances with the potential for toxicity to prioritize substances with limited or no toxicity information

USE OF HIGH THROUGHPUT/CONTENT CELL-BASED ASSAYS

- Targeted in vitro assays, Transcriptomics assays
- Protective & predictive approaches



RESEARCH-REGULATORY COLLABORATION Case Study

EXPLORING COMPUTATIONAL AND NOVEL TESTING STRATEGIES ACROSS LEVELS OF BIOLOGICAL ORGANIZATION TO DEVELOP INTERPRETATION AND APPLICATION APPROACHES TO UNDERSTAND THE DATA IN REGULATORY USE CONTEXTS

Translating Case Study Findings

- Methodology informed by proof of concept research
- Building confidence in the science

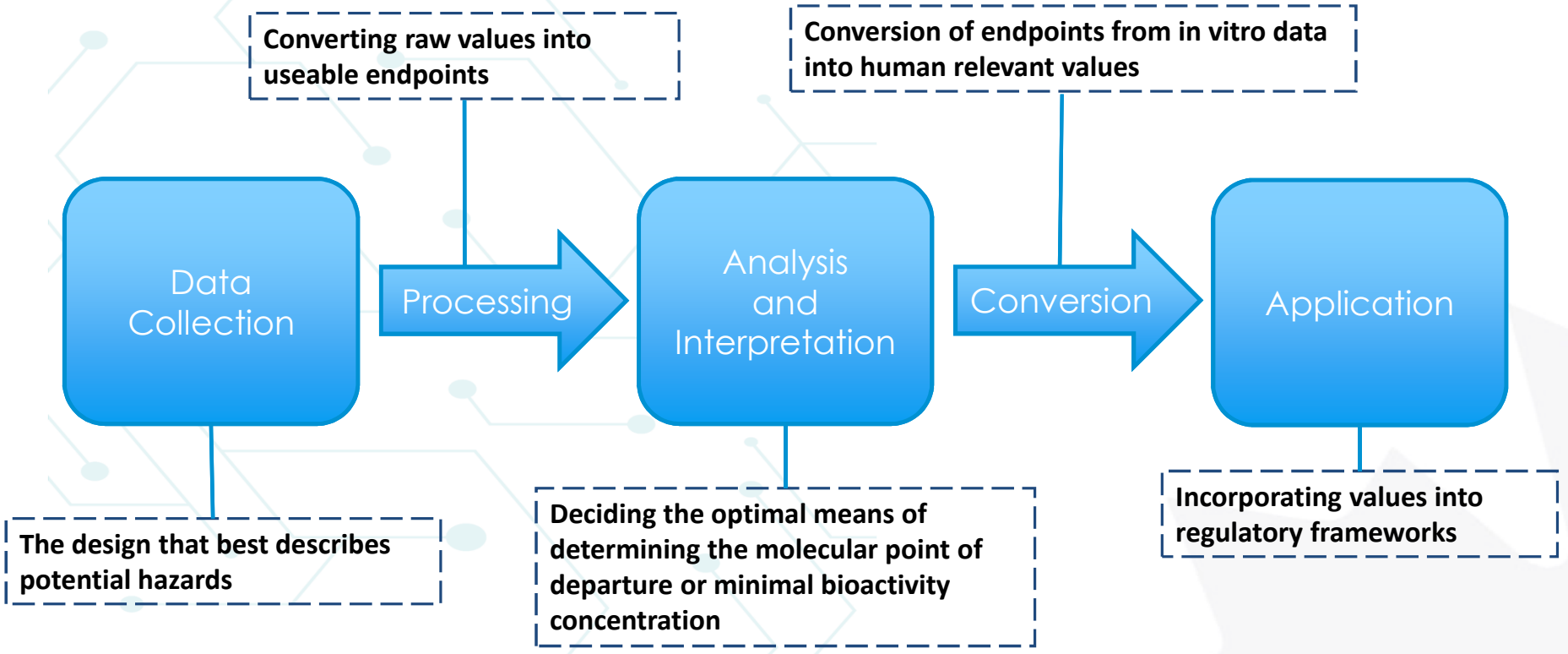
- Lessons learned
- Develop best practices & guidance

Science Approach Documents

Regulatory Acceptance

Published SciADs:
https://www.canada.ca/en/health-canada/services/chemical-substances/science-approach-documents.html

In Vitro Case Study Findings to Establish Human Relevant Endpoints



Case Study: Bioactivity Exposure Ratio (BER)Application in Priority Setting and Risk Assessment

In Vitro Bioactivity as a Conservative Point of Departure: A retrospective Case Study

- A quantitative risk-based approach to identify substances according to their level of concern to human health (low vs. high)
- Considers high-throughput *in vitro* bioactivity together with high-throughput toxicokinetic modelling to derive in vitro points of departure using ToxCast data
- Does the in vitro POD serve as a protective surrogate in the absence of traditional toxicological data

OXFORD SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight article

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
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 *

(Paul-Friedman et al., 2020)

Case Study: Bioactivity Exposure Ratio (BER)Application in Priority Setting and Risk Assessment

US EPA ToxCast/Tox21

- Assays representing targets and pathways associated with toxicity

Endpoint extraction

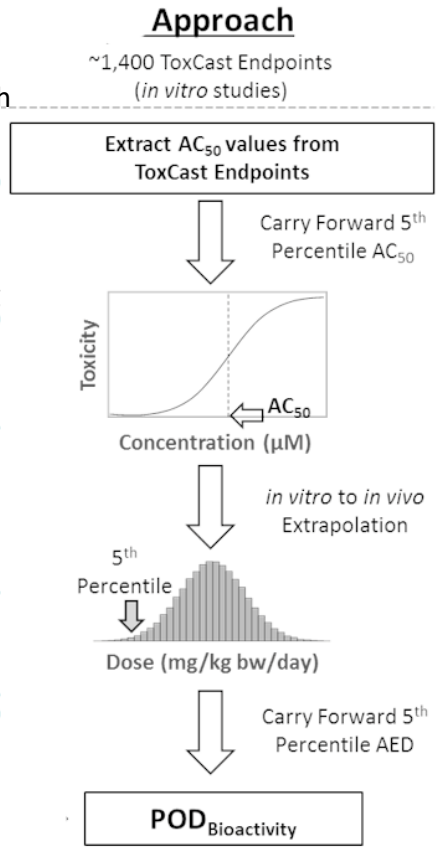
- The 5th percentile the AC50 values from all assays meeting predetermined criteria

Endpoint Conversion (IVIVE)

- Using HTTK R-based package
- µM → mg/kg/bw-day

POD determination

- The 5th percentile of all outcomes from a specific chemical



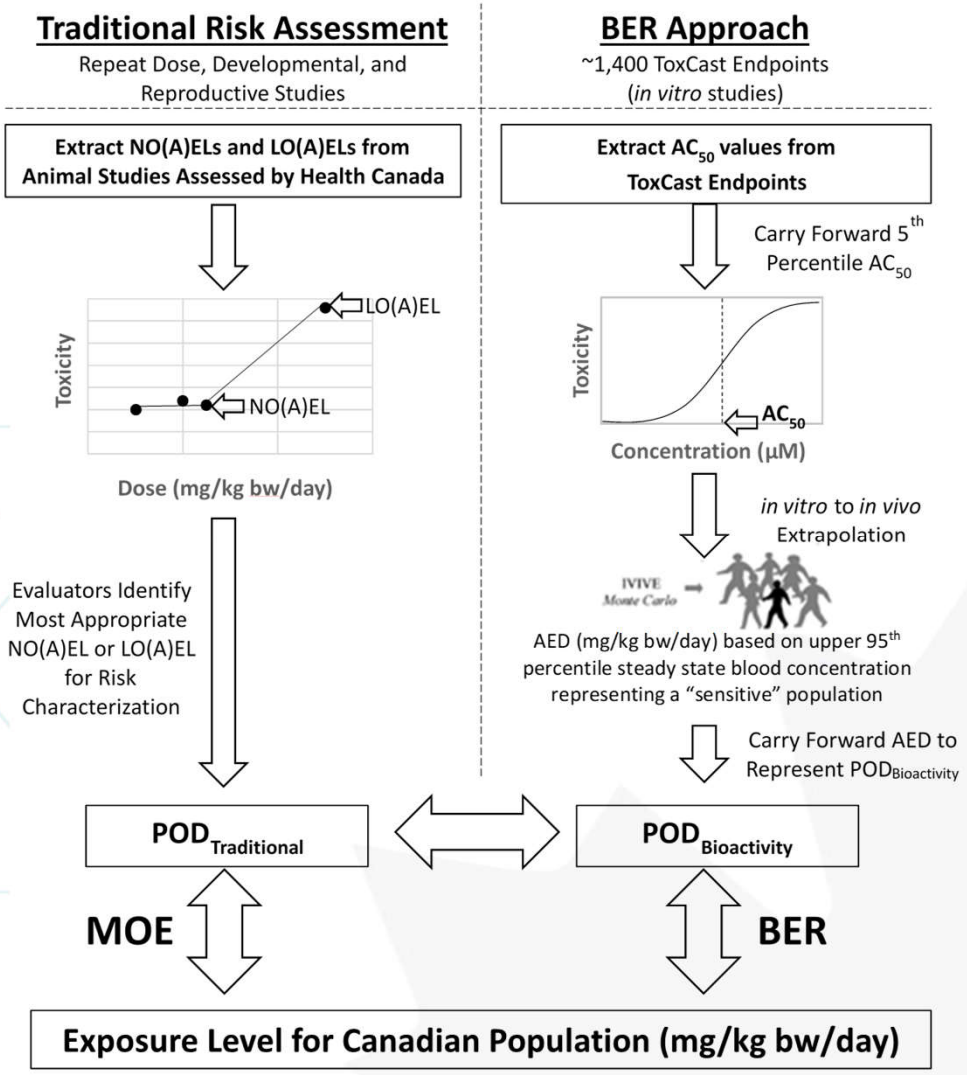
For the vast majority of chemicals the in vitro values from ToxCast represents a lower (i.e., more conservative) endpoint when compared to the POD derived from in vivo data

Science Approach Document March 2021

Health Canada SciAD Presents Comparative Analysis

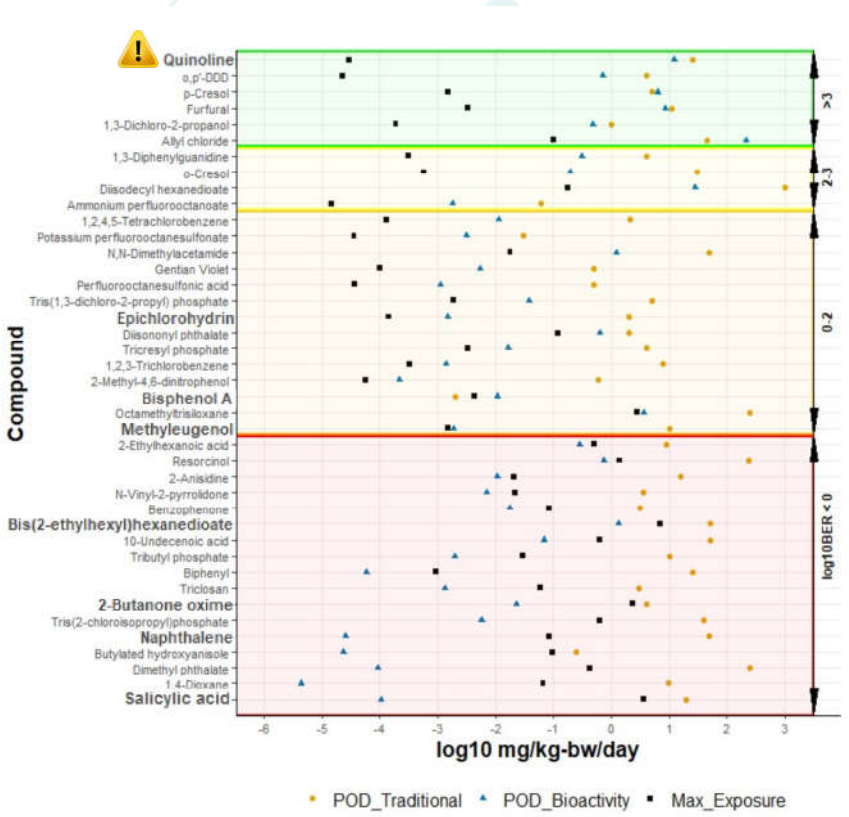
[Link to BER SciAD]

- $POD_{Bioactivity}$ values were derived for 40+ chemicals previously assessed under the Chemicals Management Plan (CMP)
- Compared $POD_{Bioactivity}$ to POD used for risk characterization as well as lowest POD identified in the Screening Assessment Report (SAR)
- $POD_{Bioactivity}$ also compared to Canadian exposure values from biomonitoring data, environmental media, and consumer products to derive Bioactivity Exposure Ratios (BERs)
- BERs were evaluated to assess the utility of bioactivity data in prioritizing chemicals for risk assessment



Establishing Toxicological Relevance

Bioactivity Exposure Ratio (BER) – Example from SciAD



Log BER

> 3 → Low concern

2 - 3 → Moderate concern

0 - 2 → Moderate concern

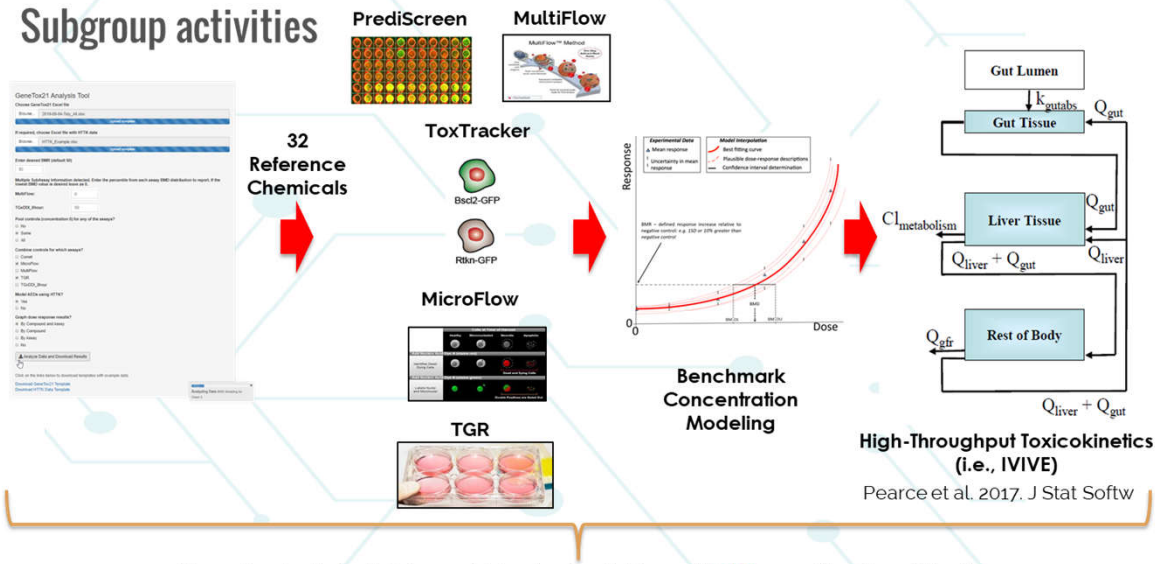
< 0 → High concern

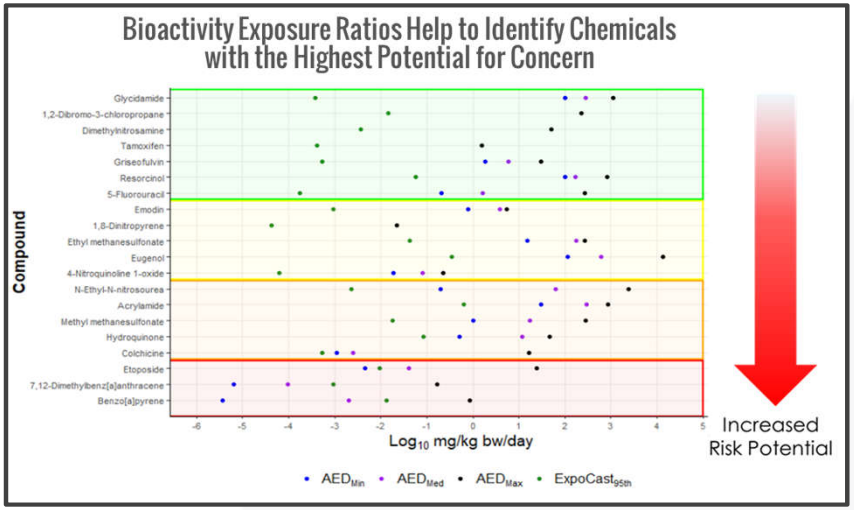
(Figure adapted from Health Canada, 2021)

Exploring In Vitro Genotoxicity Data to Estimate Points of Departure

[Health Canada Research and Risk Assessment Collaboration]
[HESI Genetic Toxicology Technical Committee]
[Accelerating the Pace of Chemical Risk Assessment (APCRA)]

Computational Toxicology increases utility of in vitro GeneTox Data for Human Health Risk Assessment



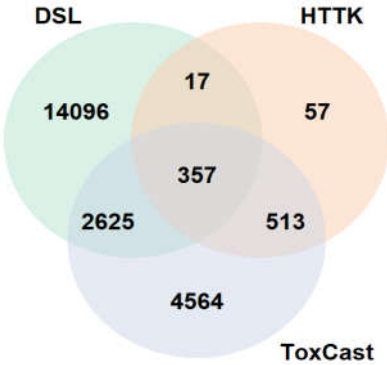


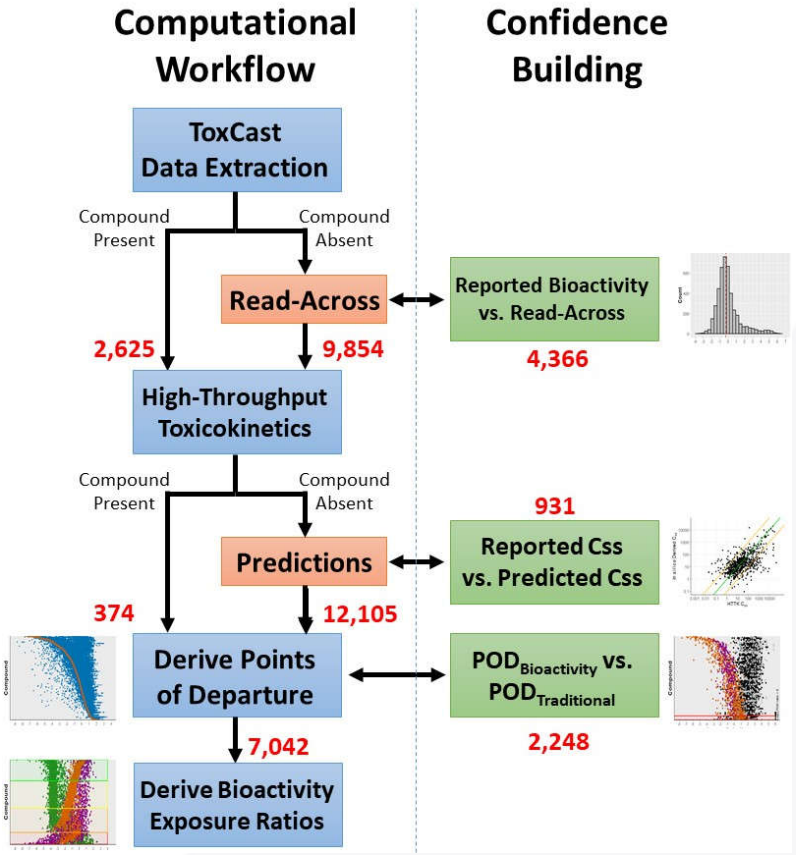
Genotoxic Administered Equivalent Dose (AED; mg/kg bw/day)
Concentration observed to be toxic in cells converted to a human dose

Courtesy of Marc Beal, Health Canada

Building on EPA Generalized Read-Across (GenRA) to Expand Scope of BER Approach for Priority Setting

- Even with more rapid *in vitro* testing available it will be difficult /impractical to test all substances and their possible transformation products
- Filling in data gaps using **read-across and computational models** allows to examine large domestic inventories of data poor substances **to enhance identification of priority substances for further action**
- It is important that workflows remain flexible and modular to use the best available science of the time



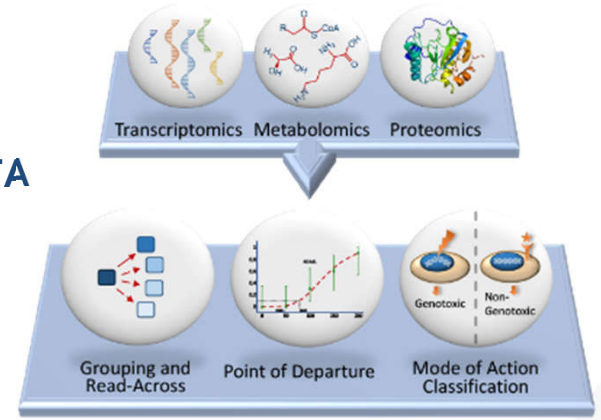


Beal et al. Implementation of *in vitro* Bioactivity Points of Departures to Address Data Gaps and Modernize Priority Setting of Chemical Inventories. ALTEX. 2022.

Gaining Experience in Deriving In Vitro Points of Departure Across Technologies

Deriving In Vitro Points of Departure Using Transcriptomic Data

- A quantitative risk-based approach to identify potentially hazardous substances
- Changes in gene expression using high-throughput screening (i.e., high-throughput transcriptomics, HTTr)
  - Examples
    - Whole transcriptome template ~20,000 genes
    - Modified transcriptomic template ~3000 known responsive genes
- Using benchmark concentration modeling and determining optimal endpoints for derived transcriptomic PODs
- Considers high-throughput in vitro bioactivity together with high-throughput toxicokinetic modelling



[OECD Omics technologies in Chemicals Testing]

Exploring Transcriptomics and Biomarker Signatures to Support Molecular-Based Points of Departure & Identification of Endocrine Modes of Action

IATA to Support Screening and Assessment of Endocrine Active Substances: Bisphenols

[Health Canada Research and Risk Assessment Collaboration]
[[OECD Integrated Approaches to Testing and Assessment (IATA) Case Study]

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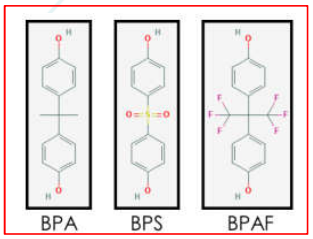
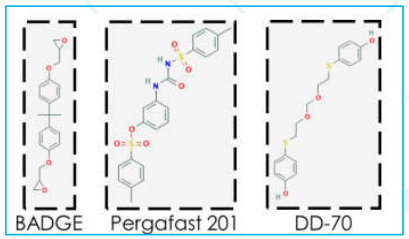
Status: Final review and approval under OECD IATA case studies project

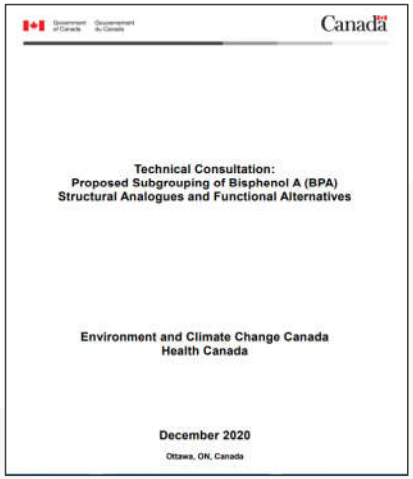
Regulatory Context - Bisphenol Priorities Identified in 2017-2018

- Certain bisphenols from the 2017-2018 assessment priorities review (IRAP) cycle included a diverse list of chemical structures
- As starting point for problem formulation there were 31 *structurally* similar and 8 *functionally* similar *substances*
- Computational approach was applied to identify a broader set of substances for further exploration (grouping approaches)





343 unique chemicals were identified


Canada

Technical Consultation:
Proposed Subgrouping of Bisphenol A (BPA)
Structural Analogues and Functional Alternatives

Environment and Climate Change Canada
Health Canada

December 2020

Ottawa, ON, Canada

Methods to identify, triage and subgroup*

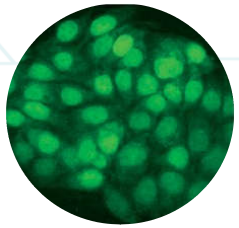
* https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/technical-consultation-proposed-subgrouping-bpa-structural-analogues-functional-alternatives.html

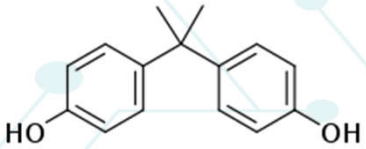
Focus on Integrating Transcriptomics and Biomarker Signatures

Health Canada Santé Canada

uOttawa

Human MCF-7 Cells



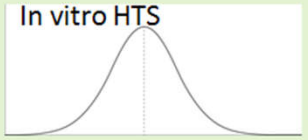


Target Chemicals (n = 25)

- **with bisphenol substructure (n = 18)**
- **potential use as a functional alternative to BPA (n = 7)**

Step 1) Data Collection / Generation

In vitro HTS



Distribution of ToxCast AC50 Values (µM)

In vitro HTTr

Distribution of BMC values in a relevant human cell line (MCF-7)

In silico

Step 2(A) Derive minimal bioactivity concentration applicable for general systemic toxicity (i.e. hazard-independent)

Step 2(B) Derive minimal bioactivity concentration applicable for ER activation (i.e. ER pathway specific)

Use in silico models to further support ER activity

Step 3) In vitro to In vivo Extrapolation to derive POD_{NAM_SYS} and POD_{NAM_ER}

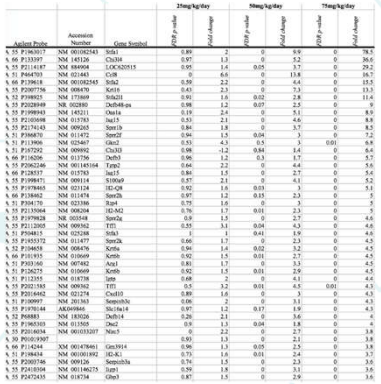
AED (mg/kg bw/day) based on upper 95th percentile steady state blood concentration representing a "sensitive" population

Not Shown in Case Study

Step 4 Compare to exposure estimates to derive bioactivity exposure ratio (BER) to identify risk assessment priorities

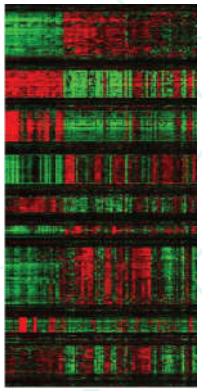
INCORPORATING TRANSCRIPTOMICS IN REGULATORY DECISION-MAKING

Data generation: Gene Lists



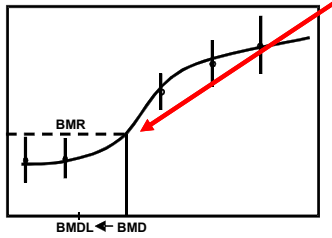
Thousands of genes per chemical and each exposure

Extraction: predictive signatures and pathways



Applied parameters identifying genes with a concentration-response

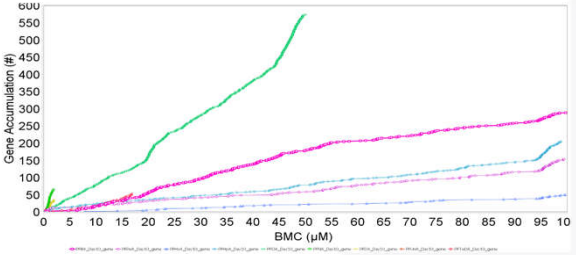
Dose-response modeling



Establish Points of Departure (PODs)

mathematical models identify defined response above background

Benchmark Concentration (BMC) accumulation plots



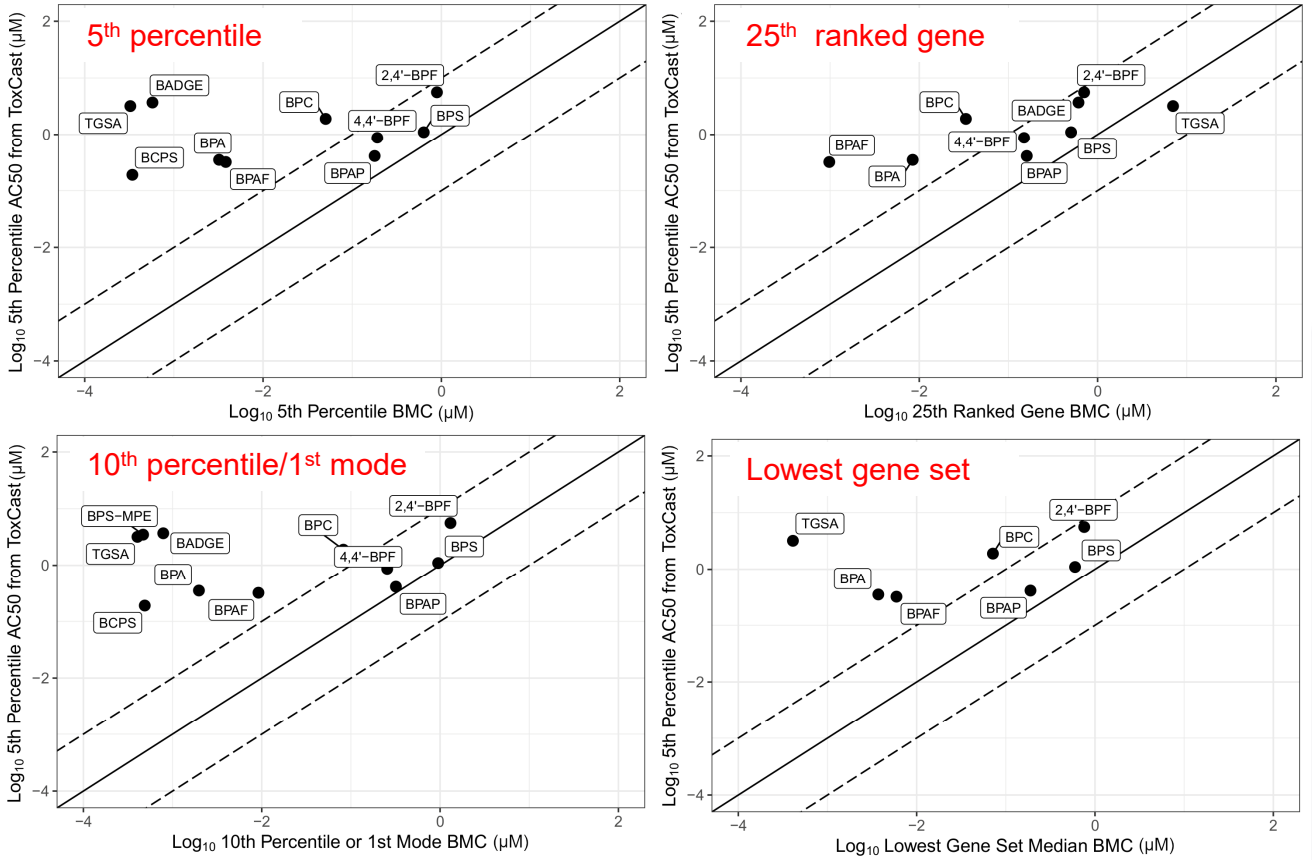
How do we define the optimal POD?

- 5th percentile
- 25th ranked gene
- 1st mode BMC
- Lowest median gene set (KEGG, GO, REACTOME)

RESULTS

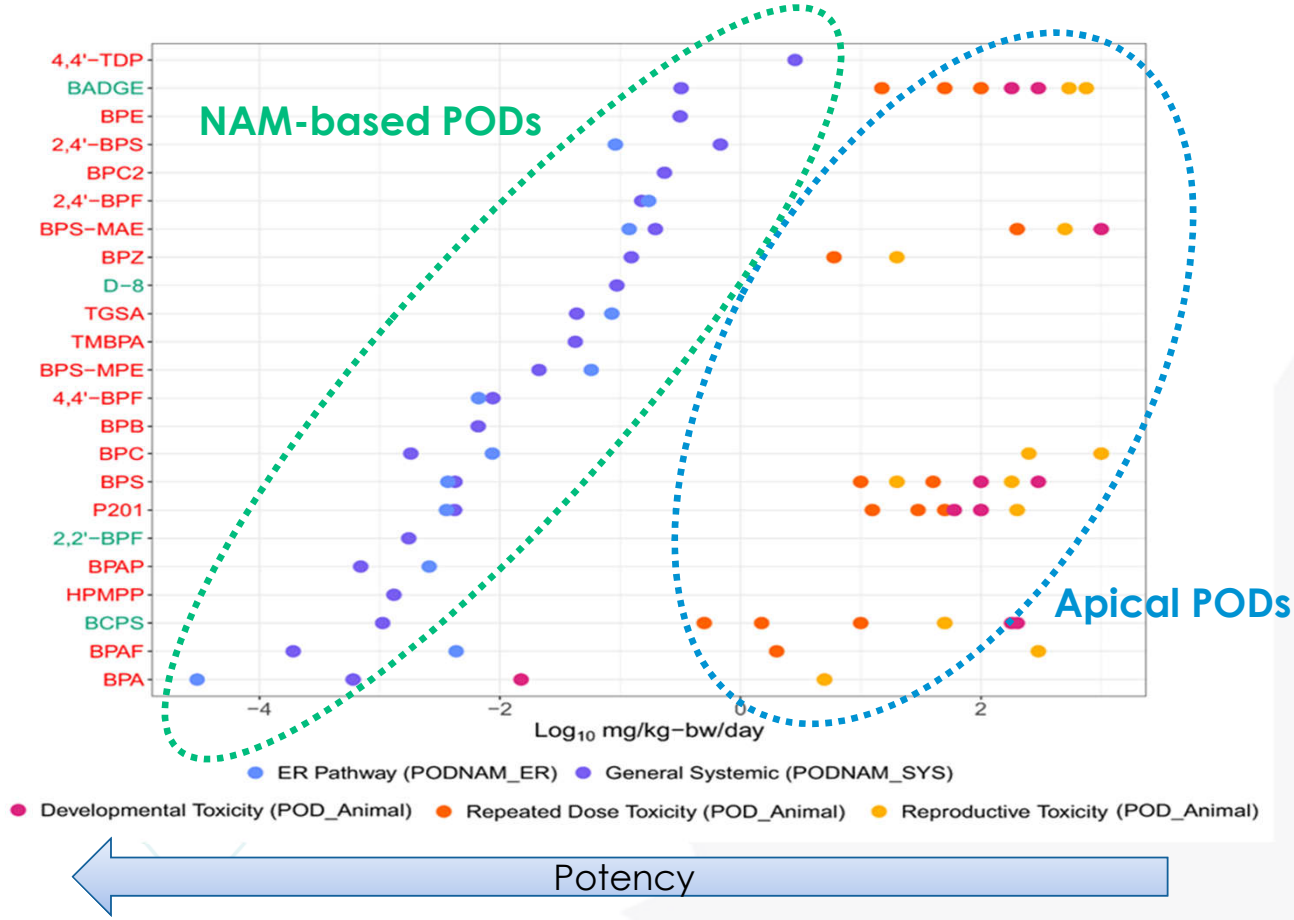
- Several approaches may be considered for establishing NAM-based points of departure for bisphenols and related chemical substances
- Specific approaches (e.g., 25th ranked gene) demonstrated robust data, as transcriptomic-derived endpoints, agreed with endpoints derived ToxCast dataset
- Substances exhibit pathway-specific activity (ERa agonists) and demonstrated to have endocrine disrupting properties

Agreement of BMCs with ToxCast AC50 values



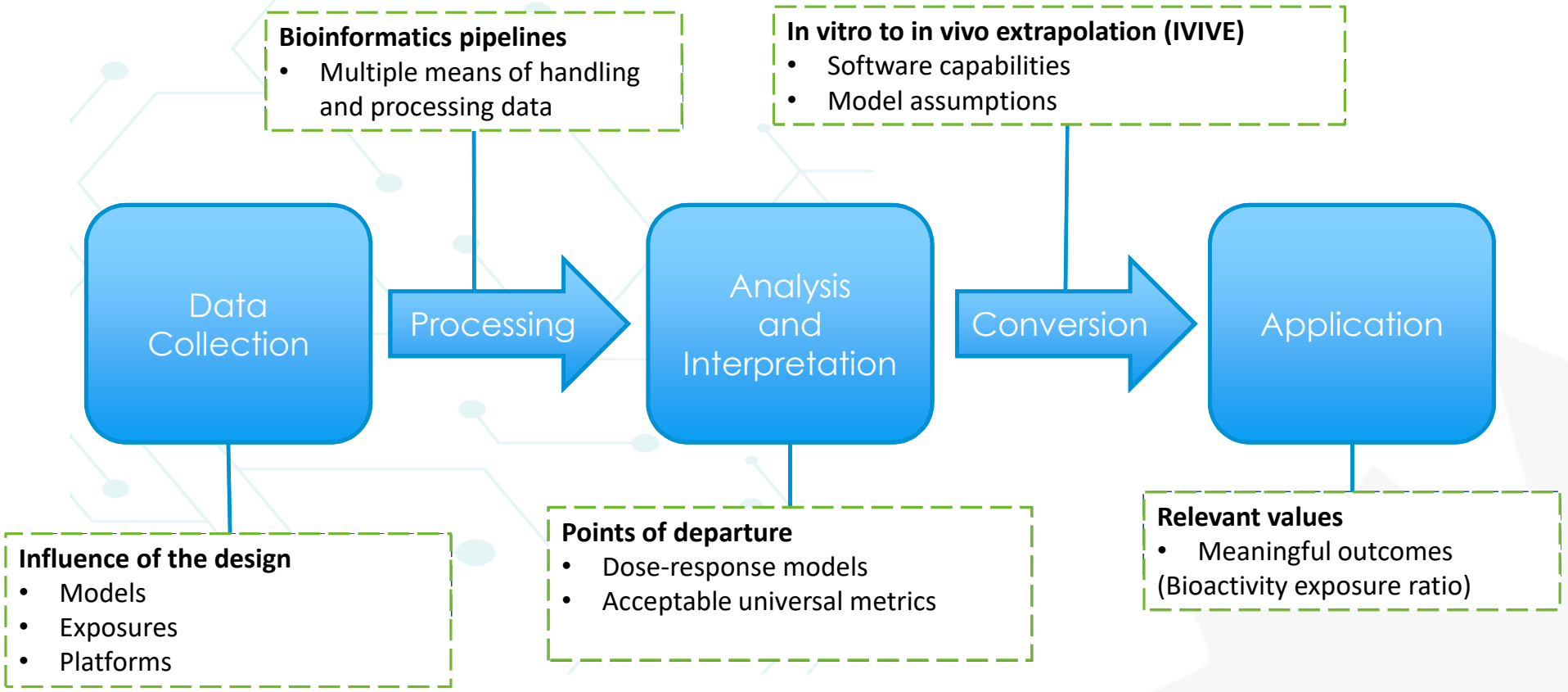
COMPARISON OF NAM-BASED POINTS OF DEPARTURE WITH APICAL ENDPOINTS

- Chemicals considered ER active based on weight of evidence from *in silico* models listed in red
- NAM-based PODs are useful endpoints for **prioritization** and **potency ranking**
  - General toxicity and pathway derived PODs are in good agreement
- Typically lower than animal-based PODs indicating that they are more conservative

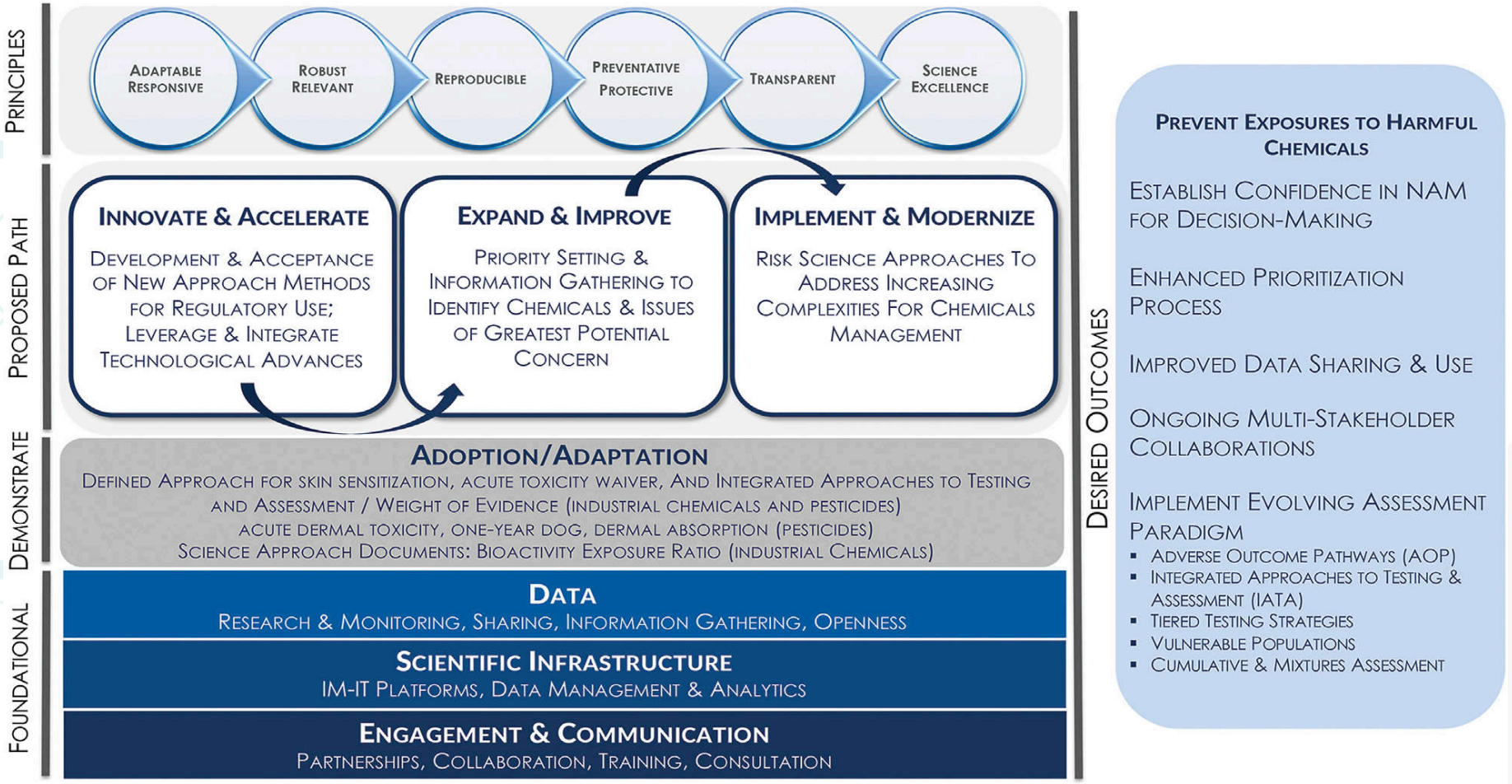


POD = point of departure

In Vitro Models – Challenges and Opportunities



Where The Rubber Meets the Road –
Achieving Results In Partnership Through Innovation and Implementation



Bhuller, Y., Ramsingh, D., Beal, M., Sunil Kulkarni, S., Gagne, M., Barton-Maclaren, TS. 2021. Canadian Regulatory Perspective on Next Generation Risk Assessments for Pest Control Products and Industrial Chemicals. Front. Toxicology 3:748406.

KEY MESSAGES

- The time is now to innovate and develop fit-for-purpose approaches to increase efficiency and improve protection.
- Key international regulatory jurisdictions are committed to reduction / elimination of animal testing – they are pivotal data sources for risk assessment; we need to keep pace and align internationally.
- Research-Regulatory collaborations are imperative.
- Continue to build a common vision and commitment to advance alternative methods and maintain excellence in science based decision making.

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- Nicole Davidson

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- Ivy Moffat
- Joleen Hanna
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- Francesco Marchetti
- Ella Atlas
- Karen Leingartner
- Mike Wade
- Andy Nong
- Byron Kuo
- Yanic Mainville
- Matthew Meier
- Andrew Williams
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