In Vitro Bioactivity Workflows for Enhanced Prioritization and Rapid Screening of Existing Chemicals in Canada: A Journey of Insights and Learnings

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EPIC Webinar

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Source: Al-Koshi Cleaning Chemicals

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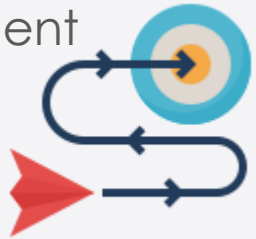
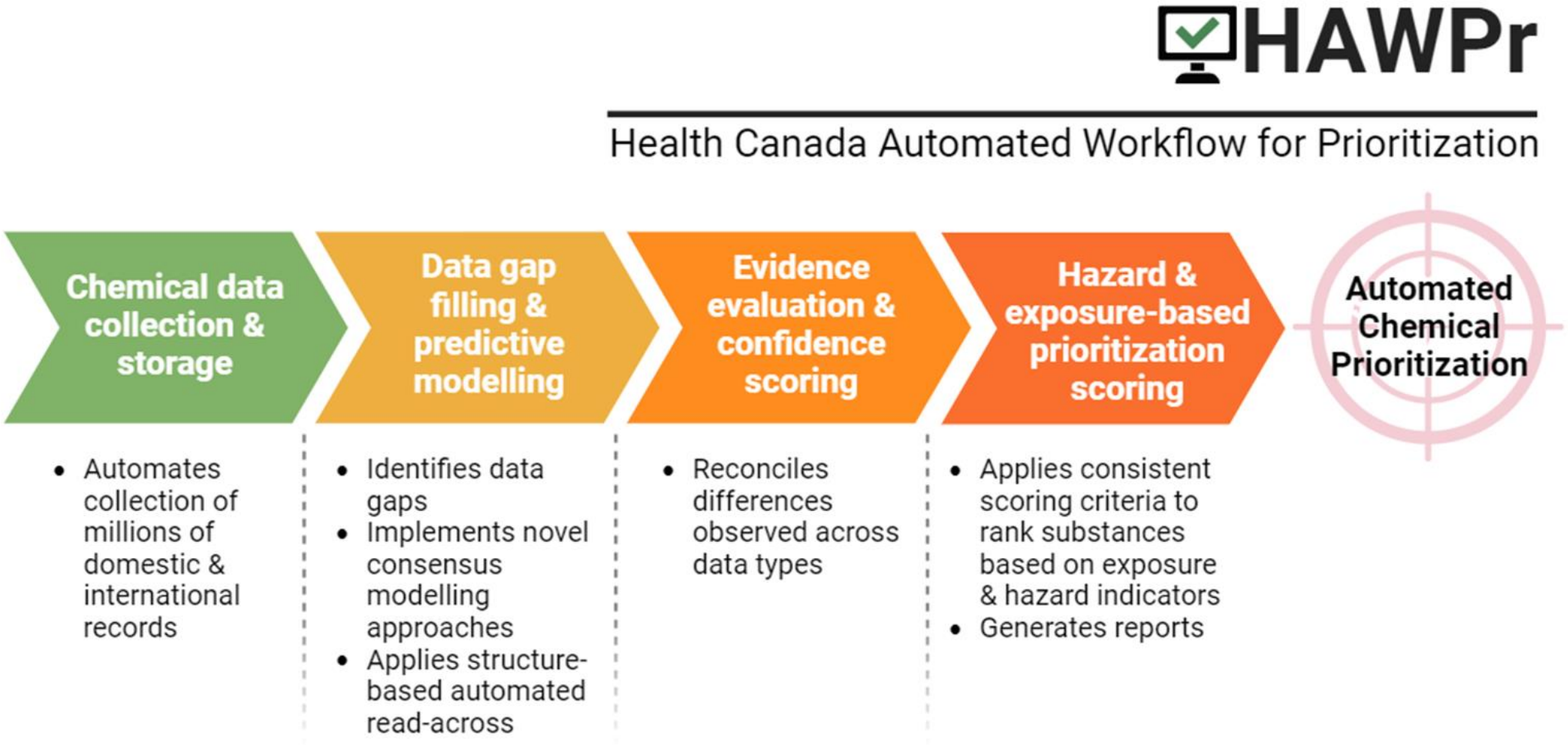
Moving Towards Integrating New Approach Methodologies (NAMs) for Chemicals Management in Canada

- Transition to NAM as a complete replacement over animals will be a challenging and a slow process
  - The better starting point may be to ask:
    - » What are the near-term opportunities for deploying currently available NAM with respect to prioritization and risk assessment activities?
    - » How can NAM help to focus the burden of assessing chemicals in Canada

- Finding a starting point -> In Canada, the focus of early development and application of NAM has surrounded program opportunities where:
  - NAM is a step above the status quo
  - where the development of fit-for-purpose NAM-based approaches help to make better informed decisions in the absence of other traditional forms of toxicity test data
    - » Addressing data poor substances
    - » Adding mechanistic or mode of action information to better inform risk management

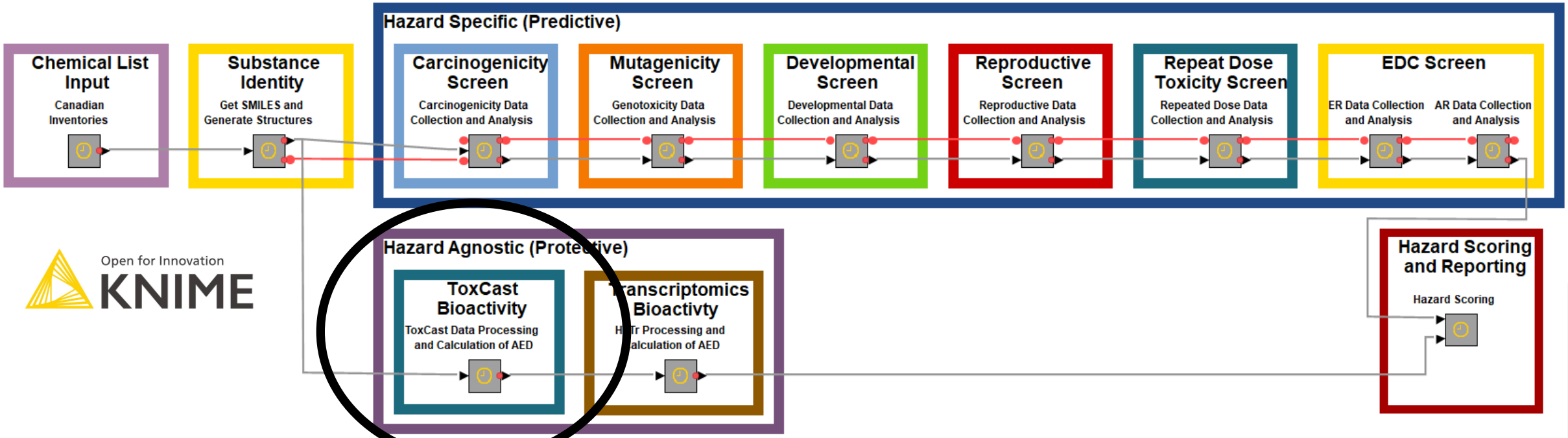


Overarching Workflow for Data Collection, Processing and Interpretation



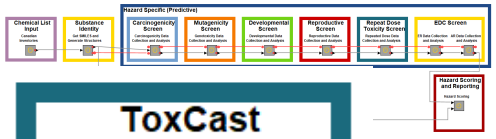
Modular Design, Flexible and can be Modified as Science and Information Sources Evolve

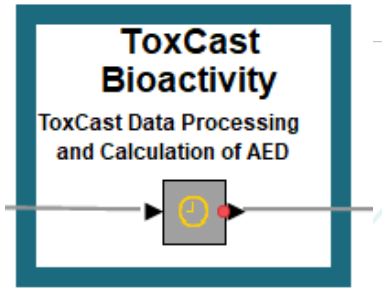
HAWPr
Health Canada Automated Workflow for Prioritization



- Collect and interpret New Approach Methods (NAM) and traditional data streams
- Workflow combines various biological levels into a decision framework
  - IATA structured nodes across toxicity endpoints
- Use best available data and processes to improve efficiency and reproducibility
- Deliver transparent and evidence-based decisions through integrated knowledge

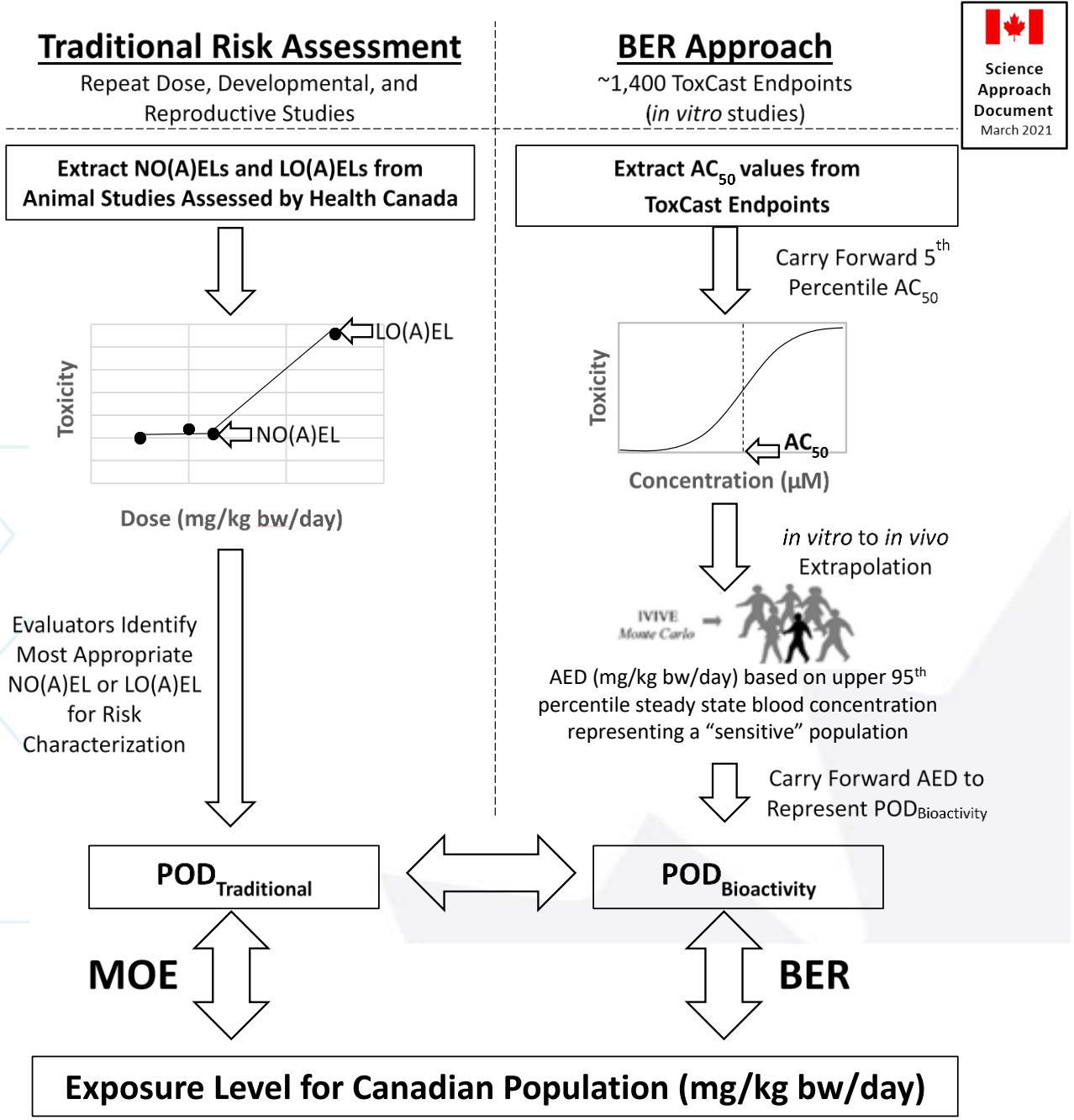
RESEARCH-REGULATORY COLLABORATION





Health Canada SciAD Presents Comparative Analysis

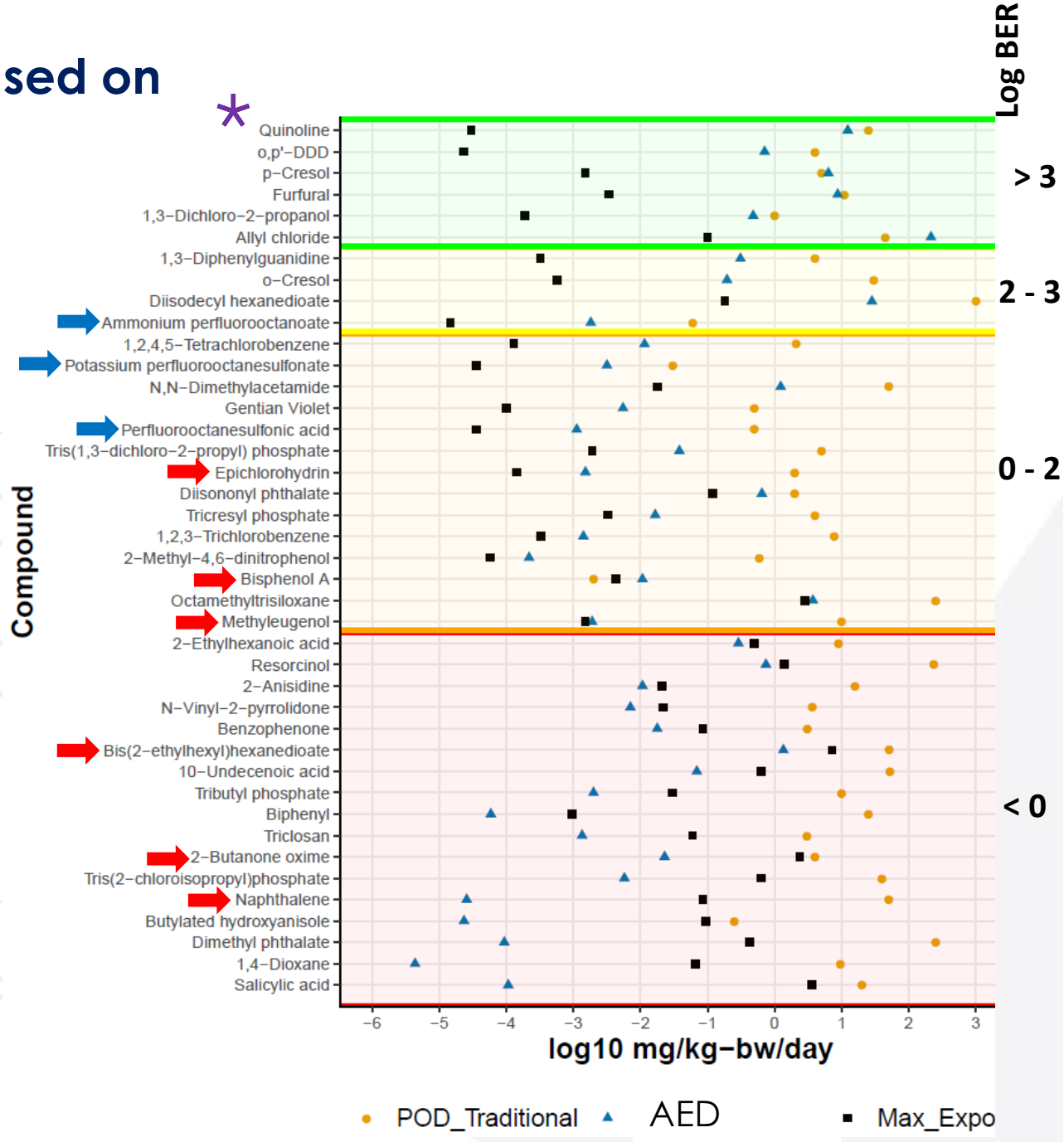
- Applied workflow to 40+ chemicals previously assessed on the Chemicals Management Plan
  - ToxCast data -> $POD_{Bioactivity}$
- Compared $POD_{Bioactivity}$ to traditional PODs used in the Screening Assessment Report (SAR)
- BERs derived: $POD_{Bioactivity}$ compared to Canadian exposure values from:
  - biomonitoring data
  - environmental media
  - consumer products
- BERs were evaluated to assess the utility of bioactivity data in prioritizing chemicals for risk assessment



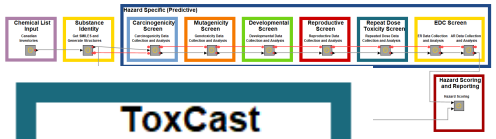
Science Approach Document March 2021

Bioactivity Exposure Ratio (BER) Based on Canadian Exposure Levels

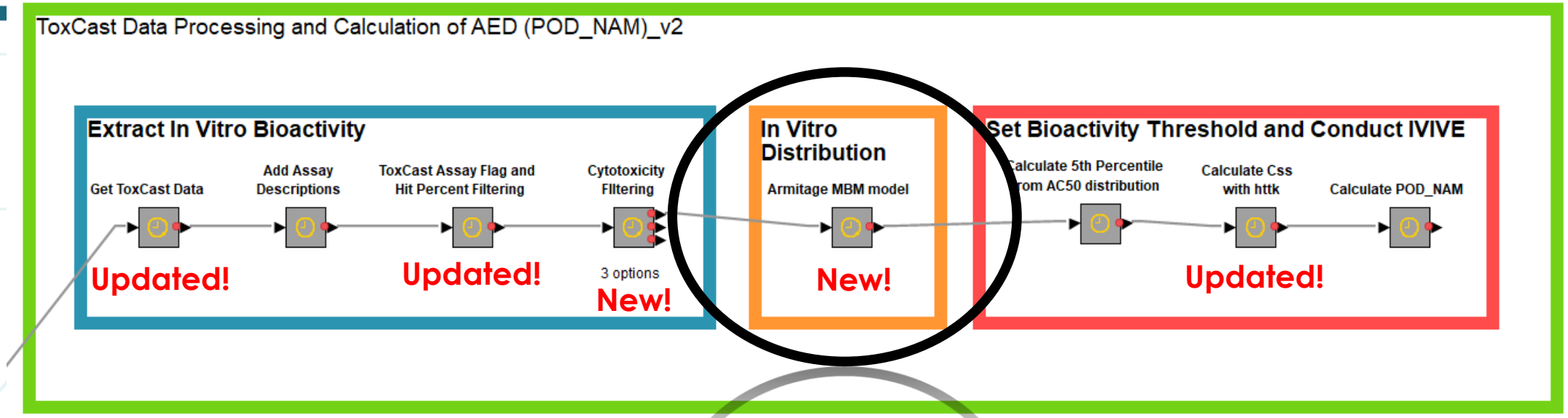
- NAM-derived AED lower than $POD_{Traditional}$ for 38 out of 41 chemicals assessed previously
- All non-genotoxic compounds assessed as toxic to human health (red arrows) had a BER < 100
- All non-genotoxic compounds assessed as ecotoxic (blue arrows) had a BER 100 -1000
- One toxic chemical (**Quinoline**; purple star), assessed as potentially genotoxic, was identified as low priority using this approach
  - Only 5 ToxCast assays measure DNA damage or stalled replication and these have low sensitivity
- **Approach for genotoxicity that builds on these experiences is needed**



Source: Health Canada Bioactivity Exposure Ratio Science Approach Document



Ongoing Refinements as Science Evolves



Can we improve our AED estimates for these data?

- In vitro disposition models

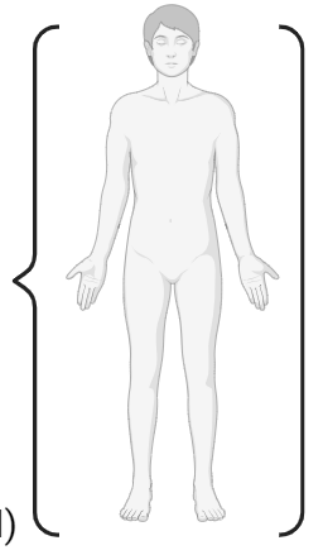
In Vitro Mass Balance Modelling (IV-MBM) Adjusts for In Vitro Toxicokinetics and In Vitro/In Vivo Bioavailability

- When doing HTTK-IVIVE we typically assume that the in vitro BMC is equivalent to:
  - the freely dissolved concentration
  - the concentration in serum/blood that exerts a toxic effect
- IV-MBM modelling uses assay & chemical specific information to adjust the NAM BMC (nominal in vitro POD) to reflect these assumptions

NAM BMC (uM) = Nominal in vitro POD

1. Assumed equal
2. Adjusted IV-MBM

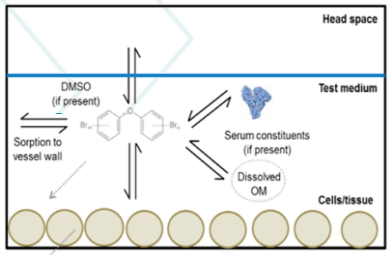
In vitro POD (uM)

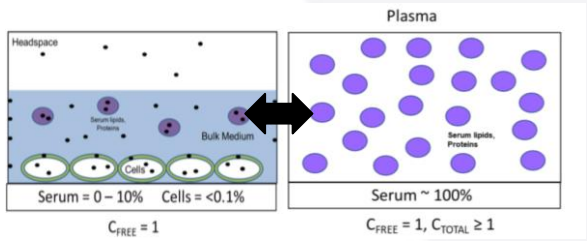


Administered Equivalent Dose (mg/kg BW/day)

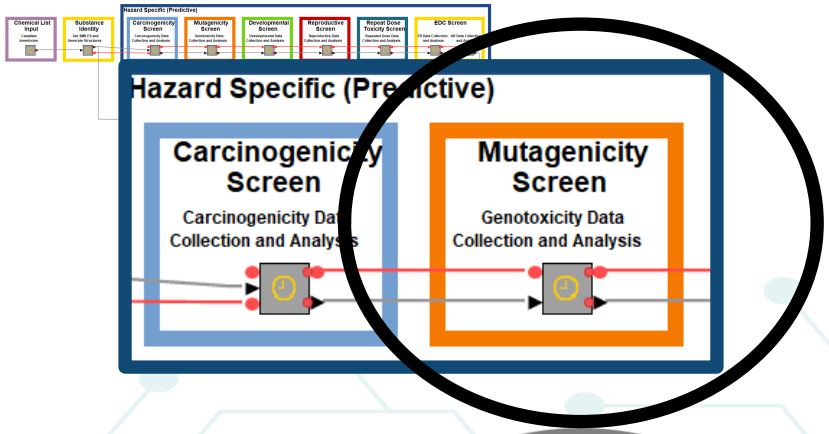
In vitro toxicokinetics
- calculates the freely dissolved concentration

In vitro/in vivo bioavailability
- corrects differences b/w exposure medium and serum





Exploring In Vitro Genotoxicity Data to Estimate Points of Departure



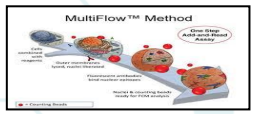
- Develop parallel approach that addresses gaps identified in the BER SciAD (2021)
- Evaluate impact of Httk refinements

Case Study Part 1

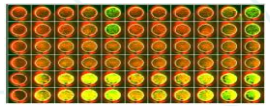
31 compounds with:

- in vivo genetic toxicity PODs
- NAM genetic toxicity data

MultiFlow



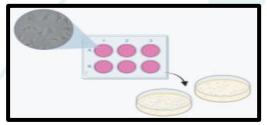
PrediScreen



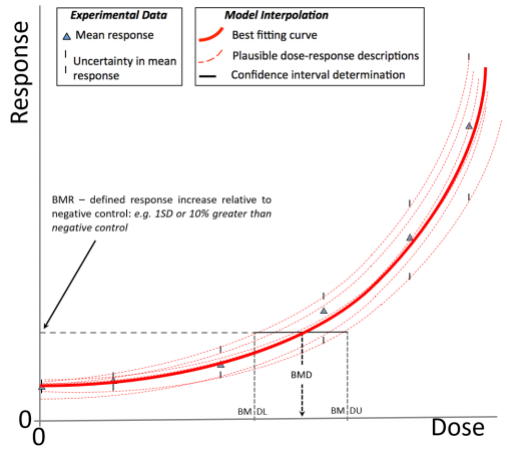
ToxTracker

MicroFlow

TGR



Genetic toxicity NAMs



Benchmark Concentration Modeling

***in vitro* to *in vivo* Extrapolation (IVIVE) using httk in R**

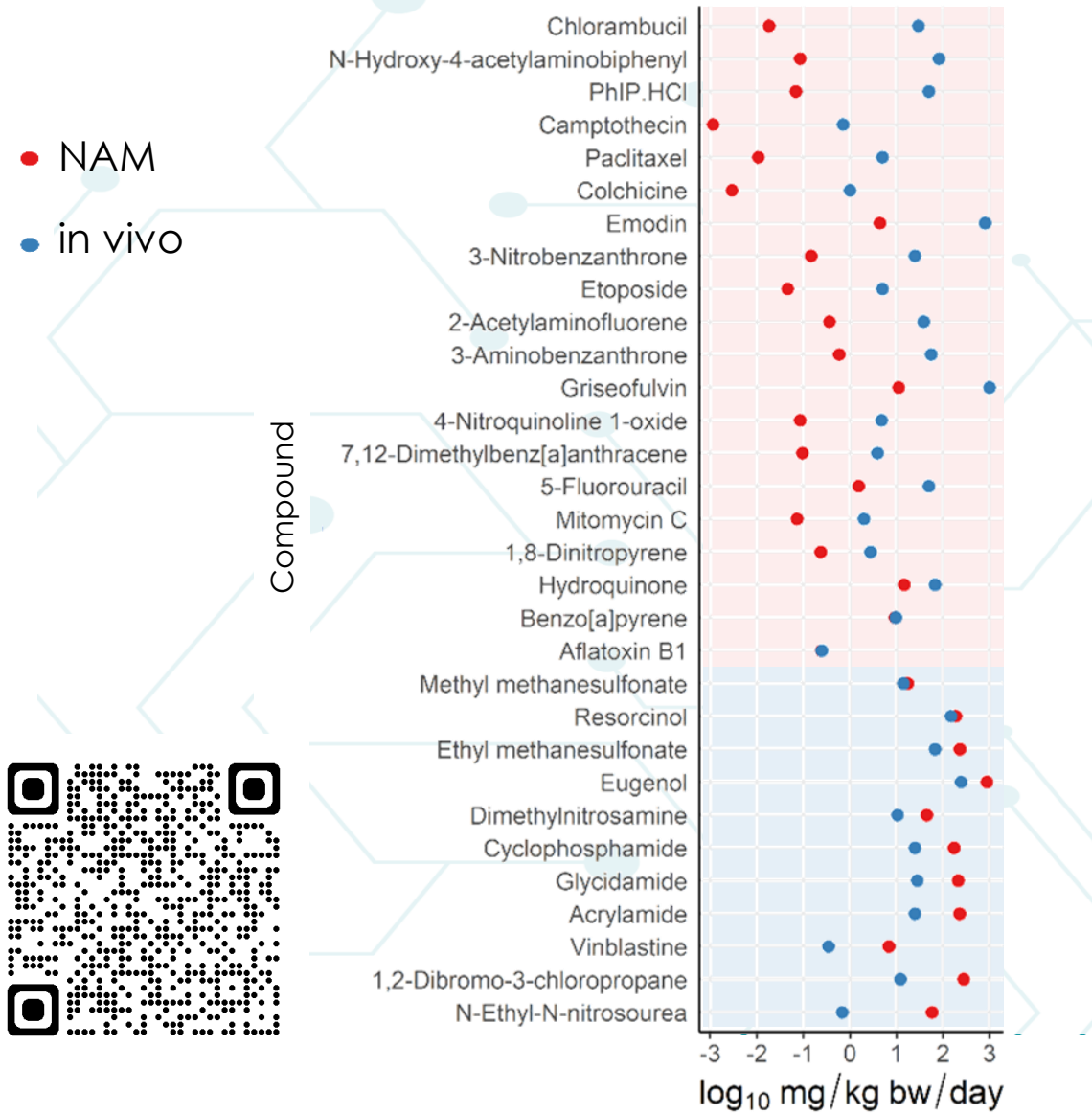
Pearce et al. 2017. J Stat Softw

Human Administered Equivalent Dose (AED; mg/kg bw/day)

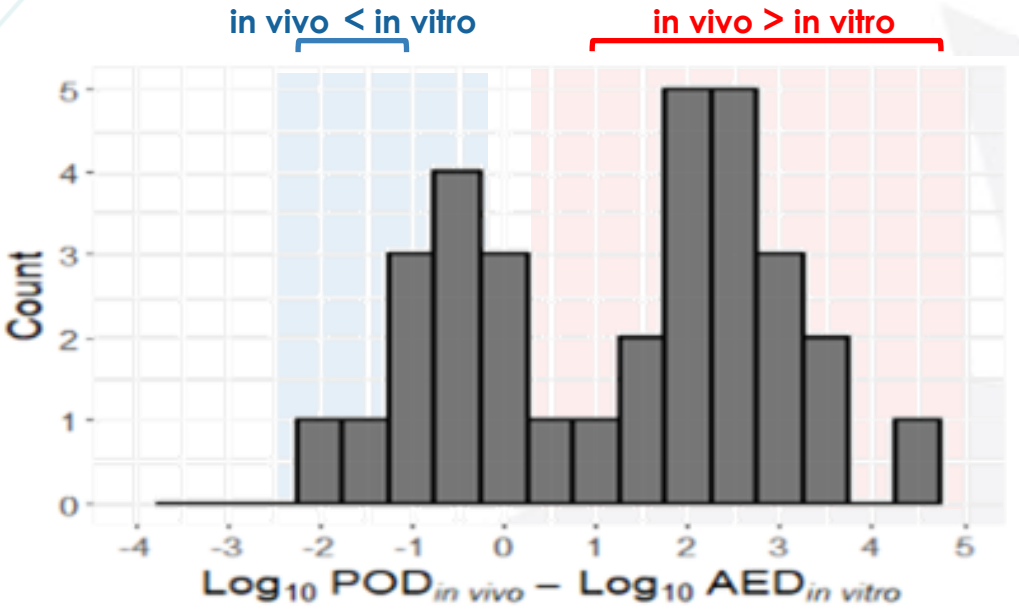
Beal et al. (2023). Quantitative in vitro to in vivo Extrapolation (IVIVE) of Genotoxicity Data Provides Protective Estimates of in vivo Dose. *Environmental and Molecular Mutagenesis* DOI: 10.1002/em.22521.

20/31 Chemicals have Median AEDs Lower than Animal-Based PODs

Median NAM-AEDs and in vivo PODs



- Across the 31 chemicals, 198 NAM-AEDs derived from in vitro genotoxicity data were compared to 321 PODs from in vivo genotoxicity data
- The NAM-derived AEDs were typically protective of human health
  - i.e., lower than animal-based PODs



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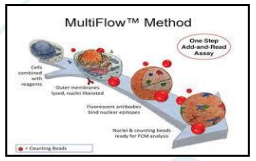
HTTK IVIVE Approach for GTTC Case Study - Part 2

31 compounds

- in vivo genetic toxicity PODs
- NAM genetic toxicity data

MultiFlow



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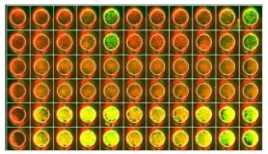
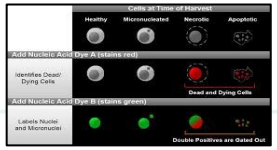
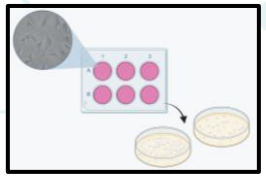


ToxTracker

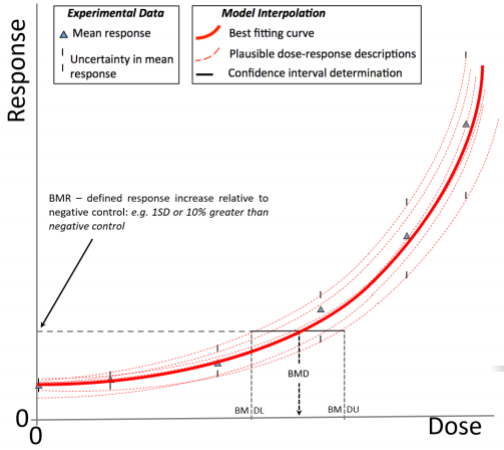
MicroFlow



TGR



Genetic toxicity NAMs



Benchmark Concentration Modeling (Nominal in vitro PoD)

Nominal in vitro POD

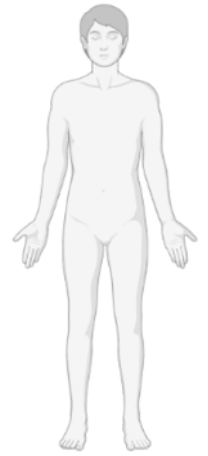
Adjusted in vitro POD

In vitro mass balance model (IV-MBM) to account for in vitro disposition

Armitage et al. (2021)

***in vitro* to *in vivo* Extrapolation (IVIVE) using httk in R**

Pearce et al. (2017)



Human Administered Equivalent Dose (AED; mg/kg bw/day)

Arnot Research & Consulting

In vitro mass balance model (IV-MBM) scaling factor reduces in vitro BMC

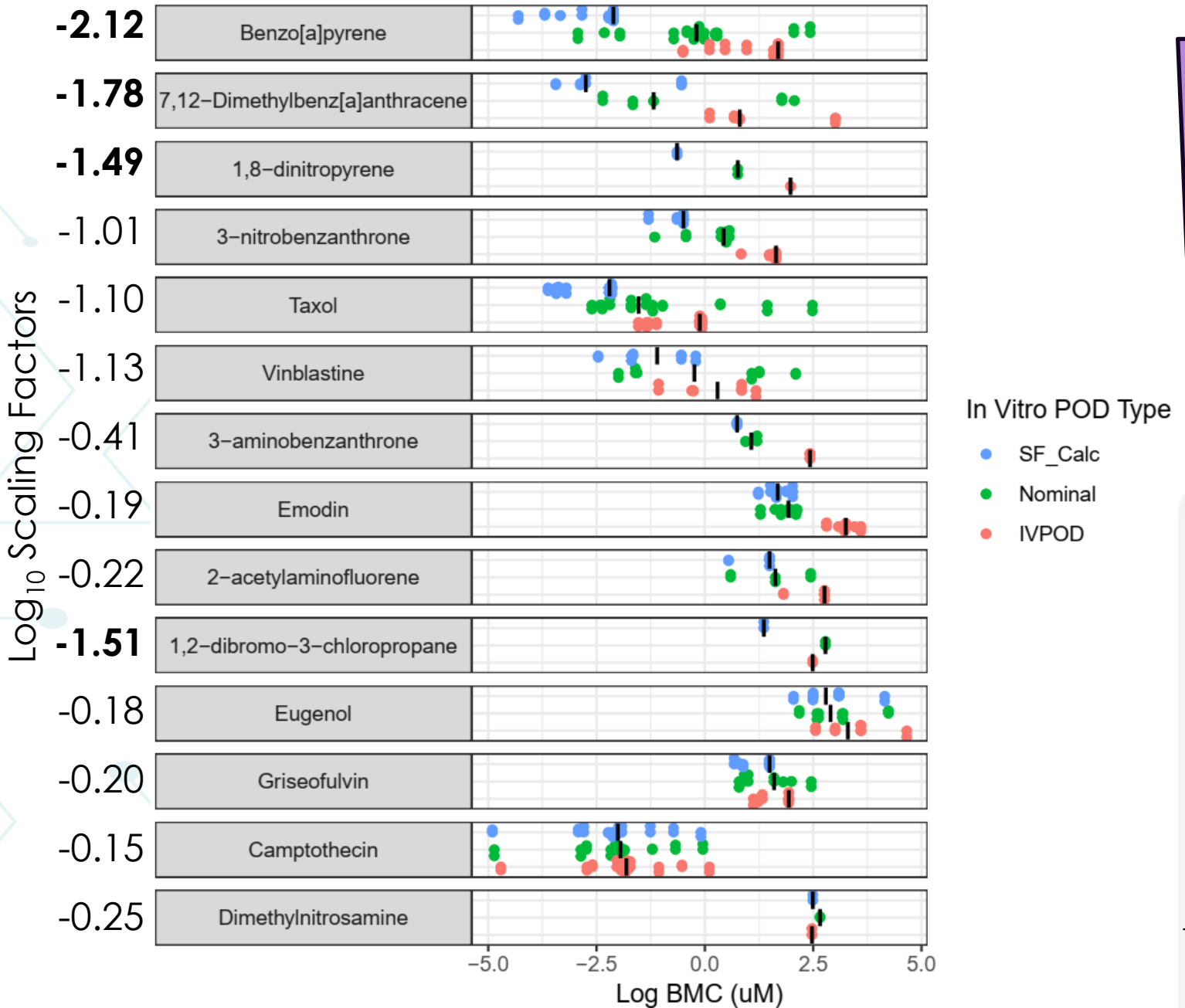
In vitro mass balance model (IV-MBM) to adjust in vitro PODs

Nominal = unadjusted BMC

Scaling Factor (SF) to correct **in vitro bioavailability** reduces the in vitro BMC

Media-plasma bioavailability differences increases the in vitro POD (IVPOD)

Bioavailability in plasma can be greatly reduced in comparison with serum-free or low serum exposure media conditions



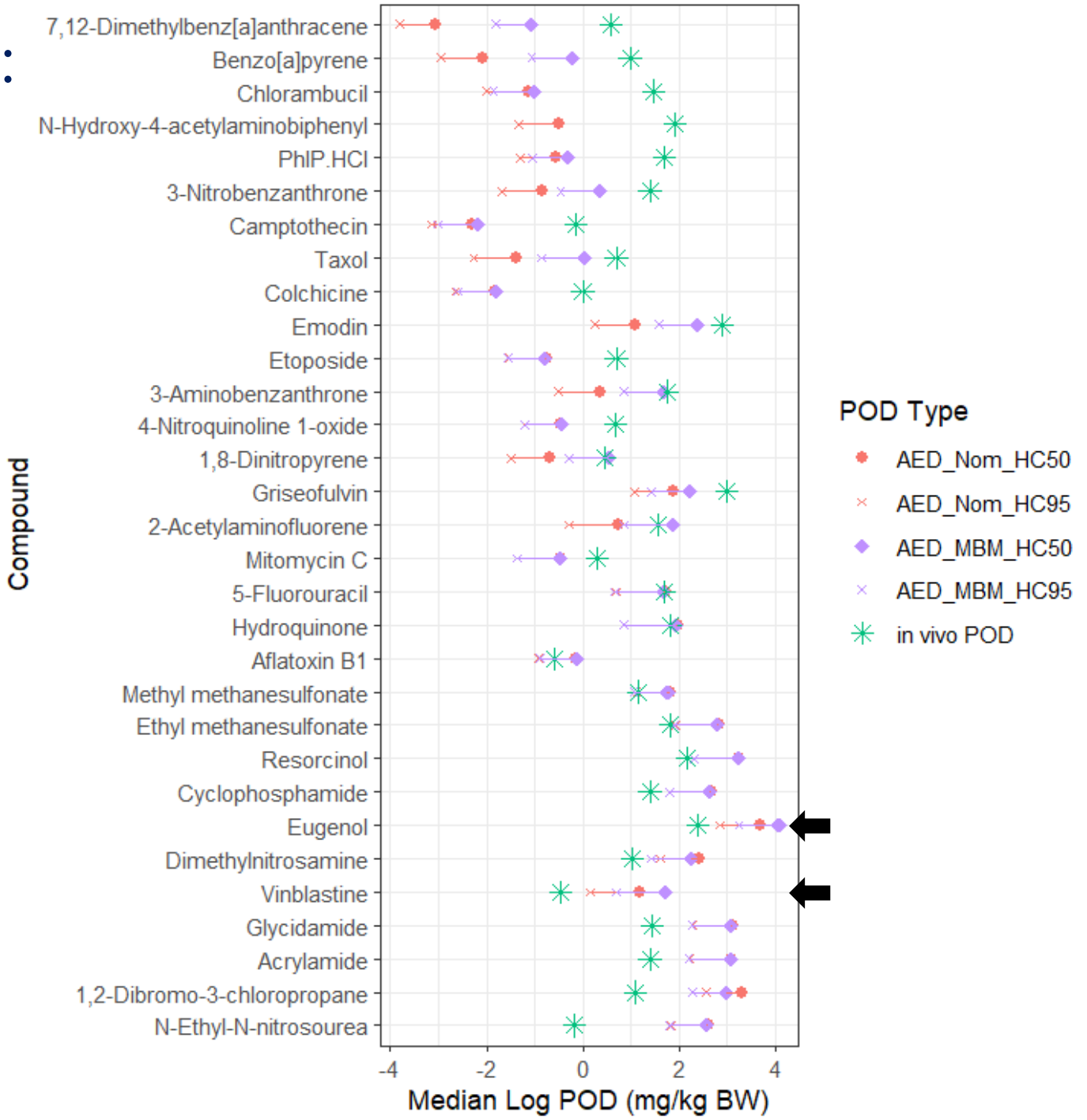
Comparing Case Study Results: Nominal & IV-MBM AEDs

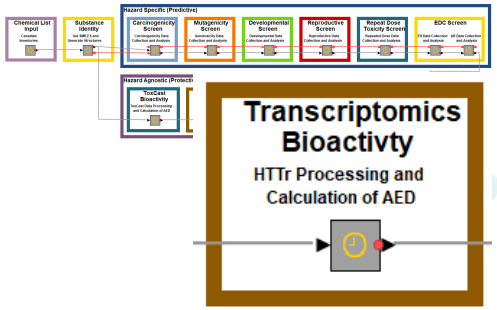
With IV-MBM:

- IV-MBM improved AED estimation when applied to the ORD httk AEDs
  - AEDs closer to in vivo PODs: **11**
  - AEDs same distance from in vivo PODs: 17
  - AEDs farther from in vivo PODs: 2

- IV-MBM tends to have greater impact on compounds with higher $LogK_{OW}$

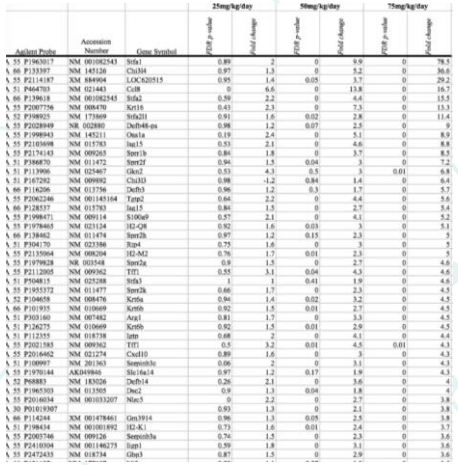
30 compounds with full data set





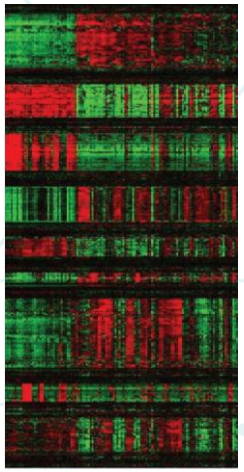
Gaining Experience in Deriving In Vitro Transcriptomic Points of Departure

Data generation: Gene Lists



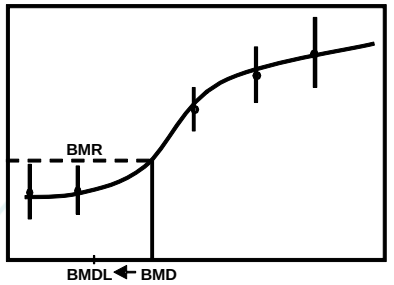
Thousands of genes per chemical and each exposure

Extraction: predictive signatures and pathways



Applied parameters identifying genes with a concentration-response

Dose-response modeling



Establish Points of Departure (PODs)

How do we define the optimal POD?

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

Evaluating In Vitro Transcriptomic PODs Using a Uniform Workflow: A Meta-analysis of Existing Datasets

1. Processing
2. Derivation
3. Conversion
4. Comparison

- 117 chemicals
- Models: MCF-7, HepaRG, liver spheroids
- Exposures: 0.25, 1, 2, 4, 10 and 14 days

Available Data (using TempO-Seq™)

- Bioinformatic Pipelines process and filter raw data:
  - The available universal R-ODAF pipeline (https://github.com/R-ODAF/)
  - The US EPA pilot pipeline (https://github.com/USEPA/httrpl_pilot)
  - In-house processing scripts

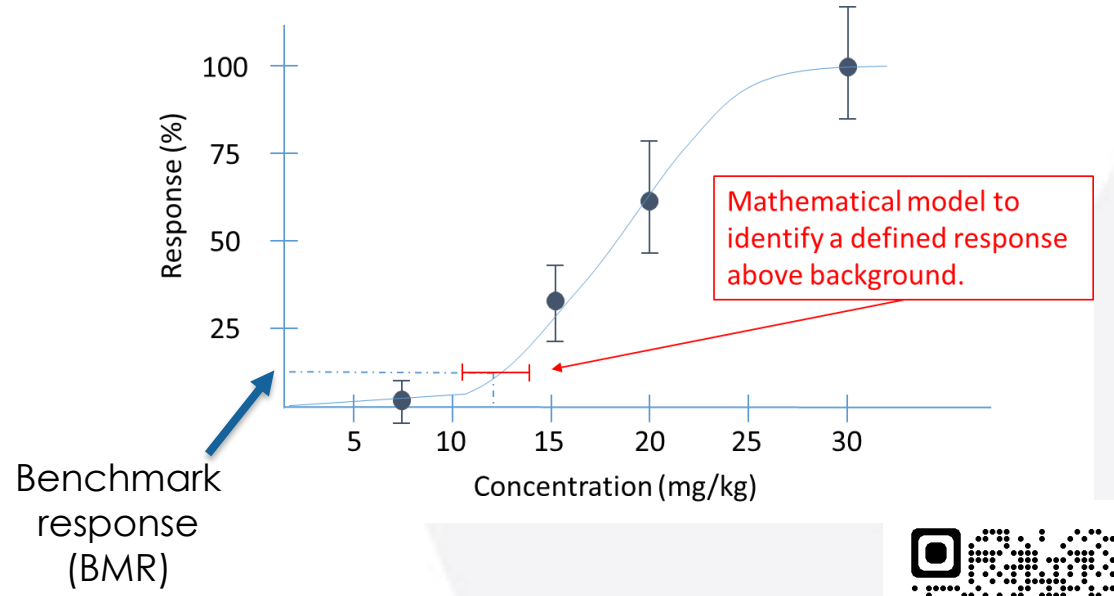
- **Step 1** → Benchmark concentration modeling
- **Step 2** → Derive transcriptomic points of departure
  - BMC distribution level metrics (LCRD, 5th percentile, 1st mode, 25th ranked gene)
  - The median of the (i.e., most sensitive) set of genes (KEGG, GO, or REACTOME)

- Convert the transcriptomic point of departure to an administered equivalent dose (httk R-package)

- Comparison to apical PODs from in vivo studies, from available databases: EPA ToxVal, REACH dossier, OECD Toolbox, and the Health Canada dossier

- Applied a uniform analysis across a diverse chemical space using different models and exposure conditions

Benchmark Concentration (BMC) modeling



Reardon AJF et al. (2023) Front. Toxicol. 5:1194895

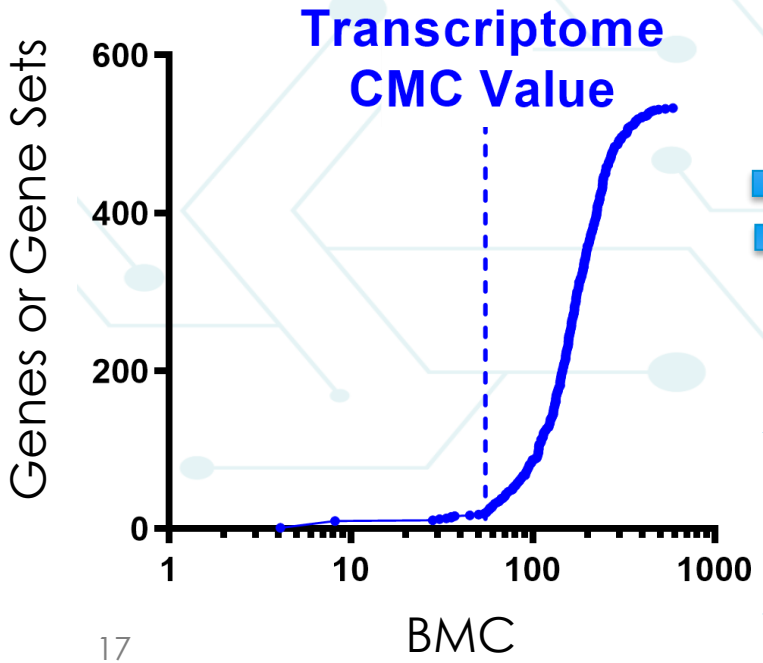
2. Derivation

- **Step 1** → Benchmark concentration modeling
- **Step 2** → Derive transcriptomic points of departure
  - BMC distribution level metrics (LCRD, 5th percentile, 1st mode, 25th ranked gene)
  - The median of the (i.e., most sensitive) set of genes (KEGG, GO, or REACTOME)

- Numerous approaches were considered to derive **tPODs** to define the point of concerted molecular change (**CMC)**

Gene Accumulation Curves

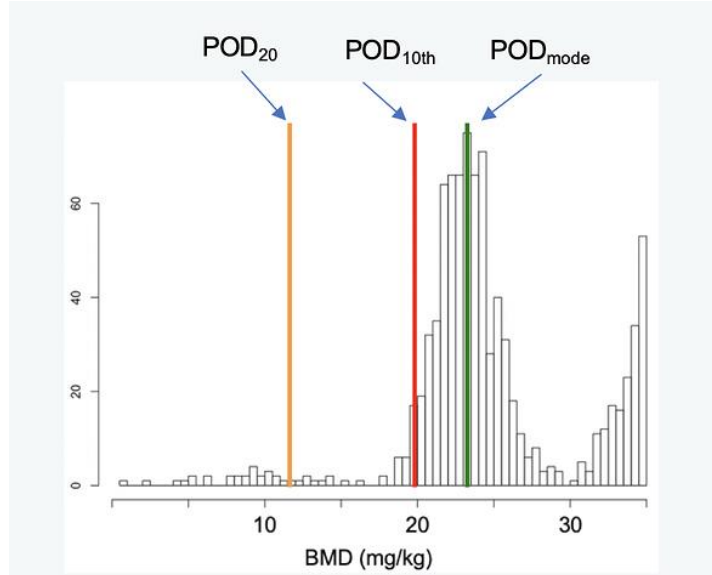
- Each chemical is depicted as a distribution of genes with BMCs to derive tPODs



1 ***Using distributions of BMCs to derive tPODs***

- $tPOD_{20}$ *(20th lowest BMD value)*
- $tPOD_{10th}$ *(10th % of gene BMD values)*
- $tPOD_{mode}$ *(mode of the first peak)*

**We used slightly different metrics*



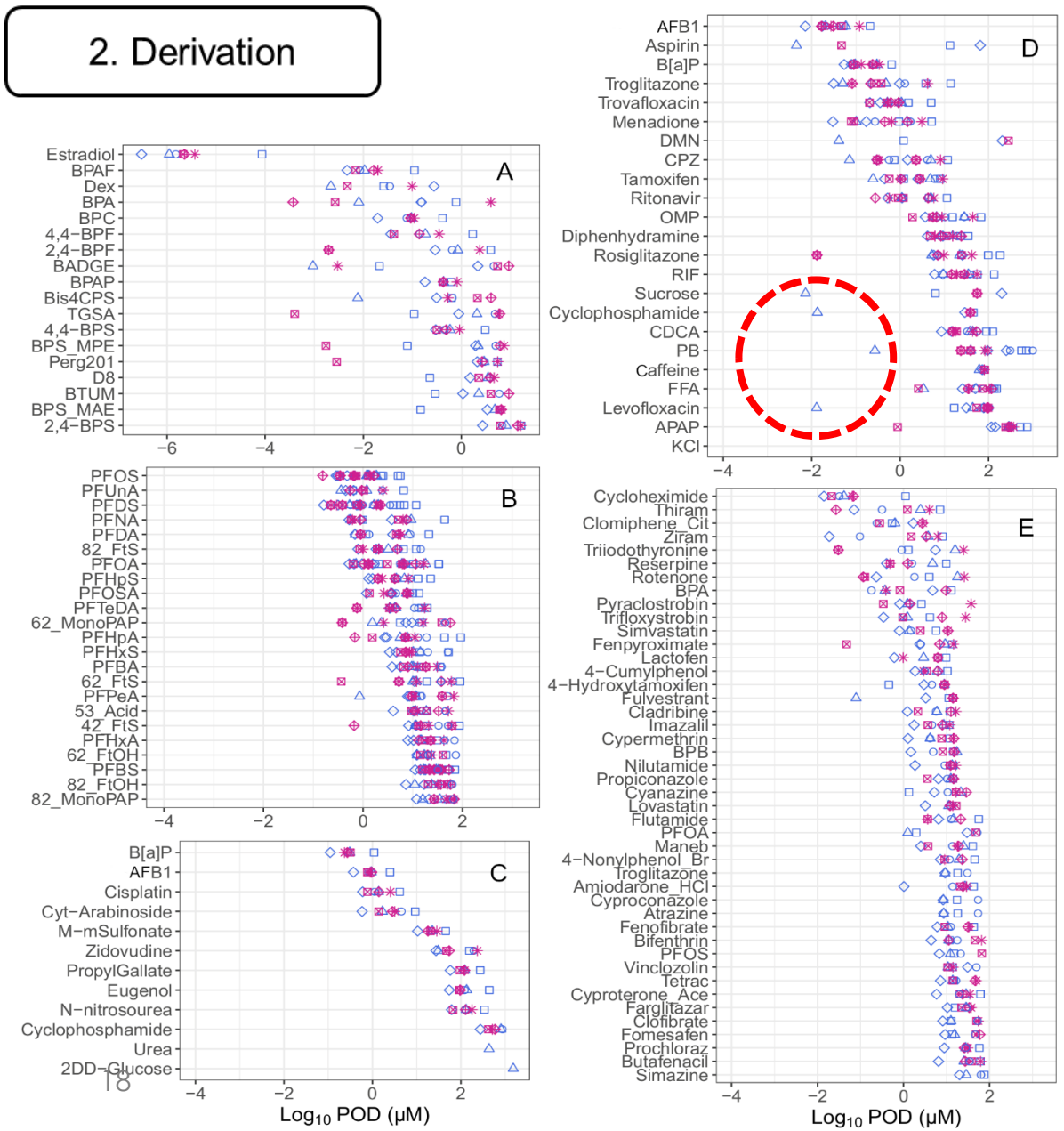
https://omicsforum.ca/t/what-is-a-transcriptomic-pod-tpod-and-how-is-it-calculated/195

2 ***Using gene sets to derive tPODs***

Assign all genes with BMCs to gene sets (eg, pathways) → Calculate median gene set BMC → Identify lowest gene set (min. 5% of pathway, 3 genes) → tPOD

A General Comparison of Approaches to Derive tPODs

2. Derivation



- Overall good agreement between data points (robust transcriptomic data)
- Commonly employed approaches may not be reliable
  - Using percentiles (e.g., 5th percentile)
- Other tPODs provide sound alternatives
  - Distribution of single genes (*e.g., the 25th Ranked Gene*)
  - Using gene sets from pathway databases (*lowest and most sensitive gene set*)

BMC Distribution

Gene Set (Pathway)

1st Mode | 25th Gene | 5th Percentile | LCRD

GO | KEGG | Reactome

Ratio of In Vitro Derived AEDs to Apical PODs

3. Conversion

- Convert the transcriptomic point of departure to an administered equivalent dose (httk R-package)

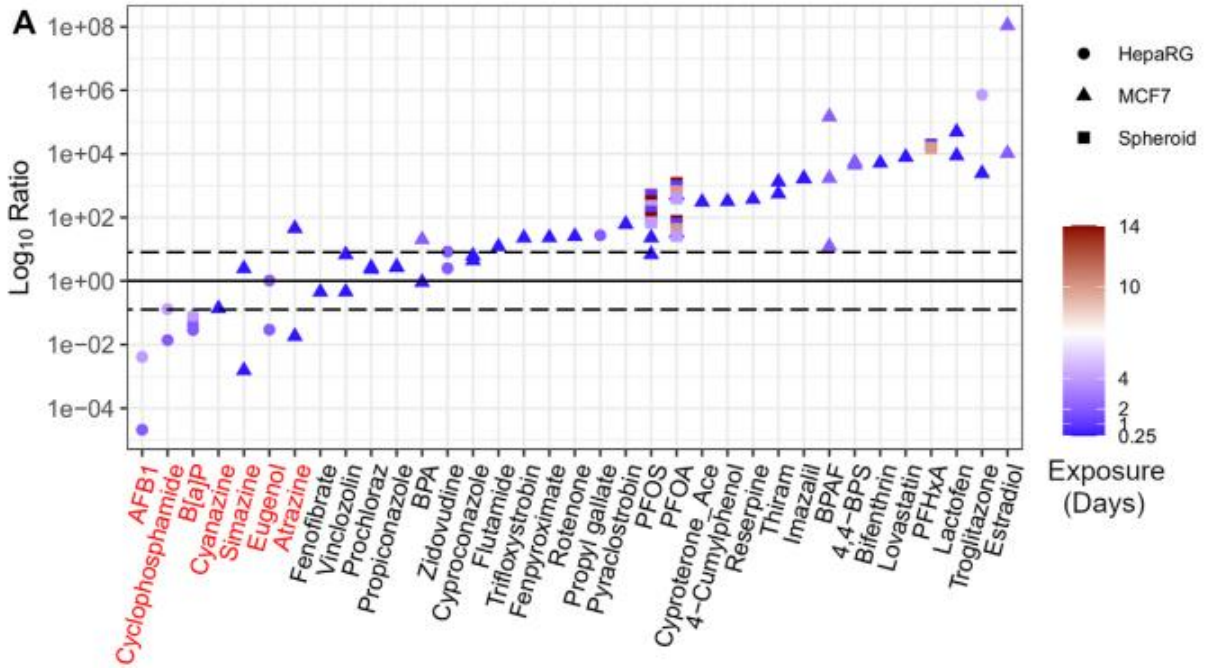
4. Comparison

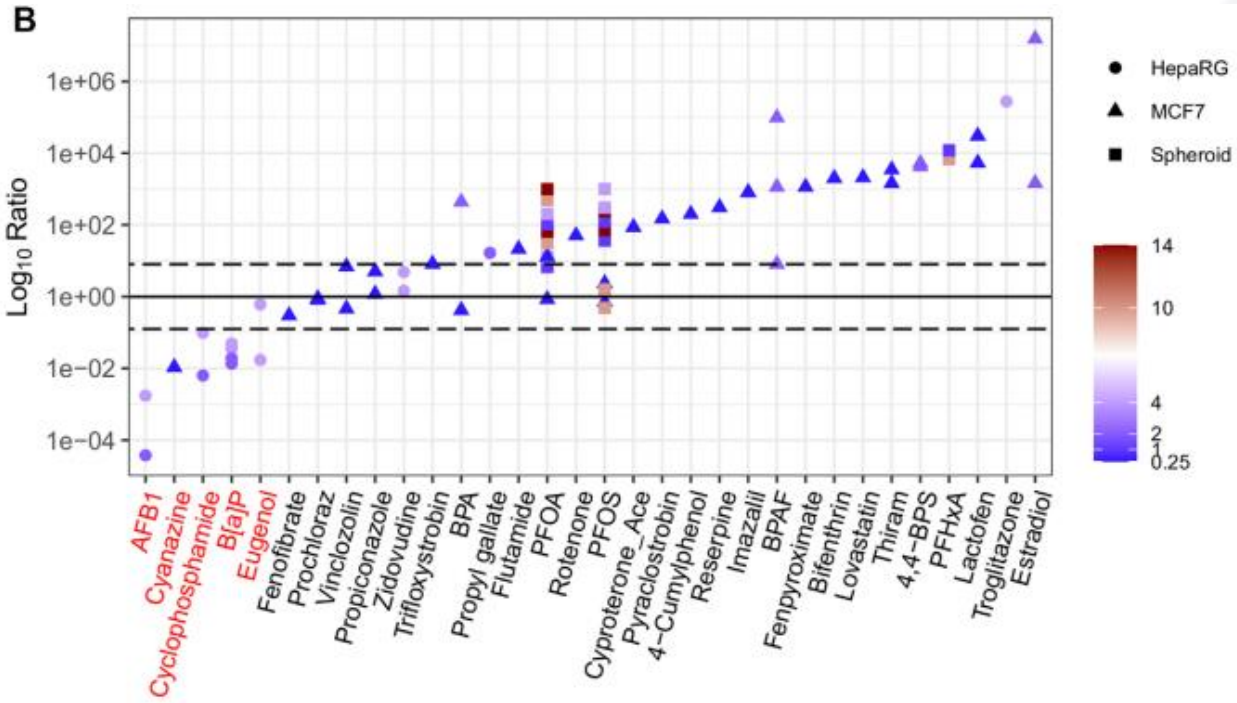
- Comparison to apical PODs from in vivo studies, from available databases: EPA ToxVal, REACH dossier, OECD Toolbox, and the Health Canada dossier

$$Log_{10}Ratio = Log_{10}POD_{Traditional} - Log_{10}AED_{NAM}$$

Identifying outliers using ratios:

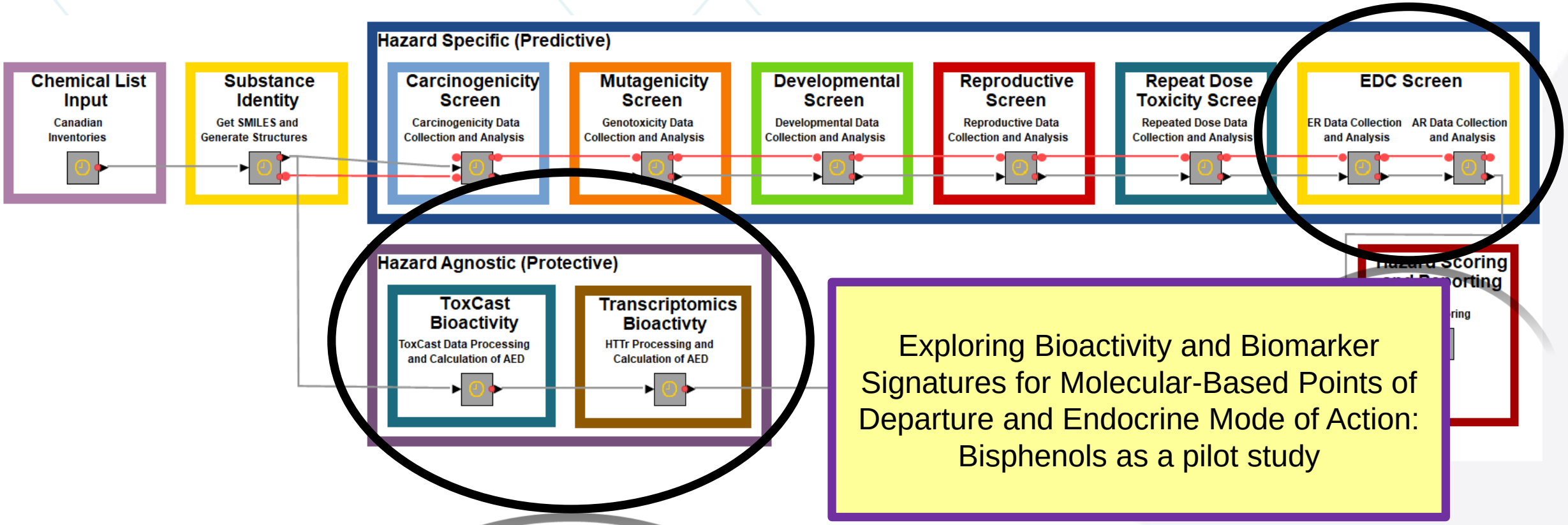
- Compared the lowest derived AED from BMC Distribution approaches (**A**) or Gene Set approaches (**B**) to apical PODs
- Select chemicals (highlighted in red) had apical PODs that were lower than in vitro estimates were flagged





Integrating Data Sources to Support Screening and Assessment

- Integrated Approaches to Testing and Assessment (IATA) represent a flexible framework
  - can include a range of different methods and sources of information;
  - can be assembled in different ways;
  - can be used in different regulatory decision-making contexts depending on assessment questions and protection goals.



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Case Study on the use of Integrated Approaches to Testing and Assessment for potential Systemic Toxicity and Estrogen Receptor Activation of a Group of Bisphenols and Select Alternatives

Series on Testing and Assessment
No. 373

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Research article

In vitro transcriptomic analyses reveal pathway perturbations, estrogenic activities, and potencies of data-poor BPA alternative chemicals

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Pilot Example: An Integrated Approach to Testing and Assessment to Evaluate BPA and Select Alternatives

Health Canada Santé Canada

uOttawa

Step 1 Chemical selection

Step 2 Identify ER activation

Step 3 POD derivation

Step 4 IVIVE extrapolation

Step 5 BER estimation

Chemical structure and properties

A list of BPs identified

In silico

ToxCast

In vitro HTTr

Estrogen activity

Yes

ToxCast

In vitro HTTr (Biomarker & IPA)

Estimate POD based on ER activation (µM)

No

Exposure estimate

AED (mg/kg bw/day)

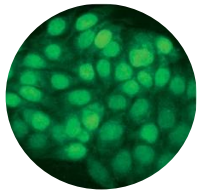
In vitro to In vivo Extrapolation

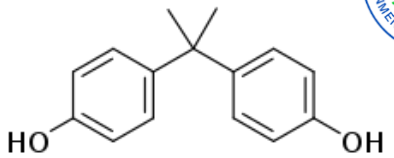
Retrieve tPOD values for all BPs based on non-specific toxicity from Matteo et al. (2023) and ToxCast data

Estimate bioactivity exposure ratio (BER) to identify risk assessment priorities

Lowest available in vivo PODs

Human MCF-7 Cells





BPA and Alternatives (n = 16)

with bisphenol substructure (n = 9)

Functional alternative to BPA (n = 7)

Farmahin and Reardon et al. In Preparation

Creating Practical Estimates for Risk Assessment with Bioactivity Data

Chemical selection

Identify ER activation

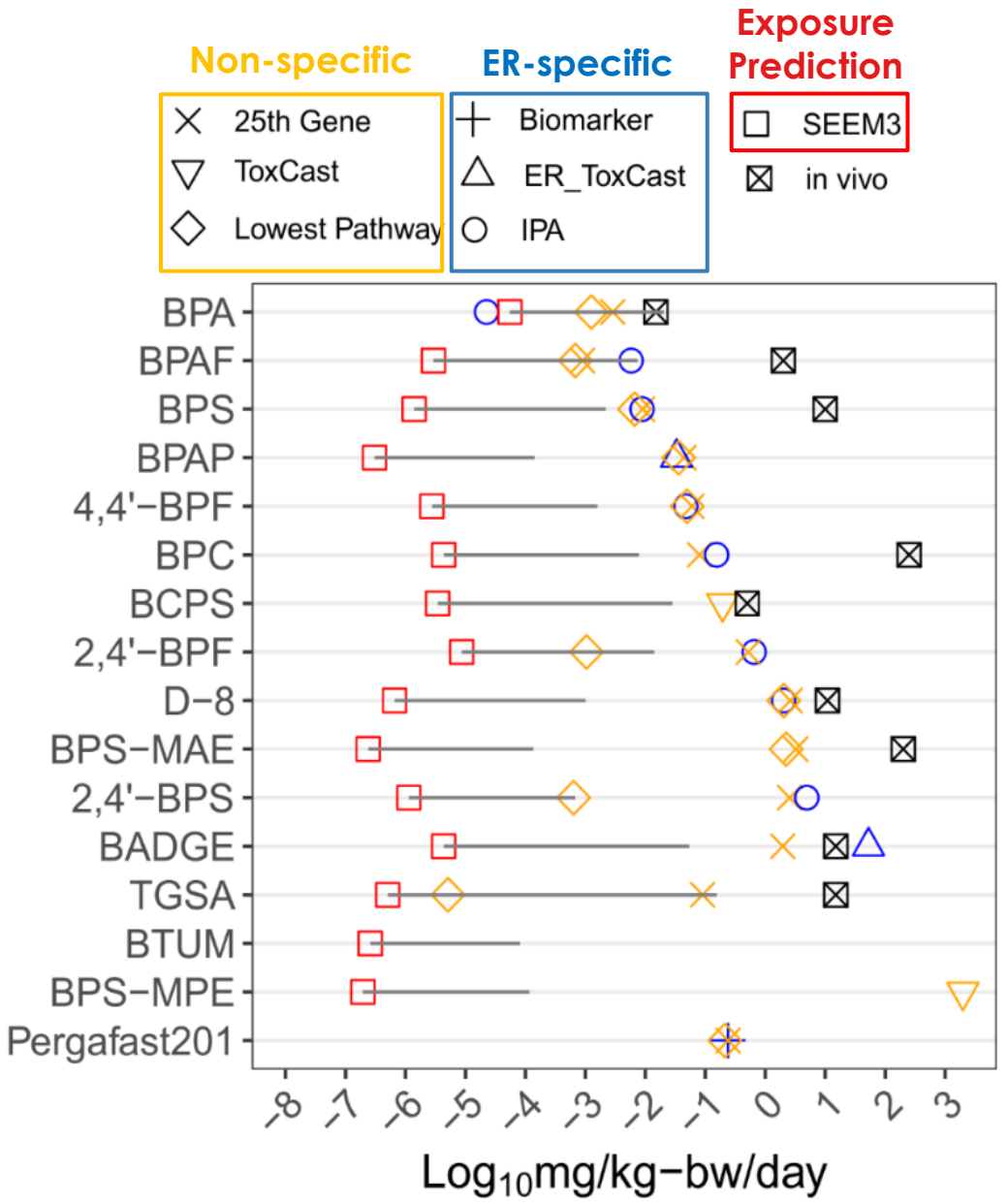
POD derivation

IVIVE extrapolation

BER estimation

- In vitro bioactivity data demonstrated to be robust, with good agreement between approaches
  - 25th ranked gene well aligned
  - lowest gene set overly conservative
- Transcriptomics AEDs for non-specific toxicity produced similar values to ER-specific estimates (when pathway specific values are available)
- Bioactivity based-AEDs typically lower than apical PODs from animal data (RDT, Repro, Dev)

The integration of bioactivity estimates provides practical information on potency and mode of action for hazard and risk characterization



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Learnings & Insights

- Data collection, interpretation and integration workflows facilitate the use of new and increasingly complex information
  - Maintain flexibility to update analysis methods and use best available science
  - Transparent, reproducible & efficient evidence-based decisions through integrated knowledge
- The IV-MBM refined the IVIVE approach for genotoxicants, providing more predictive AEDs from NAM data
  - Greater impact for compounds with higher LogKOW / LogKAW
  - Requires assay specific information often difficult to obtain; reduces throughput
- Technical and practical challenges remain
  - Access to curated datasets -> many transcriptomic studies in literature but lack common data formats and repository
  - Experimental design -> cell lines, organoids, MPS, metabolism, testing strategies
  - Standardization -> 'acceptable' practices - consistent, transparent use in risk assessment (e.g. for tPOD – R-ODAF, OORF)
  - Characterizing uncertainty -> including consideration of uncertainty factors
- Much work to be done to address toxicity endpoints of regulatory interest using predictive approaches

Final Thoughts

- Various NAM techniques are currently available and are being used for supporting hazard assessment and prioritization; context of use is important

- The combination of in vitro bioactivity data with IVIVE provides the opportunity to apply NAM-based AEDs in approaches that are protective of human health
  - Currently being targeted as an early tier assessment
  - Ongoing refinement of interpretation approaches and consideration of mechanistic/pathway analysis

- 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

Questions?

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