



Accelerating the Pace of Chemical Risk Assessment (APCRA): An International Governmental Collaborative Initiative

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

- **What is APCRA?**
- **Participants**
- **APCRA Desired Outcomes**
- **Relevance to ORD StRAPs**
- **History**
- **APCRA Case Studies**
 - Retrospective and Prospective Case Studies to Examine the Utility of NAMs in Screening-level Assessments

- **An international governmental collaboration that brings together governmental entities engaged in development of new hazard, exposure, and risk assessment methods and approaches for their chemical evaluation activities.**
 - To discuss progress and barriers in applying new tools to prioritization, screening, and quantitative risk assessment of differing levels of complexity.
 - To discuss opportunities to increase collaboration in order to accelerate the pace of chemical risk assessment.



- **United States:** EPA, California EPA, NTP, CPSC
- **Canada:** Health Canada
- **Europe:** EChA, EFSA, JRC, INERIS, RIVM
- **Asia:** Korea – Ministry of the Environment, Japan – Ministry of the Environment & Ministry of Health, Welfare and Labour, Singapore – A*STAR, Taiwan – SAHTECH
- **Australia:** NICNAS
- **OECD**

- Common understanding of current state of the science applications of New Approach Methods (NAMs), including the regulatory context.
- Increase cross-Agency collaboration to strategically address barriers and limitations of use of NAMs in a regulatory context.
- Complement member country participation in OECD, RCC, or various bi-lateral collaborations.
- Determine mechanisms to enhance data sharing capabilities.
- Increase engagement and commitment to development and sharing of case studies of mutual interest.



Relevance to the ORD StRAPs

	HTT	REMD	AOP	ETAM	CSA	ISI	HERA
The development of NAMs for specific regulatory contexts							
Approaches to chemical categorization							
Demonstration of NAMs for safety assessment							
Translation and dissemination of data							



History

**APCRA
2016
Washington, DC**

**APCRA-2
2017
Helsinki, Finland**

**APCRA-3
2018
Ottawa, Canada**

**APCRA-4
2019
RTP, NC**

Meeting Themes

- Identification of potential sources of NAM information and how such information could be shared and exploited
- Proposal of collaborative case studies
- Continuation of collaborative case studies.
- Identification of critical data gaps
- Addition of NAMs for exposure analysis
- Establishing confidence in use of NAMs both in terms of comparisons to traditional methods and integrating divergent data streams
- Addition of NAMs for ecological assessment

- **Criteria:**
 - promoting collaboration and dialogue on the scientific and regulatory needs for the application and acceptance of NAMs in clear regulatory context.
 - include international partners on topics of interest to multiple regulatory agencies.

- **Application to Risk Evaluation**
 - Bioactivity as a conservative estimate of PODs
 - Quantitative and qualitative comparison of NAMs and traditional animal toxicity testing for data poor chemicals
 - Use of transcription profiles and primary human liver cells grown as spheroids to address potency and additivity of perfluorinated alkylated substances.
- **Application to Chemical Categorization**
 - Develop NAM profiles based on available data (e.g., highthroughput in vitro assay data) for existing chemical categories
 - Evaluate the effectiveness of EcoNAMs, specifically omics technologies used in conjunction with third-wave machine learning, to derive molecular data for mechanism-driven substance grouping..
- **Application to Exposure Evaluation**
 - Use of innovative modeling and GIS approaches by various agencies for assessing lead exposures
 - Triaging chemical exposure data needs and tools for next-generation risk assessment



Retrospective and Prospective Case Studies to Examine the Utility of NAMs in Screening-level Assessments

Katie Paul Friedman

June 25, 2019

Presented as a CSS Webinar

Based on collaboration with multiple agencies

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Screening level assessment: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

- Conducted by Accelerating the Pace of Chemical Risk Assessment (APCRA)
 - “international cooperative collaboration of government agencies convened to address barriers and opportunities for the use of new approach methodologies (NAMs) in chemical risk assessment” (Paul Friedman et al., submitted)
- Two case studies including a large retrospective analysis and a prospective analysis
- A poster on these two case studies won the Top Abstract Award from the Risk Assessment Specialty Section at SOT 2019



Health
Canada

Santé
Canada



(APCRA partners for these two case studies)



Why is the retrospective case study important?

- Clear need to demonstrate in practical terms, for as many chemicals as possible, how preliminary screening level risk assessment using a new approach methodologies (NAM) based approach would perform when compared to traditional approaches to deriving points-of-departure (PODs).
- Illustrate the current state-of-the-science.
- Evaluate the specific strengths and weaknesses of rapid, screening level risk assessment using NAMs.
- Approach: Take a retrospective look at the traditional and NAM data for as many chemicals as possible (448 at the time).

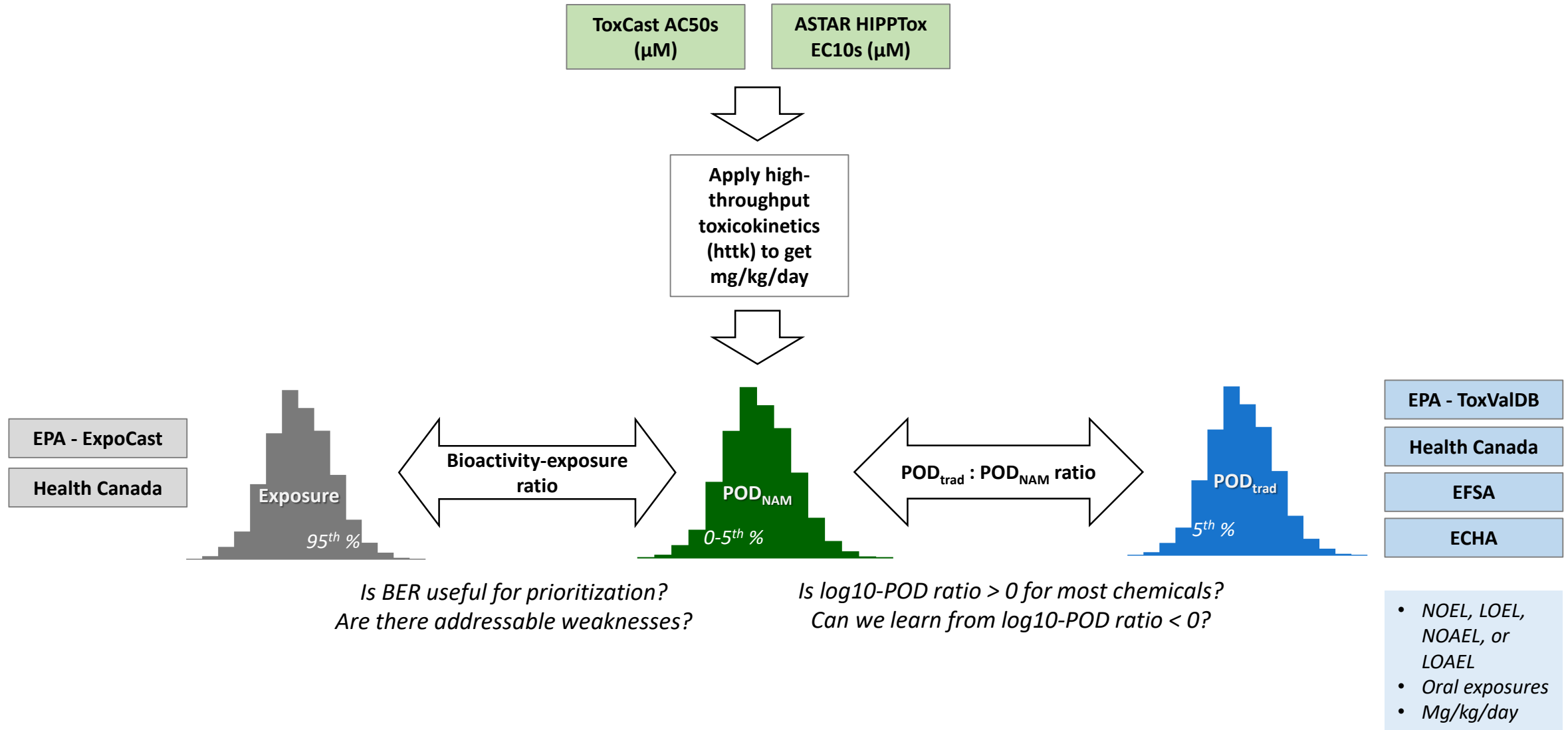


See the forest for the trees

The big question:

Can *in vitro* bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and screening level risk assessment?

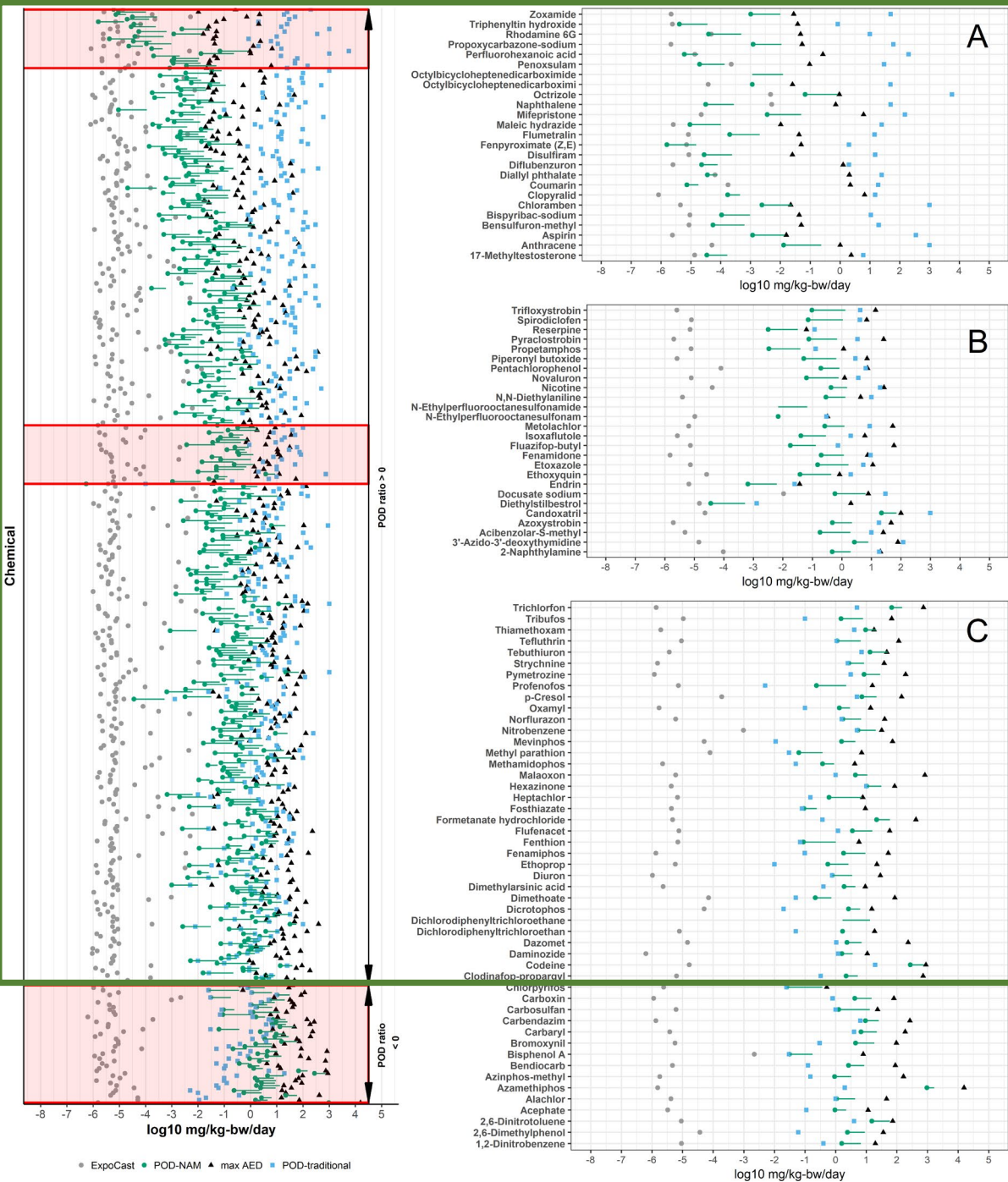
Case study workflow



$POD_{NAM} < POD_{traditional}$
(most of the time)

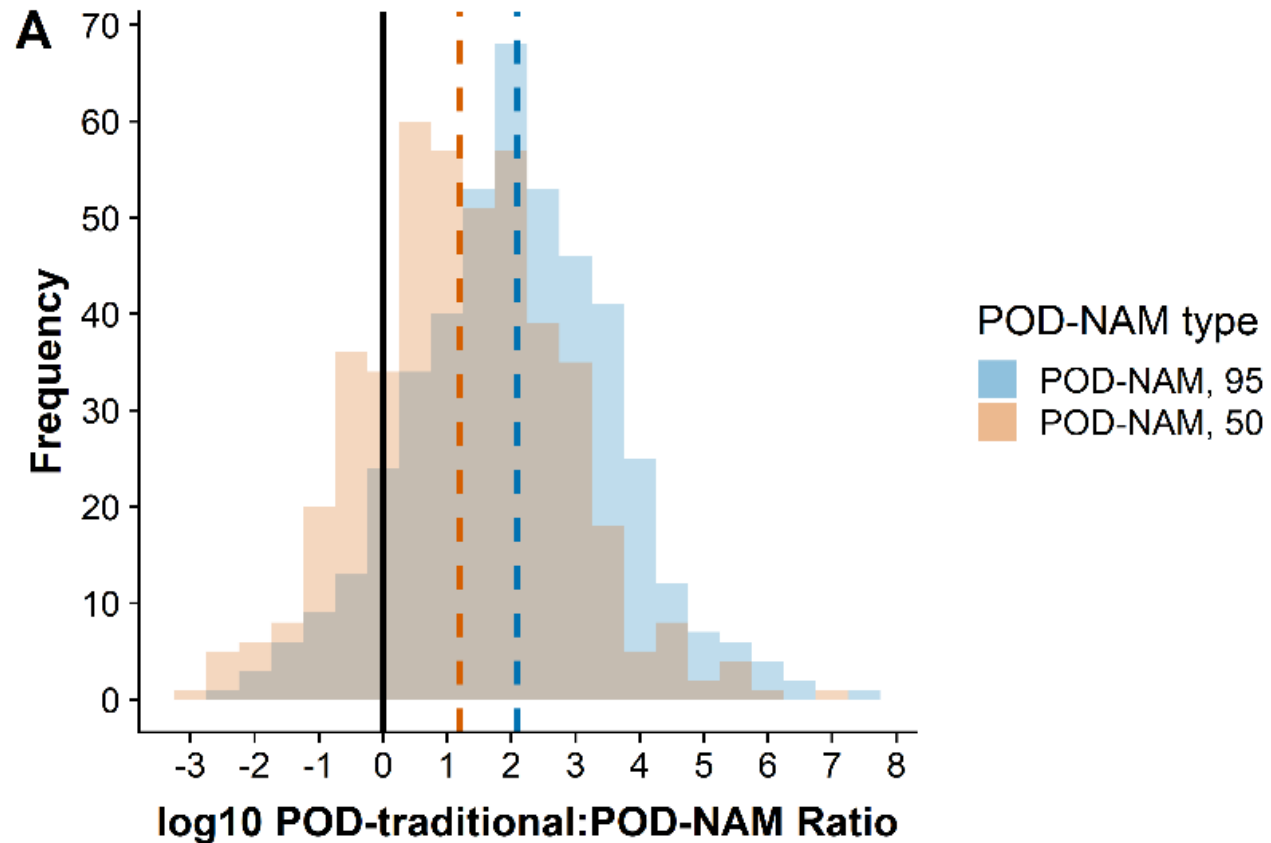
400/448 chemicals =
89% of the time this
naïve approach appears
conservative

48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$





The log₁₀-POD ratio distribution shows POD_{NAM} is generally conservative *and adjustable*.



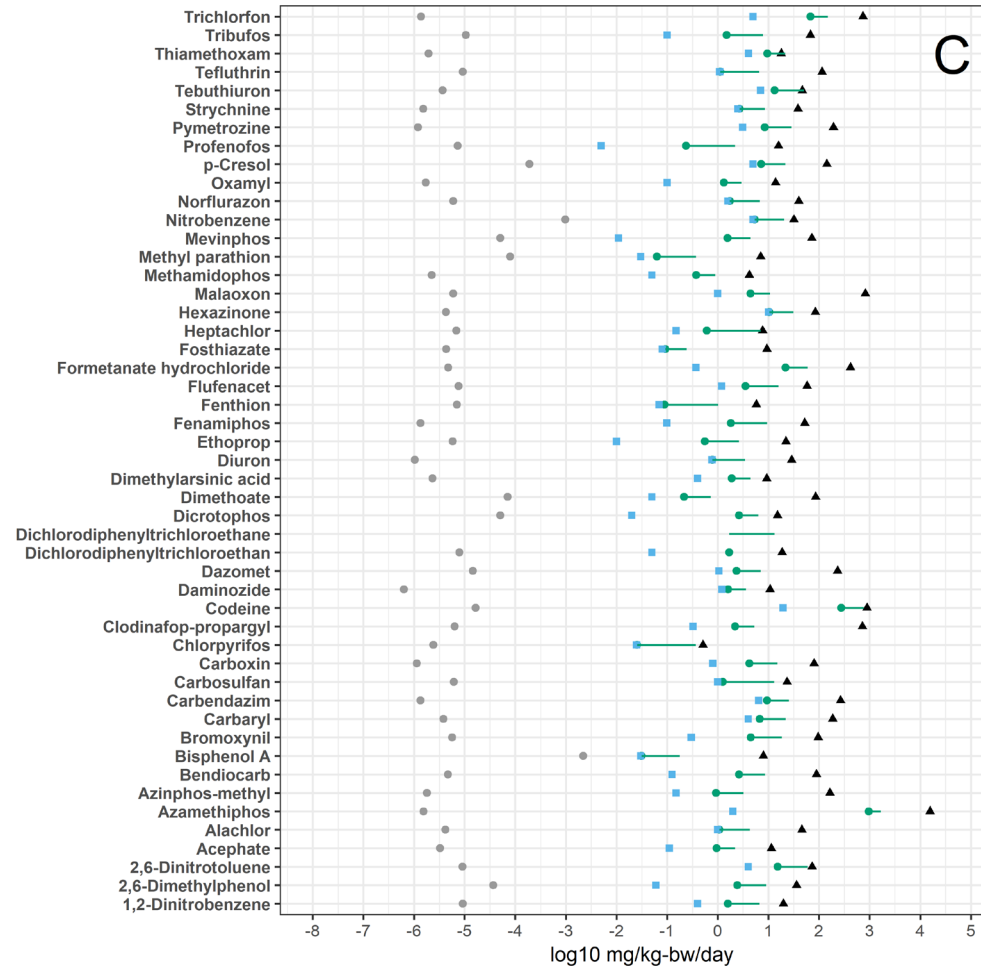
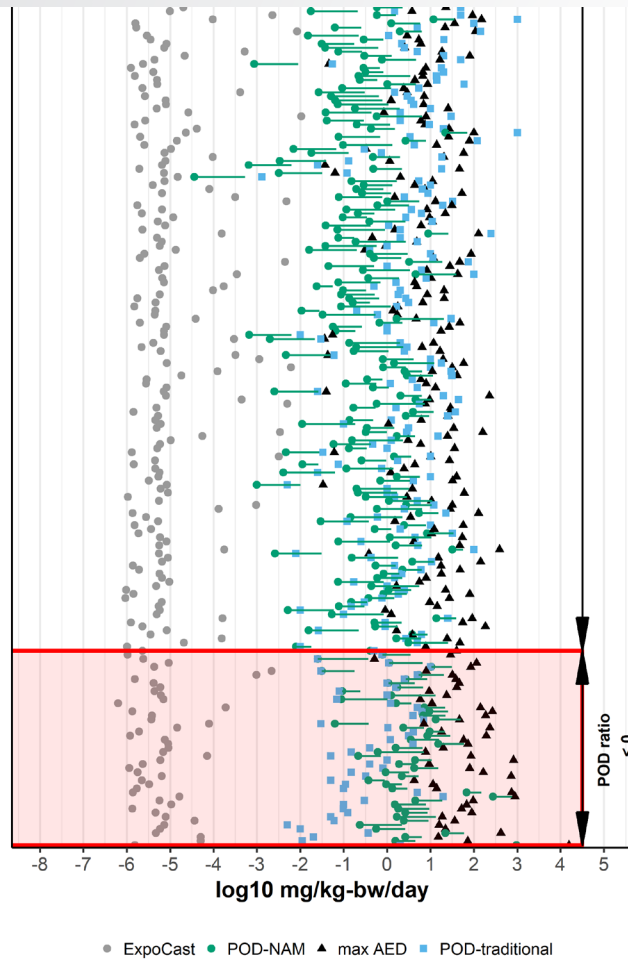
POD_{NAM,95} includes interindividual variability in the in vitro to in vivo extrapolation process to a greater extent, and is more often a conservative estimate of POD_{traditional}.

This should trigger thinking regarding uncertainty and uncertainty factors/safety factors. In the NAM-based process, we have quantitatively informed uncertainty that can be included explicitly at multiple steps in the screening assessment process.

- log₁₀POD ratio is illustrated for the POD_{NAM,95} and the POD_{NAM,50}.
- Using the more conservative (i.e., lower) POD_{NAM,95}, 48 of the 448 substances (10.7%) demonstrated a log₁₀POD ratio < 0 (to the left of the solid vertical line), whereas 92 of the 448 substances (20.5%) demonstrated a log₁₀-POD ratio < 0 using the POD_{NAM,50}.
- The medians of the log₁₀-POD ratio distributions are indicated by dashed lines for POD_{NAM,95} and POD_{NAM,50} as 2 and 1.2, respectively.



Are there key drivers of examples where $\text{POD ratio} \leq 0$?



$$\text{POD}_{\text{NAM}} : \text{POD}_{\text{traditional}} \leq 0$$

- Are some *in vivo* toxicity types poorly captured by ToxCast?
- Are some study types enriched in this space, and difficult to predict from bioactivity?





When the $\log_{10}$ POD ratio < 0 , was it driven by a specific study type (as a surrogate for phenotypes)?

Condition	Dev/Repro is min POD	Dev/Repro is not min POD
$\log_{10}$ -POD ratio, 95 < 0	3	45
$\log_{10}$ -POD ratio, 95 > 0	41	359

Condition	Chronic is min POD	Chronic is not min POD
$\log_{10}$ -POD ratio, 95 < 0	28	20
$\log_{10}$ -POD ratio, 95 > 0	244	156

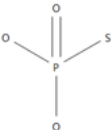
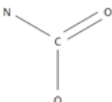
- No.
- *Based on a Fisher's exact test, when $\log_{10}$ POD ratio < 0 , it was not driven by a specific study type.*

Hypothesis	Fisher's exact test results	Caveats
Reproductive and/or developmental studies over-represented when POD ratio ≤ 0 ?	• No • p-value = 0.98; • odds-ratio = 0.26	Some ambiguity or error expected in assigning study classes; preference given to: DNT, neuro, dev/repro, acute, repeat, chronic (in that order) in the event of a min POD tie
Carcinogenicity or chronic studies over-represented when POD ratio ≤ 0 ?	• No • p-value = 0.25; • odds-ratio=1.4	



When the $\log_{10}$ POD ratio < 0 , was it driven by a specific chemical features?

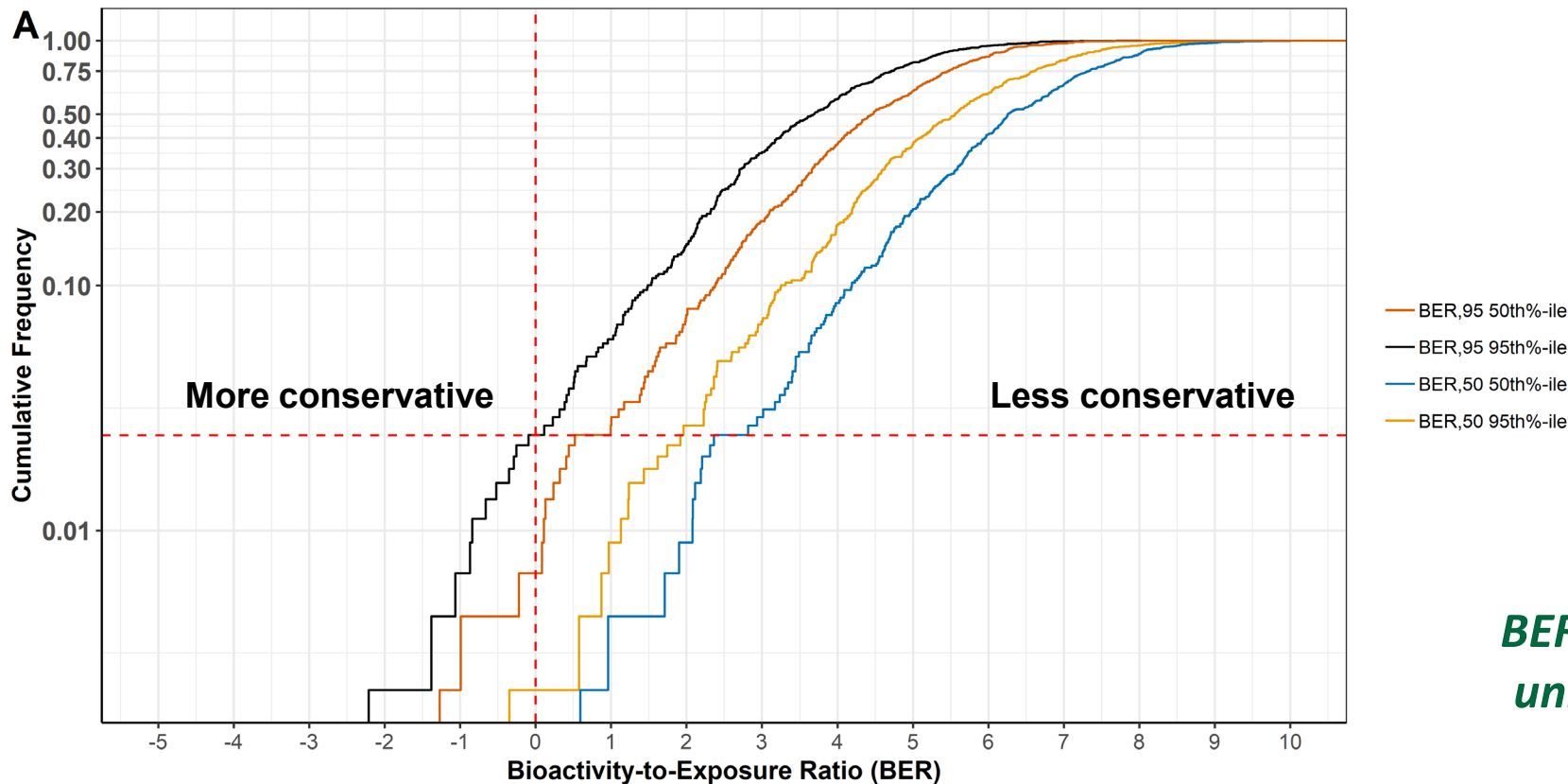
- Yes
- *Based on a Fisher's exact test, chemical features associated with organophosphate pesticides and carbamates are more likely to drive a $\log_{10}$ POD ratio < 0 .*

ChemoType Information		Appearance of the ToxPrint			Metrics			ChemoType Information		Appearance of the ToxPrint			Metrics		
Label	ToxPrint	Total	POD ratio ≤ 0	POD ratio > 0	BA	OR	p-value	Label	ToxPrint	Total	POD ratio ≤ 0	POD ratio > 0	BA	OR	p-value
bond:P=O_phosphorus_oxo		18	12	6	0.62	22	7.4E-09	bond:P~N_generic		5	4	1	0.54	36	0.00055
bond:P=O_phosphate_thio		3	3	0	0.53	NA	0.0012	bond:C(=O)N_carbamate		20	6	14	0.54	3.9	0.014
bond:P~S_generic		27	13	14	0.62	10	3.5E-7	bond:CS_sulfide		53	15	38	0.61	4.3	0.00011

So, we have a sense that a NAM-based POD can be protective of an *in vivo* POD, especially in concert with structure-based strategies like threshold of toxicological concern (TTC). How would prioritization work?



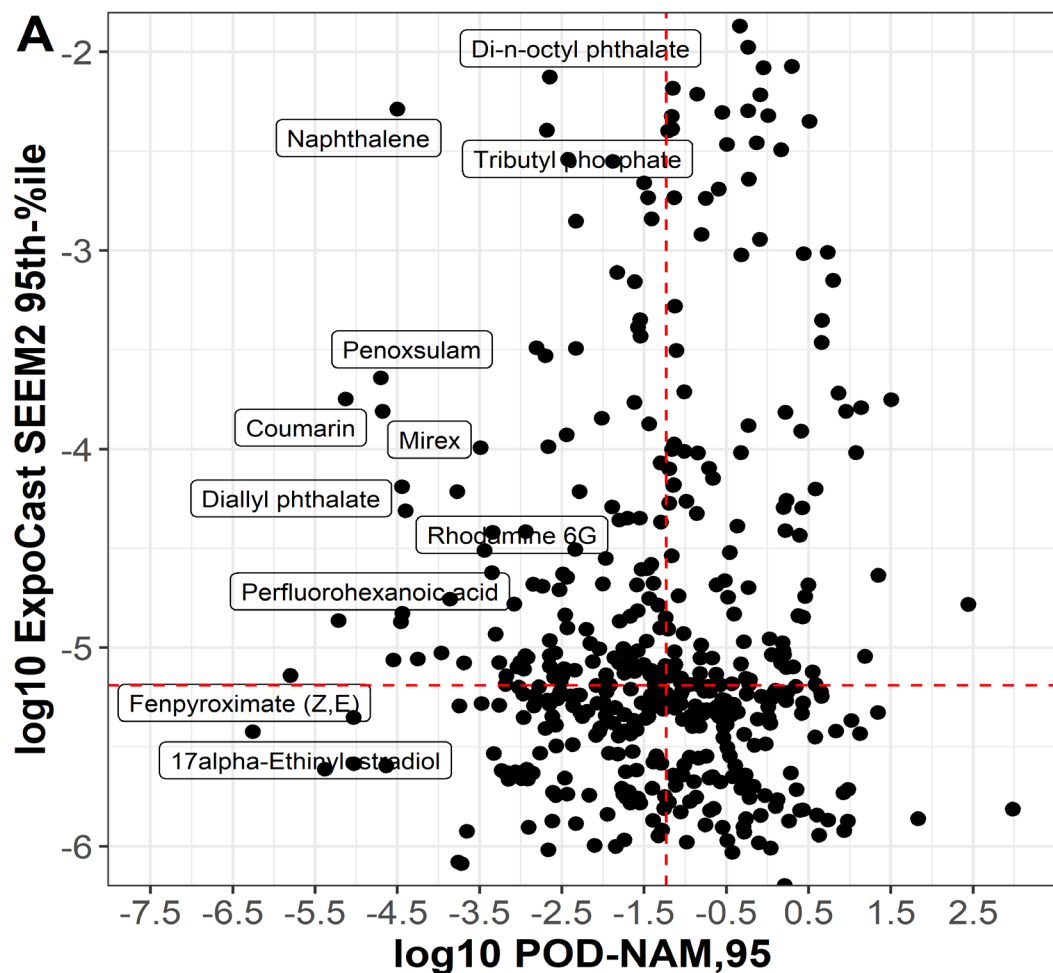
The bioactivity:exposure ratio (BER) provides a way of prioritizing substances for further review.



- Make choices based on tolerable uncertainty (i.e., based on use case).
- BER_{95} used 95th percentile from the credible interval to predict median total US population exposure (ExpoCast SEEM2); BER_{50} the 50th percentile.
- BER_{95} and BER_{50} values were calculated as the “95th%-ile” and “50th%-ile,” using the $POD_{NAM,95}$ and $POD_{NAM,50}$, respectively.

BER_{95} , 95th percentile did not prioritize an unreasonable number of substances; the BER selected reflects the level of conservatism and uncertainty considered within a screening assessment.

Did exposure or bioactivity appear to drive the BER-based priority?



- Compared 95th percentile from the credible interval to predict total US population exposure (ExpoCast SEEM2) to the $POD_{NAM,95}$.
- Dashed lines indicate the median exposure and $POD_{NAM,95}$ estimates for the 448 substances in the case study.

In general for $\log_{10}BER < 0$, the POD was relatively low. For certain substances the exposure estimates were relatively low. Both exposure or POD_{NAM} are estimates that may be refined.



Conclusions and limitations

- An approach to using *in vitro* bioactivity data as a POD appears to be a conservative estimate ~ 90% of the time for 448 chemicals.
- POD_{NAM} estimates appear conservative with a margin of ~100-fold.
- POD_{NAM} may provide a refinement of a TTC approach.
- When combined with high-throughput exposure estimates, this approach provides a reasonable basis for risk-based prioritization and screening level risk assessments.
- Specific types of chemicals may be currently outside the domain of applicability due to assay limitations, e.g., organophosphate insecticides: how do we identify these in the future?
- This is the largest retrospective look at this to-date; but what if new chemicals perform differently? What will be the prospective approach?
- Additional research to include expanded and improved high-throughput toxicokinetics and *in vitro* disposition kinetics may help improve POD_{NAM} estimates.



What about “new” or “data-poor”
chemicals?



How well does a NAM-based approach perform in the prospective case?

- This prospective case study builds upon learnings from the retrospective case study, addressing questions including:
 - Can NAM-based POD estimates be improved using additional technologies or assumptions?
 - Are reasonable NAM-based POD estimates attainable for substances with limited *in vitro* bioactivity?
 - Can BER, and additional hazard flags, be used to select substances for ***in vivo* screening**?

Step 1

Identification of substances with:

- Limited hazard information and exposure potential
- Compatibility for currently available *in vitro* screening methodology

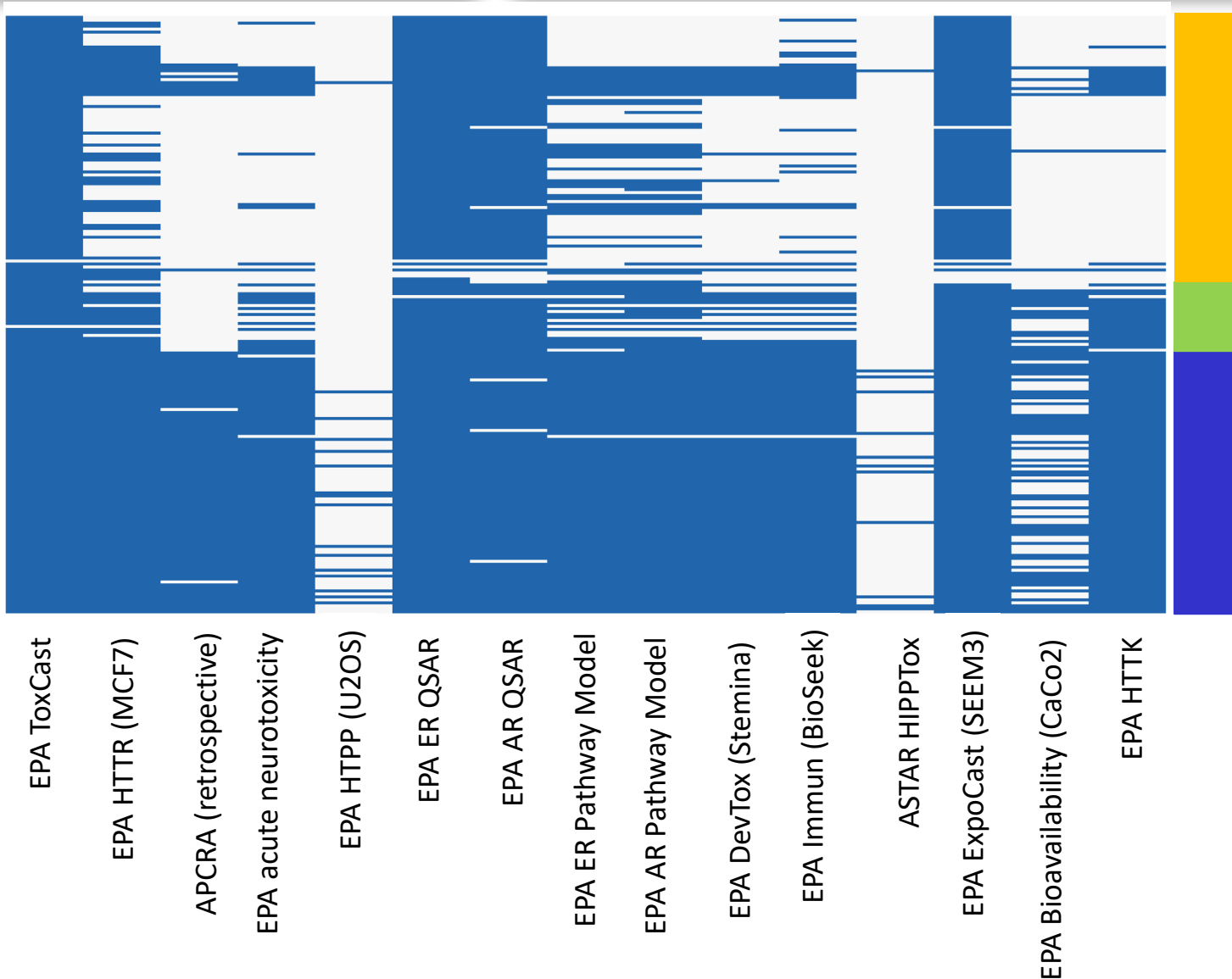
Step 2

Completion of a NAM battery for 200 substances within the substances identified

- Multiple *in vitro* platforms: ToxCast, high-throughput transcriptomics, high-throughput phenotypic profiling, Immunotoxicity assays, acute neurotoxicity assays, developmental toxicity assays, endocrine-relevant assays and models
- High-throughput toxicokinetic information for *in vitro* to *in vivo* extrapolation



Progress on Steps 1 and 2



In 2019, any gaps in this heatmap will be filled.

Scenario 1

Substance present on the EU, Canada, and/or US market, with a potential for consumer use and significant data gaps for systemic toxicity (105).

Scenario 2

Substance present on the EU, Canada, and/or US market, with known toxicity and potential interspecies differences (8).

Scenario 3

Substance selected from the retrospective case study, by sampling substances with varying log10POD ratios.

The BER ($<10^4$) from Step 2, and hazard flags based on potential endocrine, developmental, neuro, and/or immunotoxicity, will be used to advance ~20 substances to Step 3.



Advancing from *in vitro* prioritization to *in vivo* evaluation

Step 3

Confirmatory 5-day *in vivo* testing based on BER and hazard flags (performed by NTP)

- Transcriptomics in liver
- Classical *in vivo* observations and toxicokinetics
- Currently, we are working on advance selection of 3-5 chemicals for this step based on existing NAM data.

Step 4

Further confirmation of a small subset from Step 3 in a 90-day subchronic study (performed by NTP or contract)

Step 5

Evaluation

- Comparison of Step 2-4 data (if available), and any other traditional hazard information



Conclusions on the retrospective and prospective studies

- A major premise of this work is that the minimal concentration corresponding to *in vitro* bioactivity is likely to be a conservative threshold for any specific effects or toxicities that might be observed *in vivo*.
- BER may be a reasonable data-driven metric for prioritization that is tunable based on the amount of uncertainty in (1) the IVIVE that is included in development of the POD_{NAM} and (2) the exposure predictions, highlighting that for different screening applications differing amounts of uncertainty can be included in this workflow.
- The prospective case study furthers confidence, and identifies possible limitations, in NAM-based screening assessments.
- The collaborative, international consideration of these issues in screening level assessments demonstrates the current state-of-the-science and presents a transparent and adaptable basis for utilization of HTS information.



Appendix slides



Conceptual consideration of uncertainties

Uncertainty sources	ToxCast AC50 values	httk model	In vivo PODs	ExpoCast predictions
Biological and Systematic	• Incomplete biological coverage • Assay and curve modeling limitations. • In vitro disposition and/or chemical purity • Is the assay response “adverse,” compensatory, or of unknown importance?	• In vitro data for intrinsic hepatic clearance and plasma protein binding subject to assay limitations, limit of detection, and in vitro disposition issues. • Currently assume 100% bioavailability. • Inter-individual variability. • IVIVE concordance.	• The reproducibility of the PODs, and the inherent variance in POD derivation, is not described here. • Human relevance of the animal data.	• Heuristic model, trained using assumptions and limitations of NHANES data. • Specific use scenarios are not defined.
Added by interpretation and use in this case study	• Use of AC50 instead of another modeled activity level.	• Default to a model with no partition coefficients and use of steady-state concentration which may not be appropriate for all chemicals. • Evaluation of AUC and $C_{\max}$ could be added at a later date.	• Lack of a controlled vocabulary for effects. • PODs were limited to NOEL/LOEL/NOAEL/LOAEL.	NA
How it is considered	• Caution flag + hit pct filtering. • 5%-ile of the distribution of all available AC50s was taken.	• Interindividual variability in toxicokinetics is incorporated via a Monte Carlo simulation; we take the 95%-ile (lower dose).	• We derived a distribution of PODs for each chemical and took the 5%-ile.	• We take the 95%-ile on the CI for the median for the total population (adds about 2 log's of conservatism)

1. Prospective Case Study to Assess Chemicals Using New Approach Methodologies (NAMs) – EChA

- Partners: Health Canada, EPA, JRC, EC, RIVM, EFSA, A*STAR, NTP
- assess chemicals with very limited toxicological data and significant potential exposure, using both NAM and traditional repeat dose toxicological studies to inform the further development needs for NAM

2. Revisiting and Updating Chemical Categorizations with NAMs – US EPA and Health Canada

- Partners: ECCC (Environment and Climate Change Canada)
- develop the machinery to cluster and categorize chemicals based on the available bioactivity data and structural information represented in available in vitro assays.

3. Triaging Exposure Data and Modeling Needs for Exogenous Chemicals – US EPA

- Partners: : Health Canada, ECHA
- Evaluate the landscape of different levels of information required for generating defensible exposure predictions for use in RA for a set of case study chemicals.

4. NAMs for Assessing Endocrine Disrupting Properties - INERIS

- Partners: OECD, Health Canada, EPA, ECVAM
- Construct a database on New Approach Methods (NAMs) that can be actually applied for assessing endocrine disrupting properties of substances or mixtures in environmental samples.

1. Applications for read-across and additivity in risk assessment of emerging PFAS – Health Canada

- Partners: NIEHS, ASTAR
- Use of transcription profiles and primary human liver cells grown as spheroids to address potency and additivity of perfluorinated alkylated substances.

2. Substantiating Chemical Categories with Omics-derived Mechanistic Evidence (SuCCess)– ECHA

- Partners: EPA, ECCC, Japan, HC
- Evaluate the effectiveness of EcoNAMs, specifically omics technologies used in conjunction with third-wave machine learning, to derive molecular data for mechanism-driven substance grouping..

3. Evaluation of the zebrafish (*Brachydanio rerio*) model as an in vivo NAM that serves as an alternative to rodent assays for validating in vitro assays in the assessment of chemicals for general toxicity and endocrine disruption– Health Canada

- Partners: NTP, ECCC
- Evaluate the performance of the National Research Council (NRC) of Canada zebrafish larval and embryo assay, relative to conventional repeated-dose rodent assays, for predicting the potential of chemicals for general (systemic) toxicity and endocrine disruption, using conventional hazard assessment parameters and transcriptomics.

4. Investigating the applicability of bioactivity data to inform quantitative hazard assessments for ecological species using bioactivity-to-exposure ratios (eco-BER)- ECCC

- Partners: Health Canada, EPA, JRC, USGS, US ACE, ECHA, Germany
- inform how in vitro bioactivity data could be leveraged as a quantitative line of evidence to estimate maximum acceptable toxicant concentrations (MATCs) and to evaluate how those compare to MATCs derived from traditional aquatic toxicity studies.

1. Retrospective Case Study Examining the Utility of In Vitro Bioactivity as a Conservative Point of Departure:– US EPA and Health Canada

- Partners: EChA, EFSA, A*STAR
- elucidate whether a “region of safety” (ROS), i.e. a threshold below which no bioactivity or toxicity would be anticipated, can be identified using NAMs for a list of chemicals with existing human health evaluations.

2. Linking Exposure to Toxicology Using Lead as Case Study – US EPA

- Partners: EFSA, CalEPA, INERIS
- Advancing the science and pace of multimedia chemical risk assessments using higher-tier exposure models and biomonitoring information through two data-rich case studies: aggregate multi-pathway lead exposures.

1. Practitioner Insights: Bringing New Methods for Chemical Safety into the Regulatory Toolbox; It is Time to Get Serious, R.J. Kavlock (2016) BNA Daily Environment Report,
http://news.bna.com/deln/DELNWB/split_display.adp?fedfid=100707248&vname=dennotallissues&split=0
2. Accelerating the Pace of Chemical Risk Assessment, R.J. Kavlock, T. Bahadori, T.S. Barton-Maclaren, M.R. Gwinn, M. Rasenberg, and R.S. Thomas (2018) Chem. Res. Toxicol. 31 (5):287-290 <https://pubs.acs.org/doi/10.1021/acs.chemrestox.7b00339>
3. Insight: New Approaches to Chemical Assessment: A Progress Report. T.S. Barton-Maclaren, M.R. Gwinn, R.S. Thomas, R.J. Kavlock, M. Rasenberg (2019) BNA Daily Environment Report,
<https://news.bloombergenvironment.com/environment-and-energy/insight-new-approaches-to-chemical-assessment-a-progress-report>