Session 1189
Abstract 1192
9:05 am Pacific
Remote presentation

Integration of New Approach Methodologies for Prospective Selection of Chemicals for Additional Study

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Presentation in "Computational Toxicology Workflows for Prioritization and Safety Assessment of Environmentally Relevant Chemicals"

Society of Toxicology 61st Annual Meeting in San Diego, CA

March 30, 2022

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 or any other members of APCRA.

Conflicts of interest

- 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
- No additional conflicts of interest

APCRA prospective case study has 3 major phases and aims to bridge new approach methods (NAMs) to the need for any additional data in an international context

In silico and *in vitro* NAMs for toxicodynamics and toxicokinetics

~200 substances

Goal: Point of departure (POD) estimates and insights into hazard

5-day rodent studies using transcriptomics in liver/kidney

~20 substances

Goal: Greater certainty in POD

Development of a NAM-enhanced 90-day study?

# of substances tbd

Goal: Confirmation of POD from 5-day studies and/or hazard profile, if needed

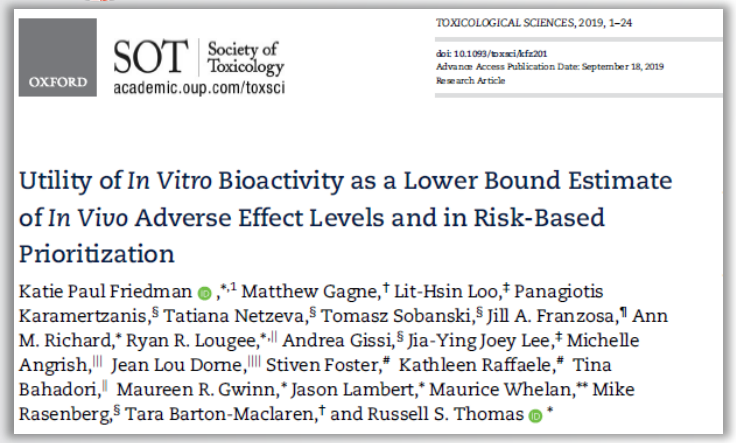
- Building confidence in the connections between NAMs and traditional toxicology studies
- Inform needs for data-poor substances in an international context

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

Led by Tomasz Sobanski at ECHA with contributions from scientists across the world

Can we build a broad NAM-informed framework that is protective and predictive of *in vivo* effects, with more biological information?

Thesis statement: we can use toxicodynamic and toxicokinetic NAMs to inform selection of "data-poor" chemicals for additional screening in models such as a 5-day assay.


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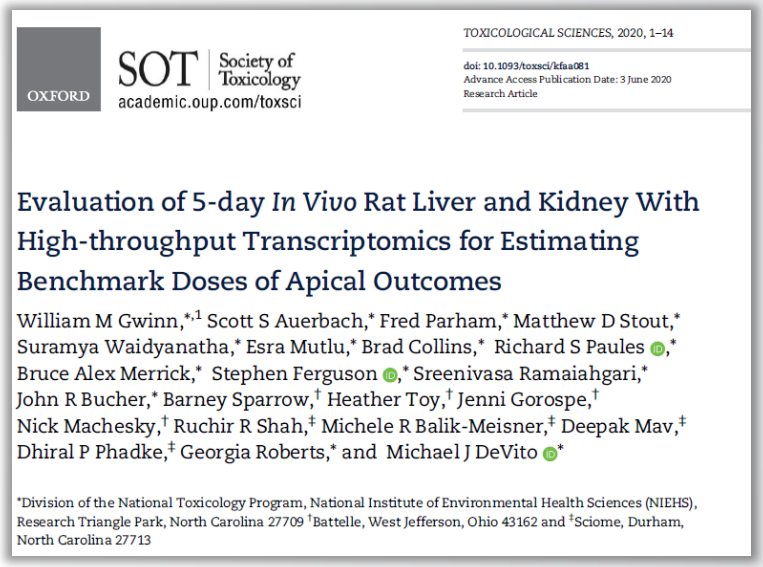
TOXICOLOGICAL SCIENCES, 2019, 1–24
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

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 *

Several key retrospective learnings for 448 data-rich chemicals included:

- A protective point of departure (POD) based on *in vitro* new approach methods (NAMs) could be derived for most chemicals
- PODNAM informed bioactivity:exposure ratios for prioritization
- PODNAM was useful as a comparator to threshold of toxicological concern (POD_{TTC})


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Research Article

Evaluation of 5-day *In Vivo* Rat Liver and Kidney With High-throughput Transcriptomics for Estimating Benchmark Doses of Apical Outcomes

William M Gwinn,*,[1] Scott S Auerbach,* Fred Parham,* Matthew D Stout,* Suramya Waidyanatha,* Esra Mutlu,* Brad Collins,* Richard S Paules ,* Bruce Alex Merrick,* Stephen Ferguson ,* Sreenivasa Ramaiahgari,* John R Bucher,* Barney Sparrow,[†] Heather Toy,[†] Jenni Gorospe,[†] Nick Machesky,[†] Ruchir R Shah,[‡] Michele R Balik-Meisner,[‡] Deepak Mav,[‡] Dhiral P Phadke,[‡] Georgia Roberts,* and Michael J DeVito *

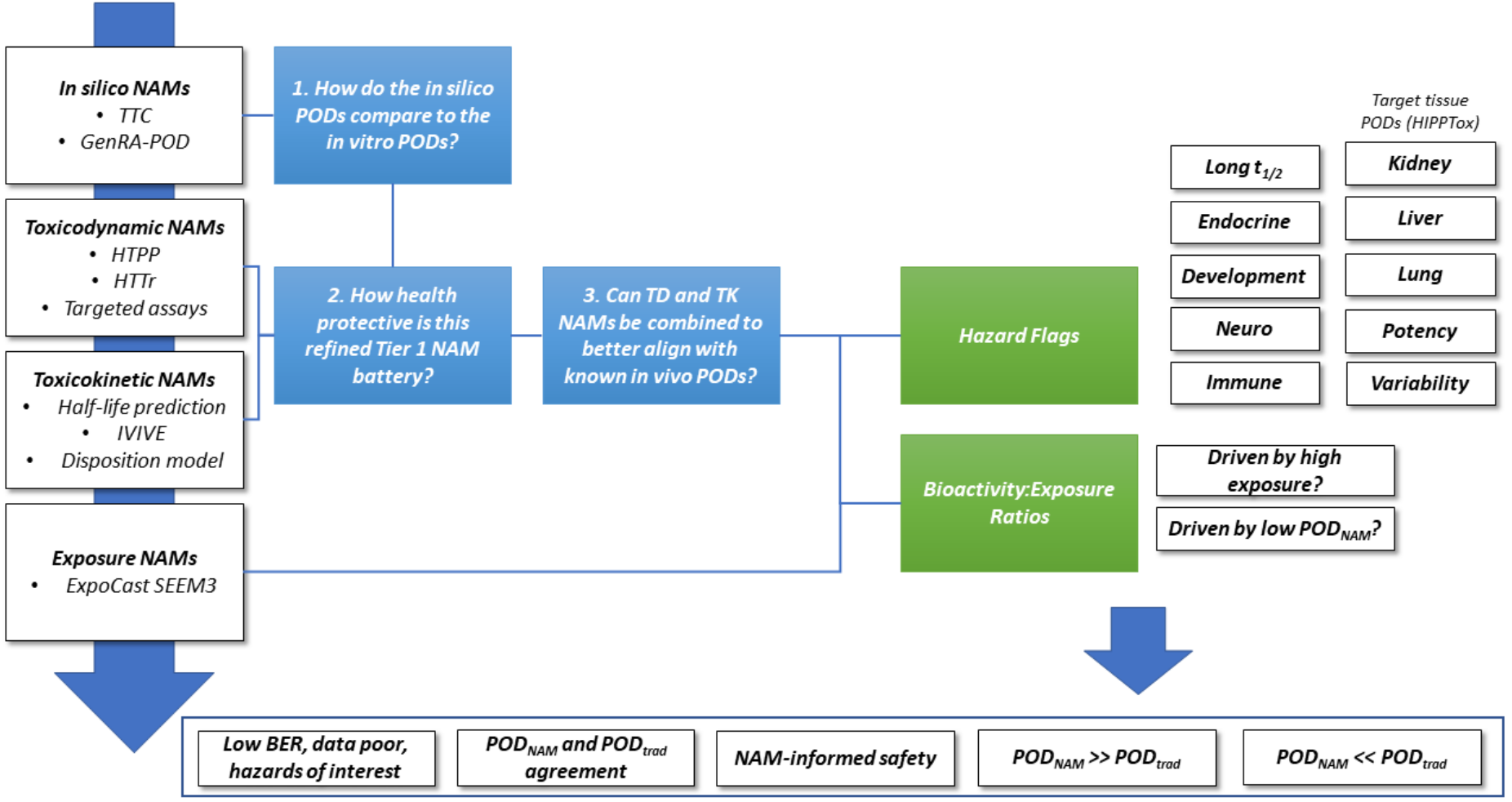
*Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina 27709 [†]Battelle, West Jefferson, Ohio 43162 and [‡]Sciome, Durham, North Carolina 27713

Several key learnings for 18 data-rich chemicals included:

- A lower bound POD based on high-throughput transcriptomic data in the liver and kidney were largely within a factor of 5 for the lowest *in vivo* histopathological PODs from 90d and 2yr repeat dose studies in rodents
- This 5-day paradigm could inform estimates of chemical exposure that produce minimal bioactivity

In silico and *in vitro* NAMs are combined prospectively to identify chemicals with putative hazard and BER based prioritization

Figure 1. Mapping the approach.

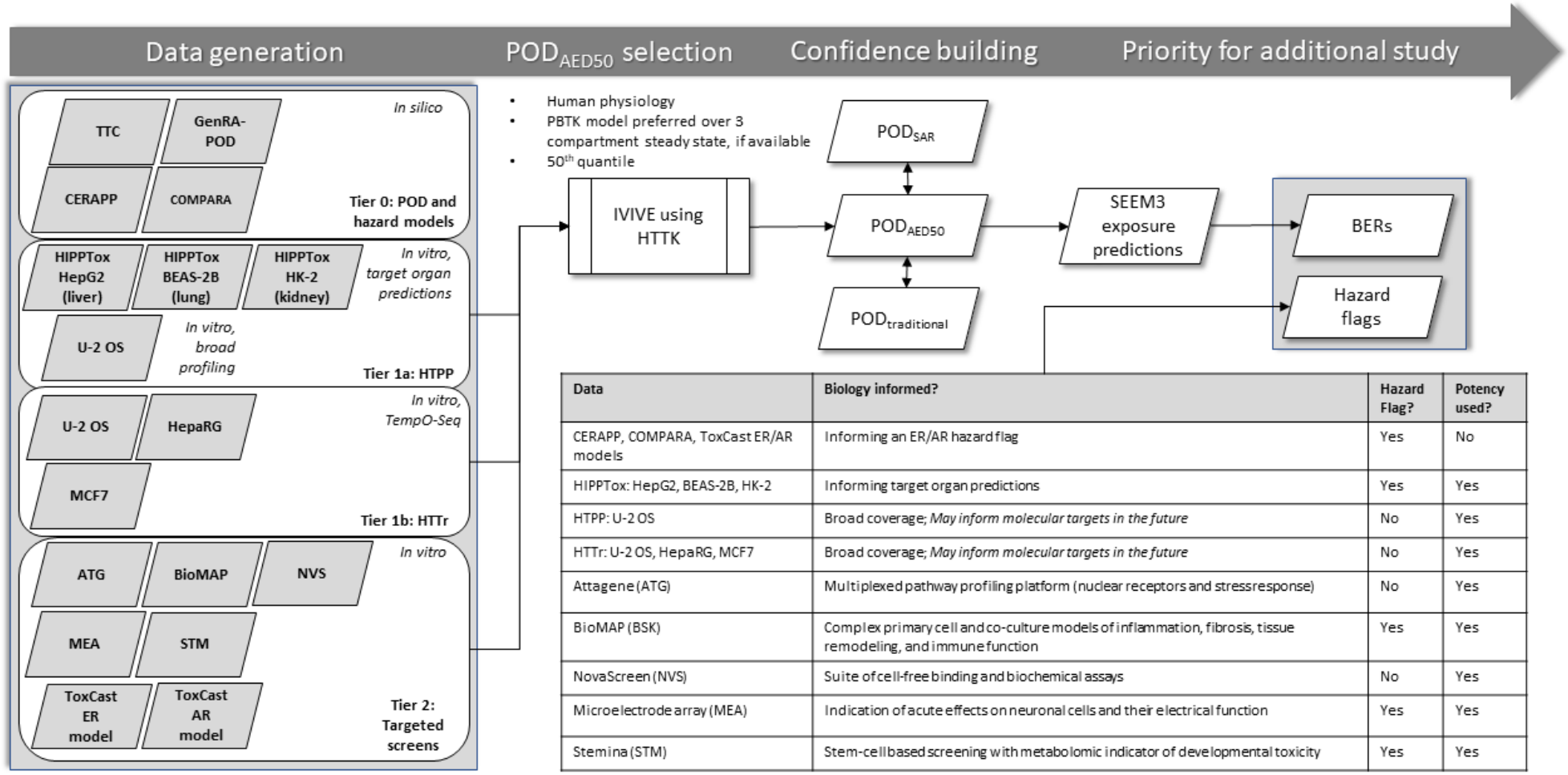


- Refine assay battery and include assays with broad biological coverage
- Refine IVIVE approach
- Experiment to understand which data may be most informative of $POD_{traditional}$
- Include indicators of putative hazard and related interests (hazard flags)
- Include updated exposure predictions for BER

5 potentially overlapping groups that the NAM data can inform for selection of chemicals for additional screening

More detail on the data generated and how it informed the workflow

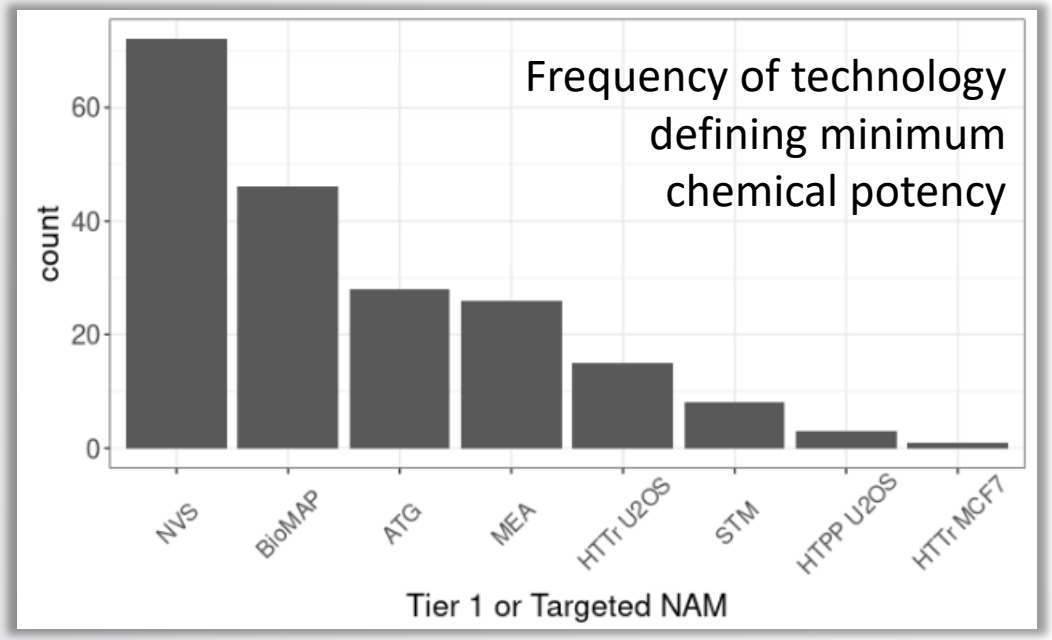
Figure 2. Combining the toxicodynamic and toxicokinetic NAMs.

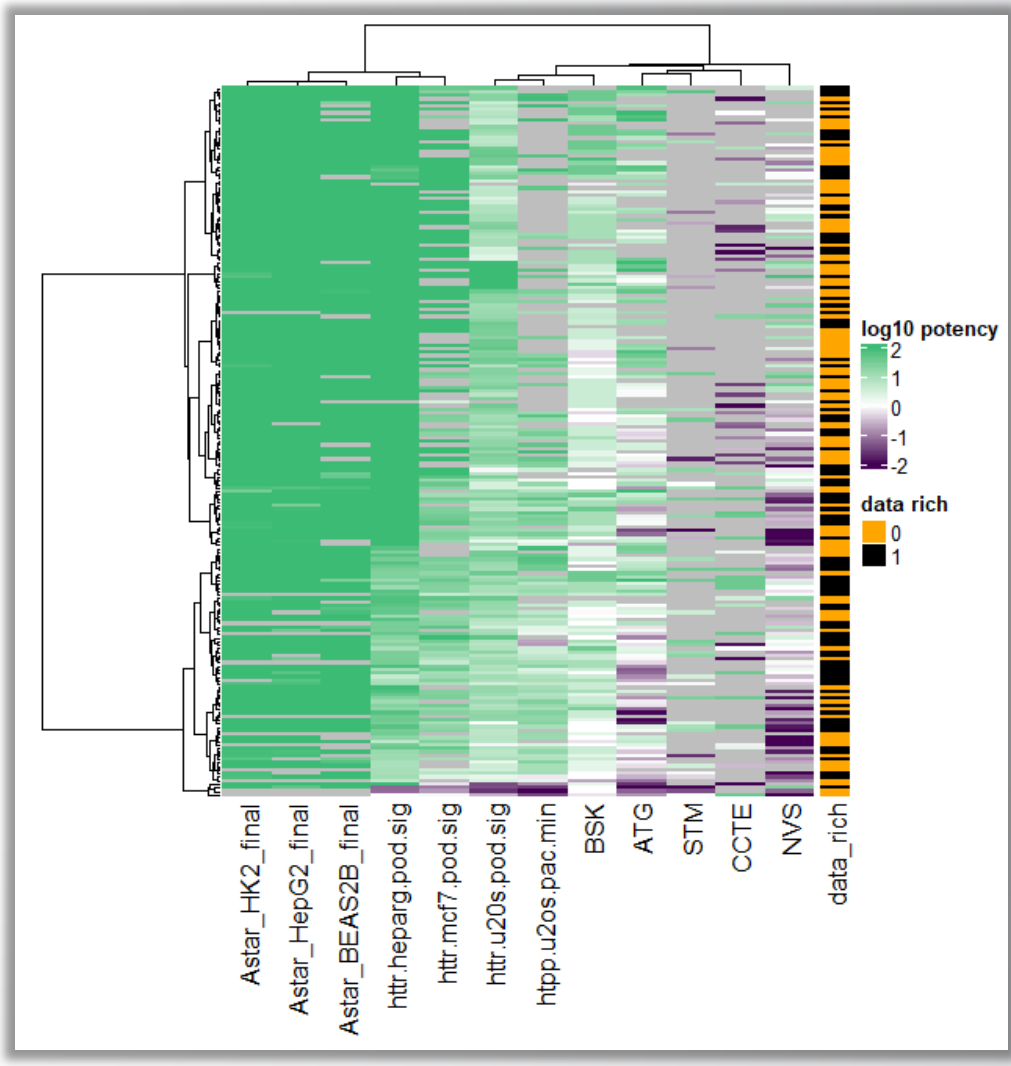


Data	Biology informed?	Hazard Flag?	Potency used?
CERAPP, COMPARA, ToxCast ER/AR models	Informing an ER/AR hazard flag	Yes	No
HIPPTox: HepG2, BEAS-2B, HK-2	Informing target organ predictions	Yes	Yes
HTPP: U-2 OS	Broad coverage; *May inform molecular targets in the future*	No	Yes
HTTr: U-2 OS, HepaRG, MCF7	Broad coverage; *May inform molecular targets in the future*	No	Yes
Attagene (ATG)	Multiplexed pathway profiling platform (nuclear receptors and stressresponse)	No	Yes
BioMAP (BSK)	Complex primary cell and co-culture models of inflammation, fibrosis, tissue remodeling, and immune function	Yes	Yes
NovaScreen (NVS)	Suite of cell-free binding and biochemical assays	No	Yes
Microelectrode array (MEA)	Indication of acute effects on neuronal cells and their electrical function	Yes	Yes
Stemina (STM)	Stem-cell based screening with metabolomic indicator of developmental toxicity	Yes	Yes

In vitro bioactivity screening data were generated for ~200 chemicals, including 96 "data-rich" chemicals and 104 relatively "data-poor" substances

- Large data collection exercise was undertaken with EPA, ASTAR, and JRC using chemicals identified from the ToxCast chemical library that were "data-poor" and/or were of regulatory interest
- Overlap with APCRA retrospective project allows for evaluation of results
- *In vitro* potency generally spanned 3 orders of log10 magnitude, with most potencies in the 1-100 micromolar range.
- Some technologies defined the minimum potency more frequently.



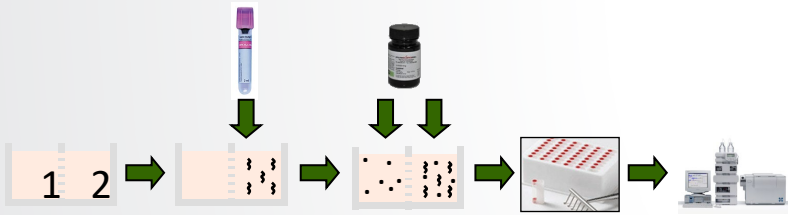


IVIVE approach based on R library 'httk'

in vitro data

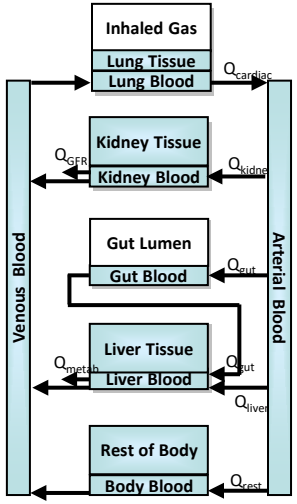
Hepatic clearance from suspended hepatocytes





Plasma protein binding

Generic toxicokinetic models



= *httk*

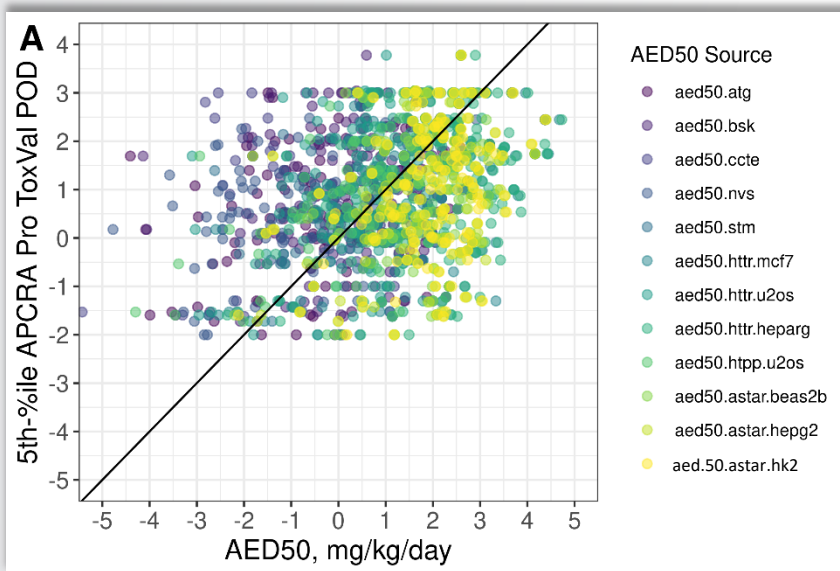
- Preference to PBTK model over 3 compartment steady state model
- Preference to in vitro HTTK data over in silico HTTK predictions
- Predictive modeling of available estimates of a lower bound *in vivo* POD using AEDs from 3 compartment steady state or PBTK modeling failed to show unique improvement

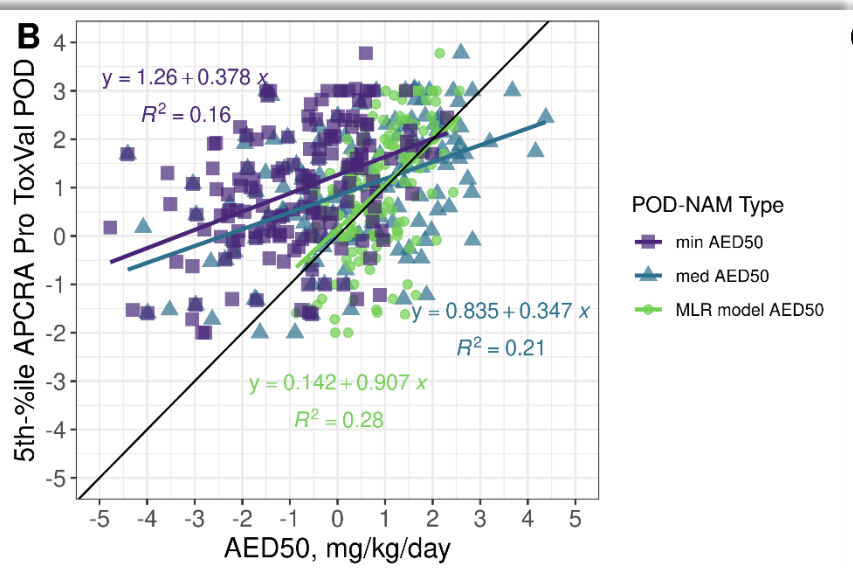
Using 156/195 chemicals with POD_{AED50} and $POD_{traditional}$ suggest approaches for selecting a benchmark POD_{AED50}

(A) Minimum AED50s by assay technology fail to suggest that a single technology can accurately predict estimates of $POD_{traditional}$

(B) The median from the set of minimum AED50s by assay technology performs fairly well in predicting estimates of $POD_{traditional}$

(C) Predicting estimates of $POD_{traditional}$ with TD and TK NAMs resulted in RMSE that approach 1 to 1.2 log_{10}-mg/kg/day





C

	Min AED50 Model	Med AED50 Model	MLR AED50 Model
RMSE	1.182	1.147	1.098
R-squared	0.164	0.213	0.279

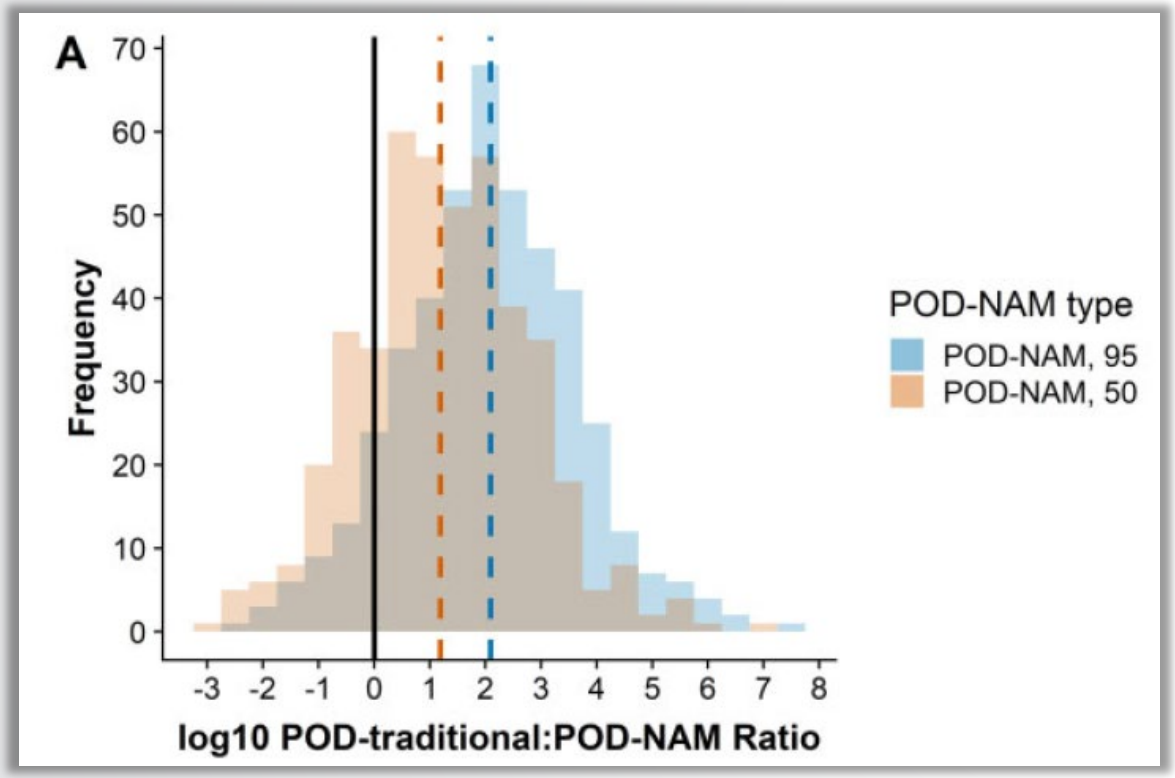
HIPPTox data from BEAS2B, HepG2, and HK2 cells are designed to yield estimates of tissue adversity and tend to be higher than other *in vitro* technologies

A multi-linear regression model performs slightly better than the median. Other machine learning models failed to reduce the RMSE below 1.1 log_{10}-mg/kg/day.

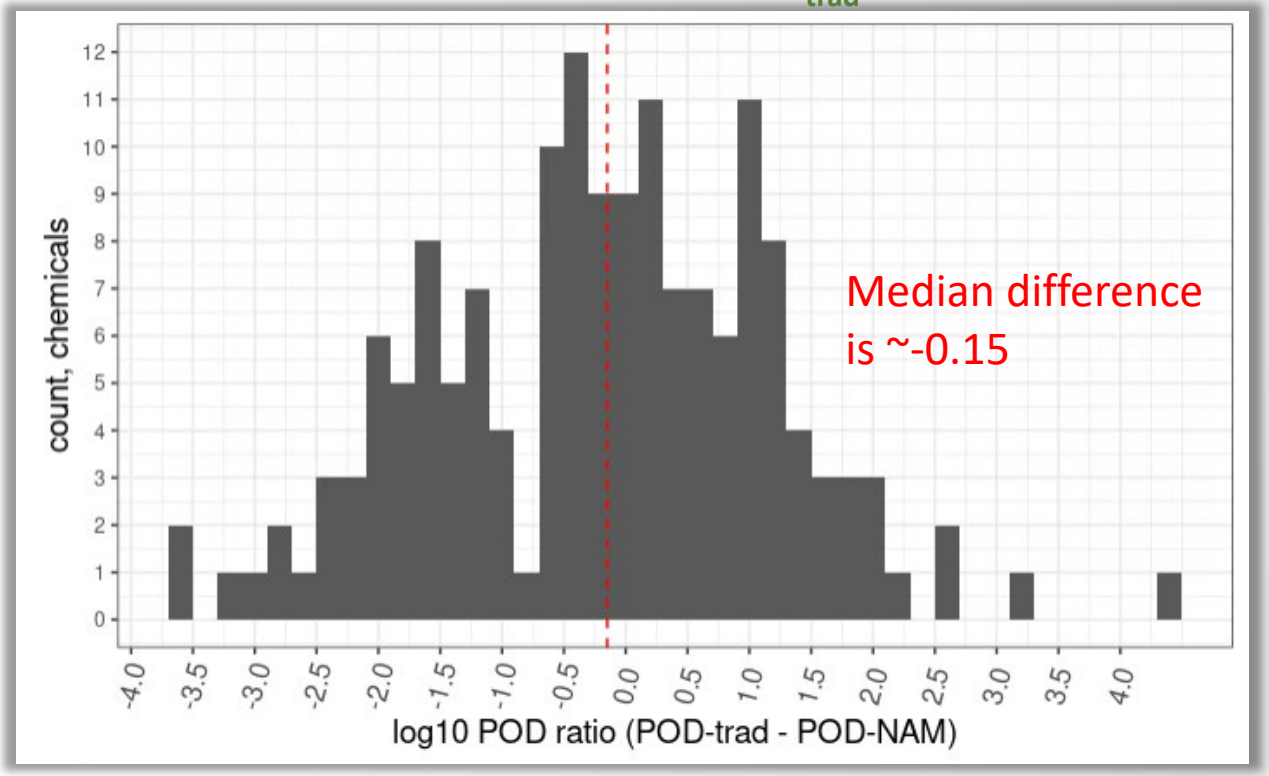
If no other data were available, a possible adjustment factor to ensure conservatism for using POD_{AED50} could be ~ 1 log_{10}-mg/kg/day

How does the overall level of conservatism of POD compare to the retrospective case study?

Retrospective paper (Fig 7)

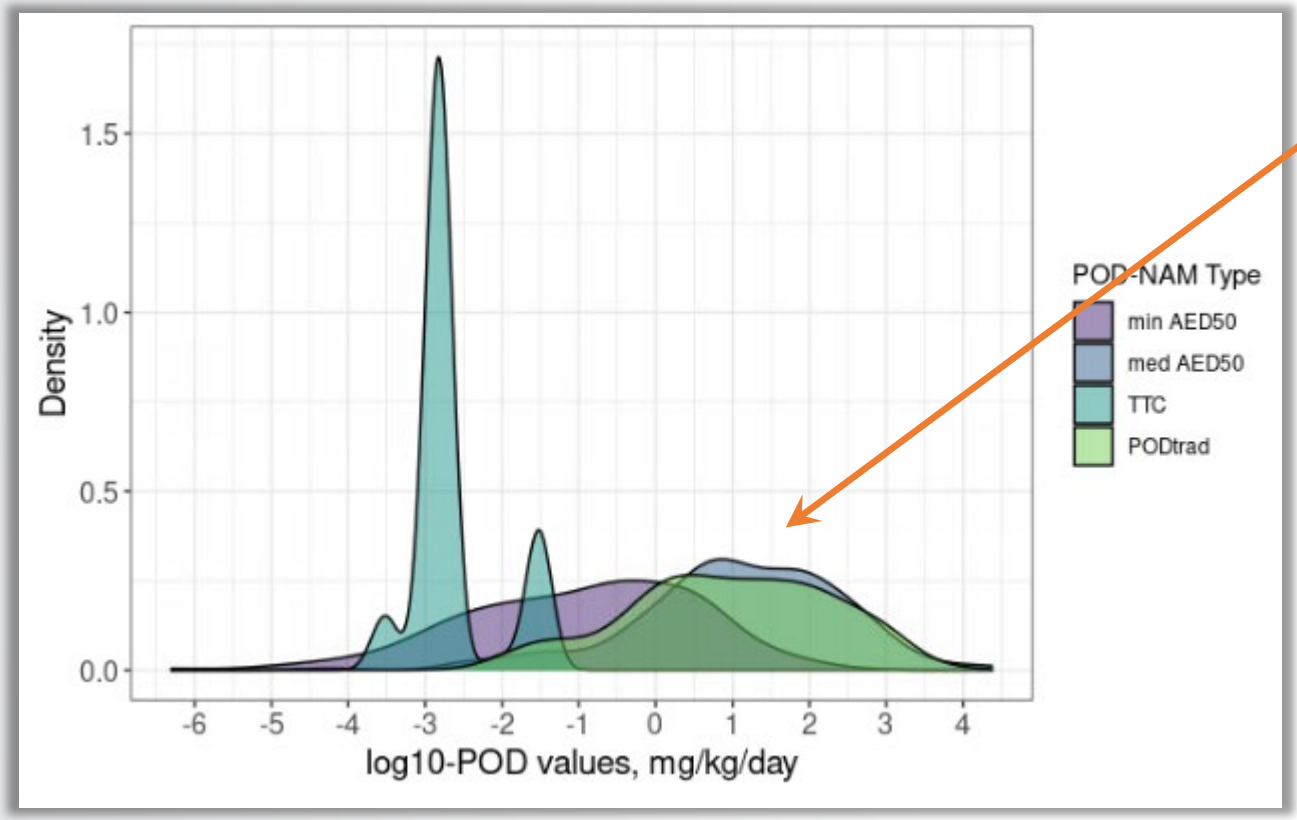


Preliminary prospective analysis for the 157/200 chemicals with POD_{trad}



It seems we've eliminated the ultra-conservative POD_{NAM} values and somewhat improved the median comparison of $POD_{NAM, 50}$ to POD, noting that this is not the matched-chemical analysis (different chemicals in the left and right figures).

Building confidence: In silico POD_{SAR} and in vitro POD_{AED50}



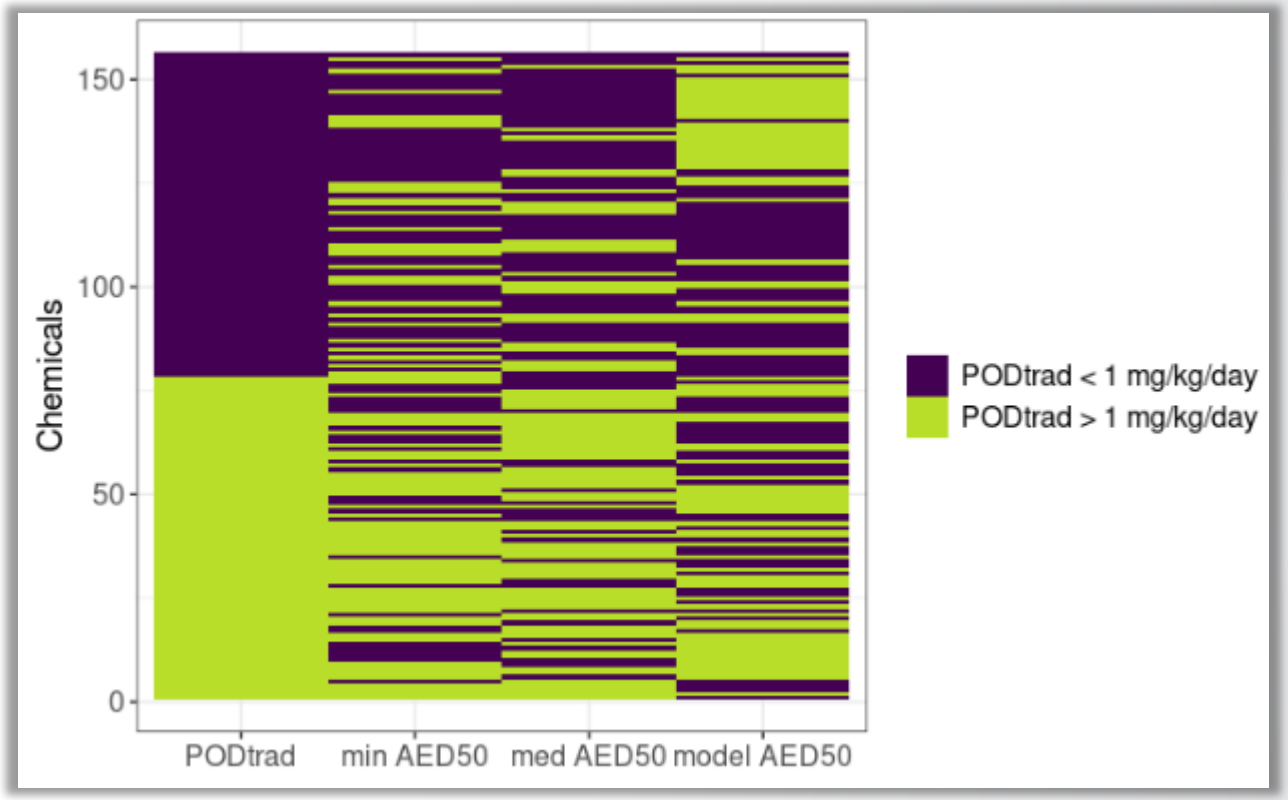
- A POD_{AED50} based on the median of minimum AED50s by assay technology is an empirical and less conservative estimate of POD than TTC that overlaps with the distribution of $POD_{traditional}$
- Min AED50 is more conservative/overlapping with TTC
- TTC may appear more conservative because safety/uncertainty factors are built into the approach

TTC values were based on Cramer classes, including a specific class for organophosphates and carbamates.

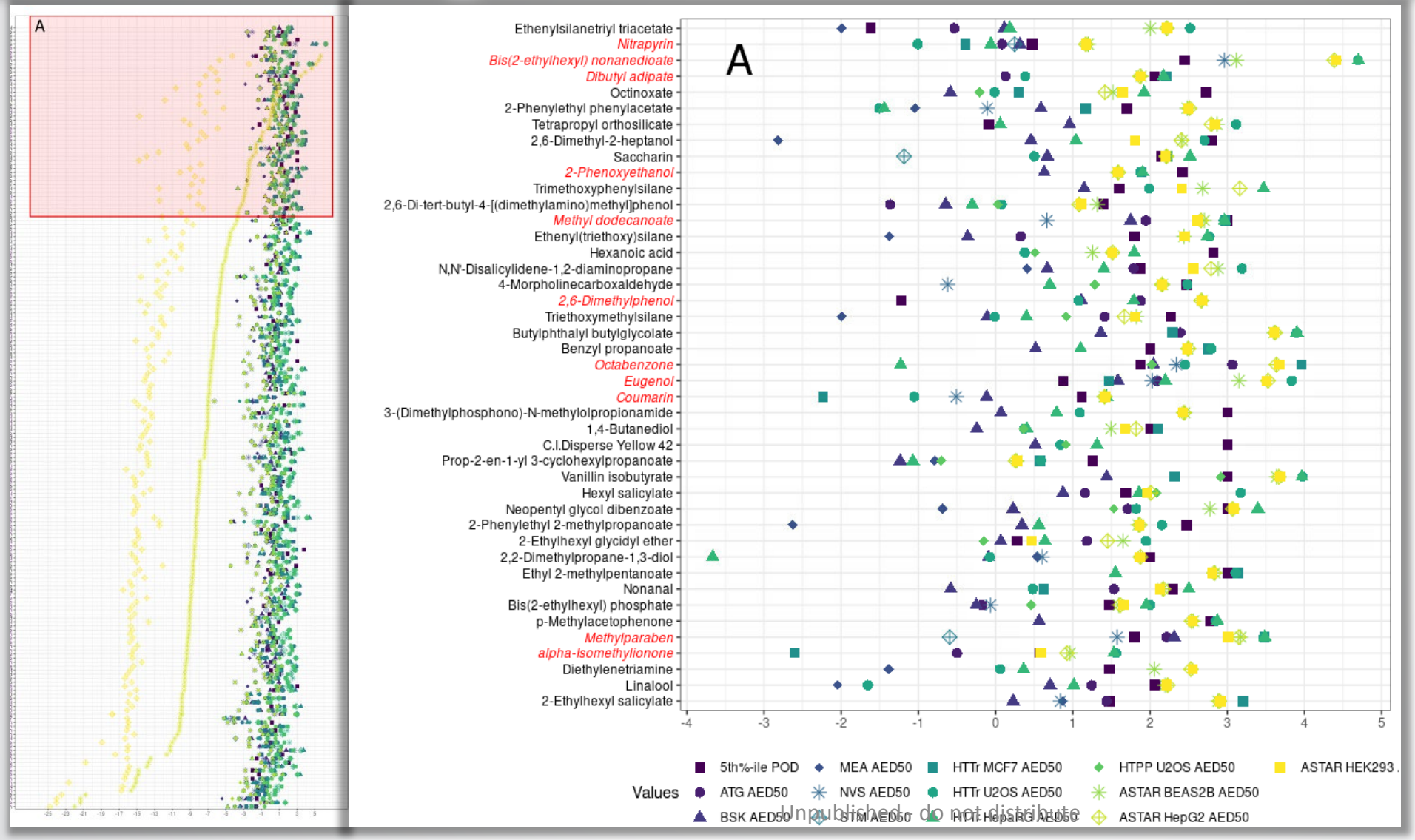
No TTC values for genotoxic carcinogens were used.

How well does POD_{AED50} recapitulate the order of $POD_{traditional}$?

Condition	% of chemicals with $POD_{traditional} < 10$
$POD_{medAED50} < 10$	61%
$POD_{medAED50} < 100$	85%
$POD_{medAED50} < 1000$	99%



We use median BER as an indicator for triaging in screening, but we can also examine the assays (and biology) driving minimum potency values.

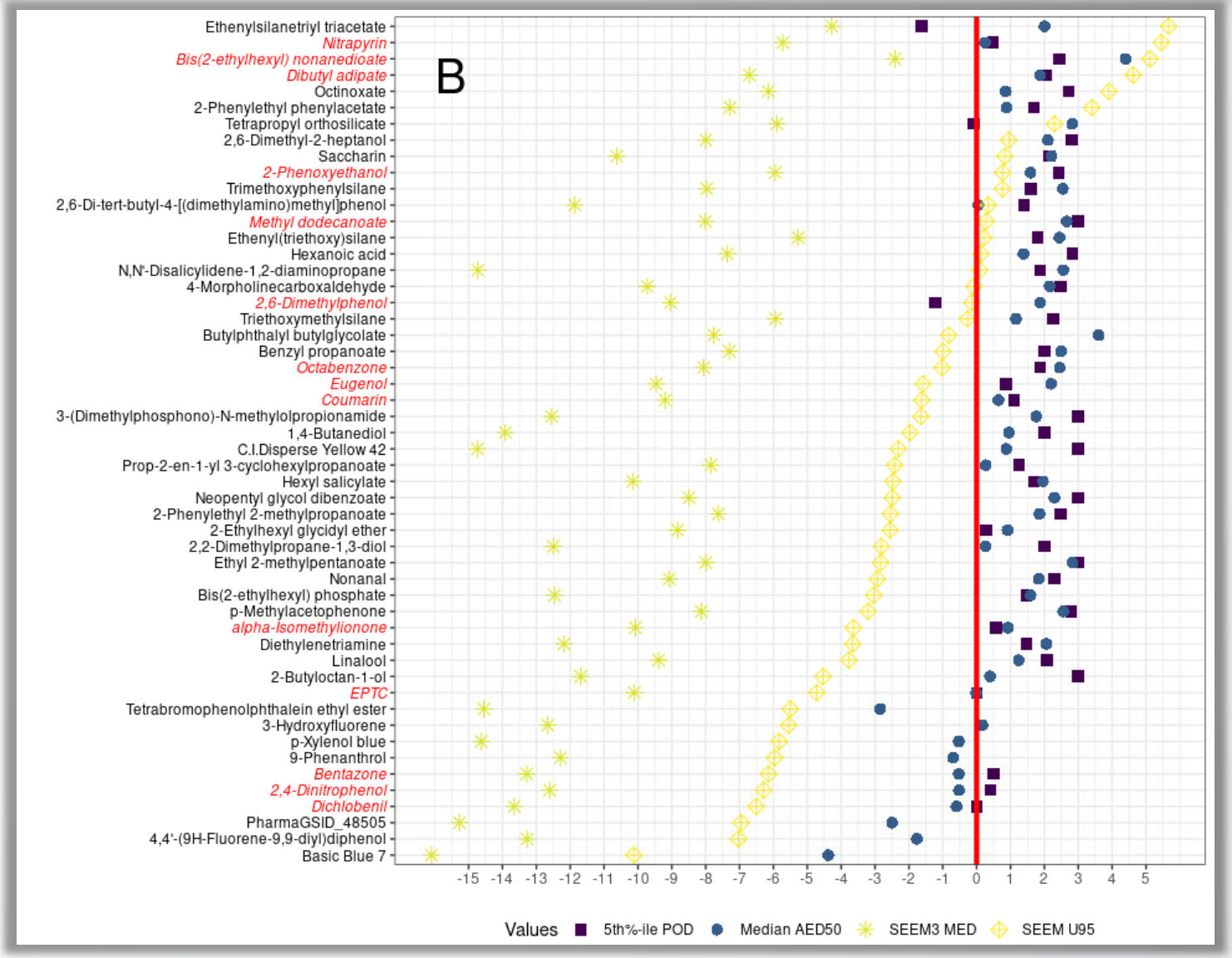


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We use median BER as an indicator for triaging in screening, but we can also examine the assays (and biology) driving minimum potency values.

- POD_{NAM} < 1 mg/kg/day may have some importance in prioritization
- Recall that using the median AED50 to predict the *in vivo* 5th percentile $POD_{traditional}$ appeared reasonable
- 49/195 substances have a BER < 6

Red italic indicates APCRA retrospective chemical



Possible flags to identify priority chemicals for further information

Endocrine flags

In silico | *In vitro*

CERAPP < ToxCast ER model

COMPARA < ToxCast AR model

Immunosuppression flag

In vitro

BioMAP
- Markers of ↓ T-cell proliferation
- Decreased IgG and B-cell proliferation
- Selective cytotoxicity to PBMCs

Serum half-life flag

In silico/in vitro

HTTK, plasma $t_{1/2}$ > 90 days

Median POD_{NAM} < 1 mg/kg/day

In vitro

Median AED50 < 1 mg/kg/day?

Developmental flag

In silico | *In vitro*

TEST

STM

STM, selective

Acute neuroactive flag

In vitro

Acute MEA bioactive concentration = minimum bioactive concentration

Technologies with min POD_{NAM}

In vitro

Minimum AED50 from all technologies?

Are certain target tissues indicated as more sensitive from HIPPTox?

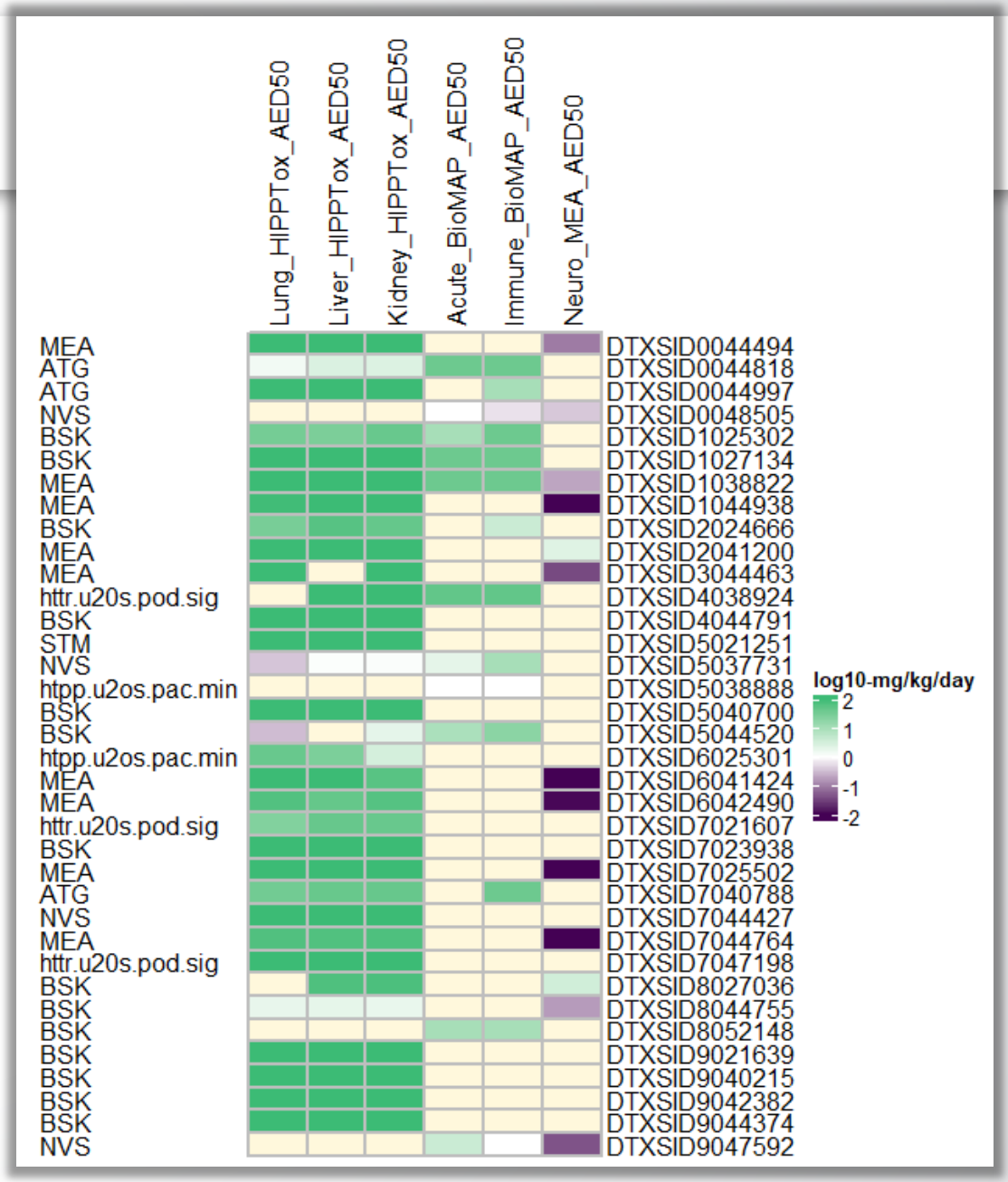
Large variance in POD_{NAM}

In silico/In vitro

Standard deviation > 1 log10-mg/kg/day?

NAM-based target organ hazard flags

- 36 substances are in the “data poor” group (prospective case study only) and have log10-BER < 6 (shown to the right).
- Bioactivity in models of organ-based toxicity can be used as hazard flags.
- These hazard flags can be reviewed by potency.

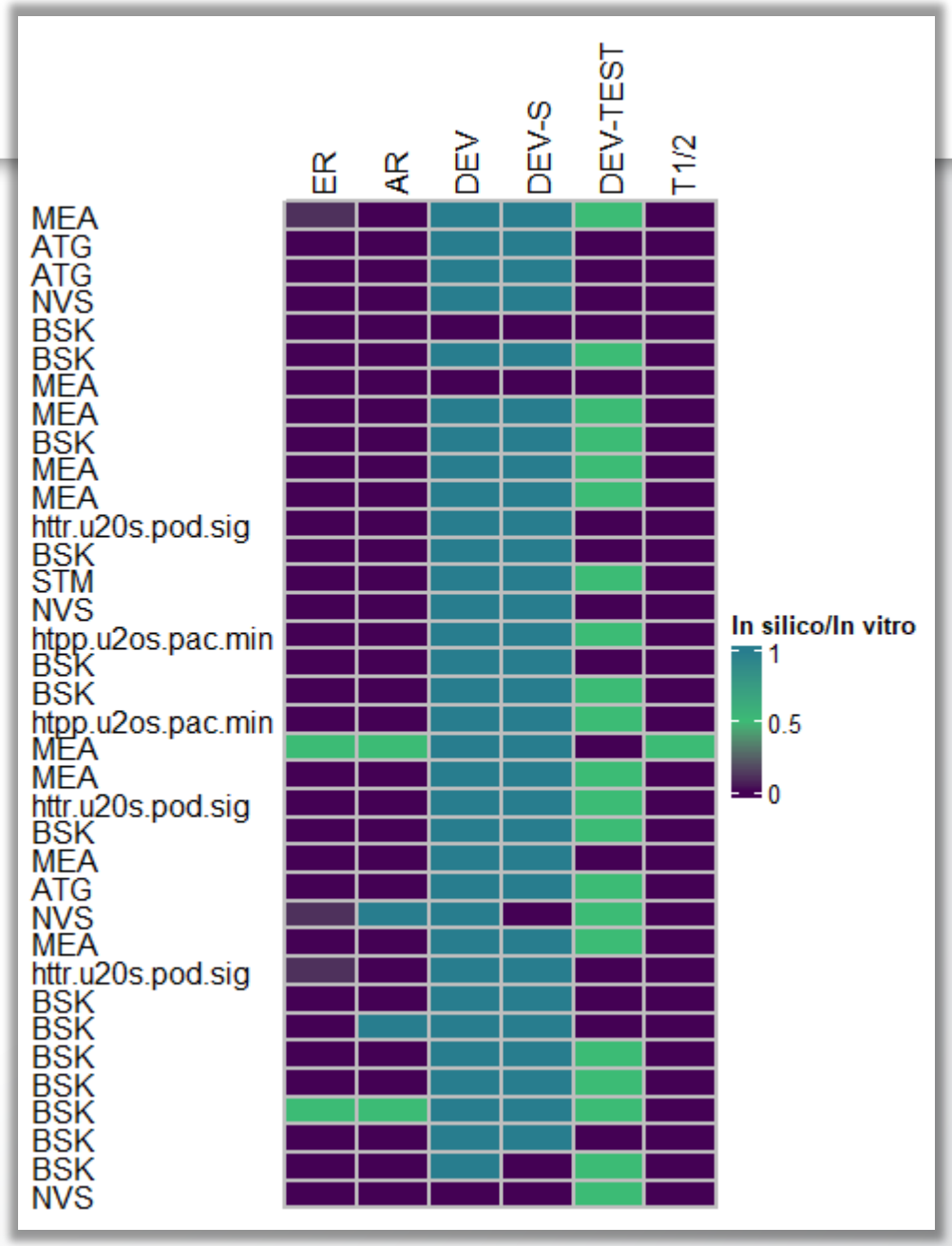


Hazard flags for endocrine and developmental toxicity

- 36 substances are in the “data poor” group (prospective case study only) and have log10-BER < 6 (shown to the right).

- Bioactivity in *in silico* (0.5) and *in vitro* (1) NAMs can be used to indicate putative endocrine and/or developmental hazard.

- DEV = STM positive
- DEV-S = STM positive that is selective
- DEV-TEST = TEST model prediction > 0.7
- T1/2 = half-life predicted to be > 90 days (8 substances in the case study)

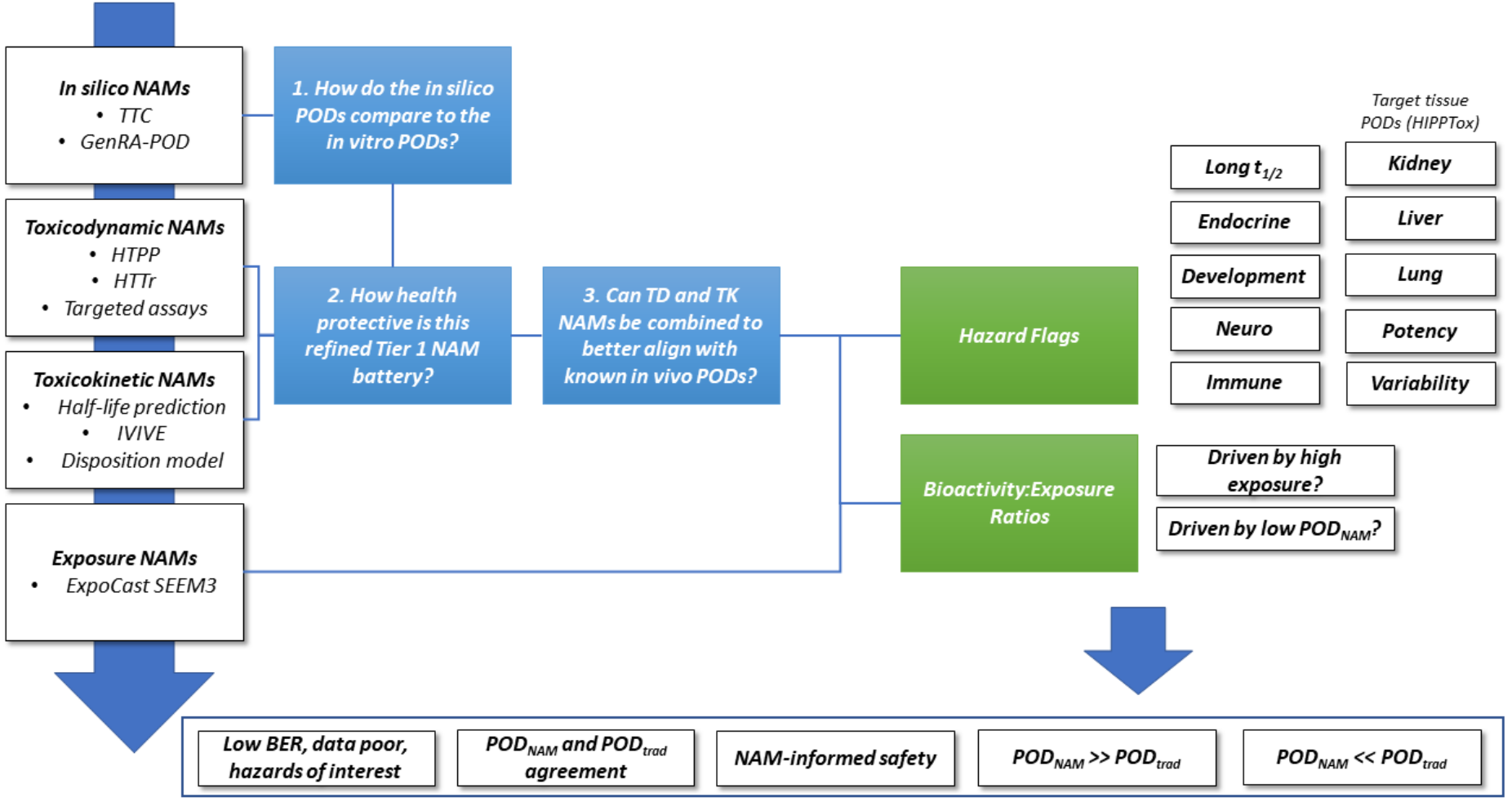


Will these be useful general decision cases for understanding chemical behavior in Tier 1?

General decision case for further screening	Preliminary read-outs
Low BER, data poor, hazards of interest	<12 substances after refined searches
POD_{NAM} and POD_{trad} agreement	48 substances (out of 157 with POD_{trad}) have a difference within ± 0.5 log10-mg/kg/day; 82 within ± 1 log10-mg/kg/day
NAM-informed safety	29 substances have a POD_{NAM} > 300 mg/kg/day; 15/29 have a BER > 7; most of these have limited flags
POD_{NAM} >> POD_{trad}	7 substances with log10-POD ratio > 2 (> 100-fold different)
POD_{NAM} << POD_{trad}	17 substances with log10-POD ratio < -2 (> 100-fold different)

In silico and *in vitro* NAMs are combined prospectively to identify chemicals with putative hazard and BER based prioritization

Figure 1. Mapping the approach.



- Refine assay battery and include assays with broad biological coverage
- Refine IVIVE approach
- Experiment to understand which data may be most informative of $POD_{traditional}$
- Include indicators of putative hazard and related interests (hazard flags)
- Include updated exposure predictions for BER

5 potentially overlapping groups that the NAM data can inform for selection of chemicals for additional screening

Thank you to the entire prospective case study crew, especially:

EPA	Health Canada	ECHA	NTP
Katie Paul Friedman John Wambaugh Josh Harrill Richard Judson Rusty Thomas	Matthew Gagne Marc Beal Tara Barton-Maclaren	Tomasz Sobanski Mounir Bouhifd Lidka Maslankiewicz Mike Rasenberg	Scott Auerbach John Bucher
A*STAR	**JRC**	**Uni Birmingham**	
Lit-Hsin Loo	Thomas Cole Maurice Whelan	Mark Viant	