# APCRA Prospective Study

## Assessment of chemicals, using and developing New Approach Methodologies (NAMs)

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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 any participating government organization.*

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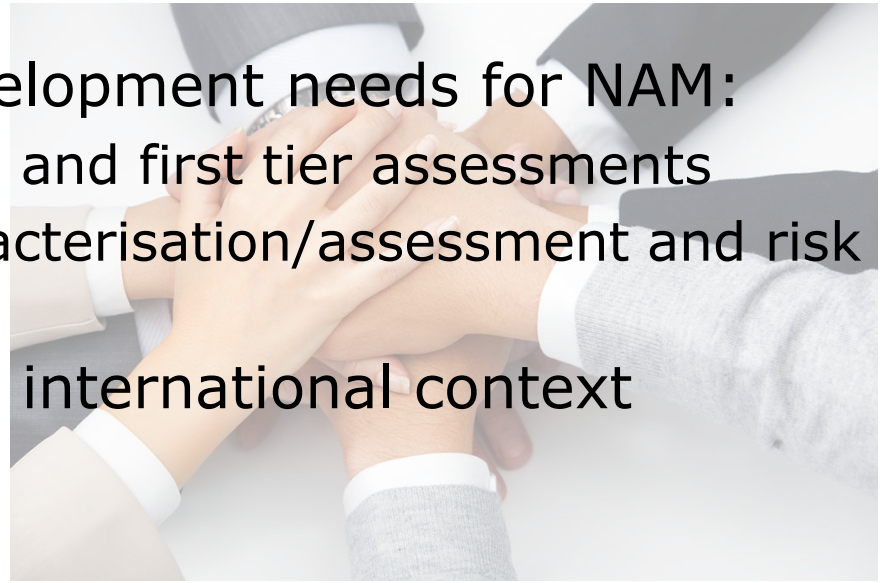
# APCRA prospective study

## Project Goals

- To assess chemicals with limited/unclear toxicological data, using both NAM type of data and classical toxicological studies;
- To inform the further development needs for NAM:
  - for screening, prioritisation and first tier assessments
  - for conclusive hazard characterisation/assessment and risk management
- To assess chemicals in an international context



*This prospective case study also builds upon findings and learnings from the APCRA retrospective case study…*

# APCRA retrospective study

*See the forest for the trees*

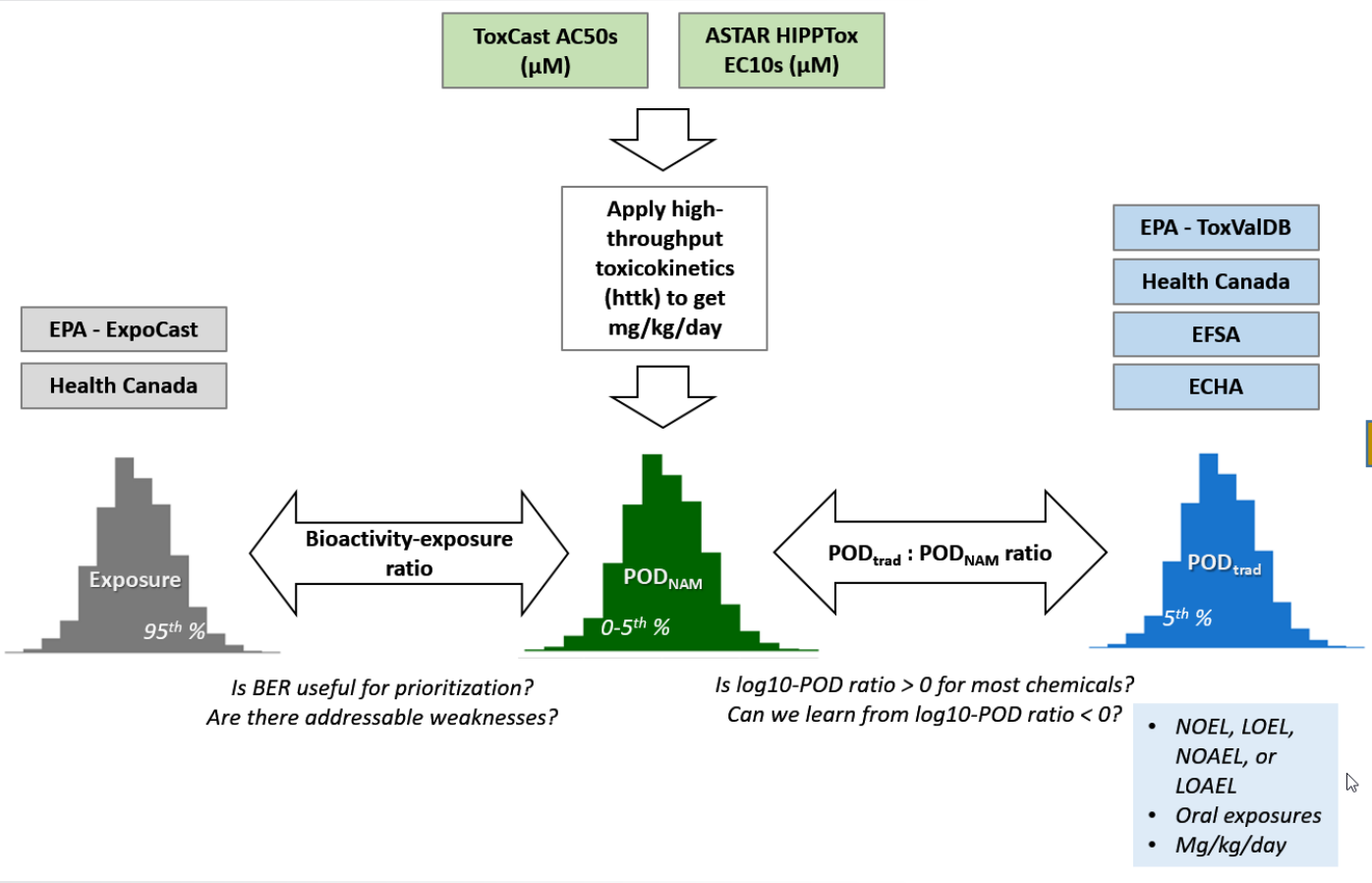
The big question:

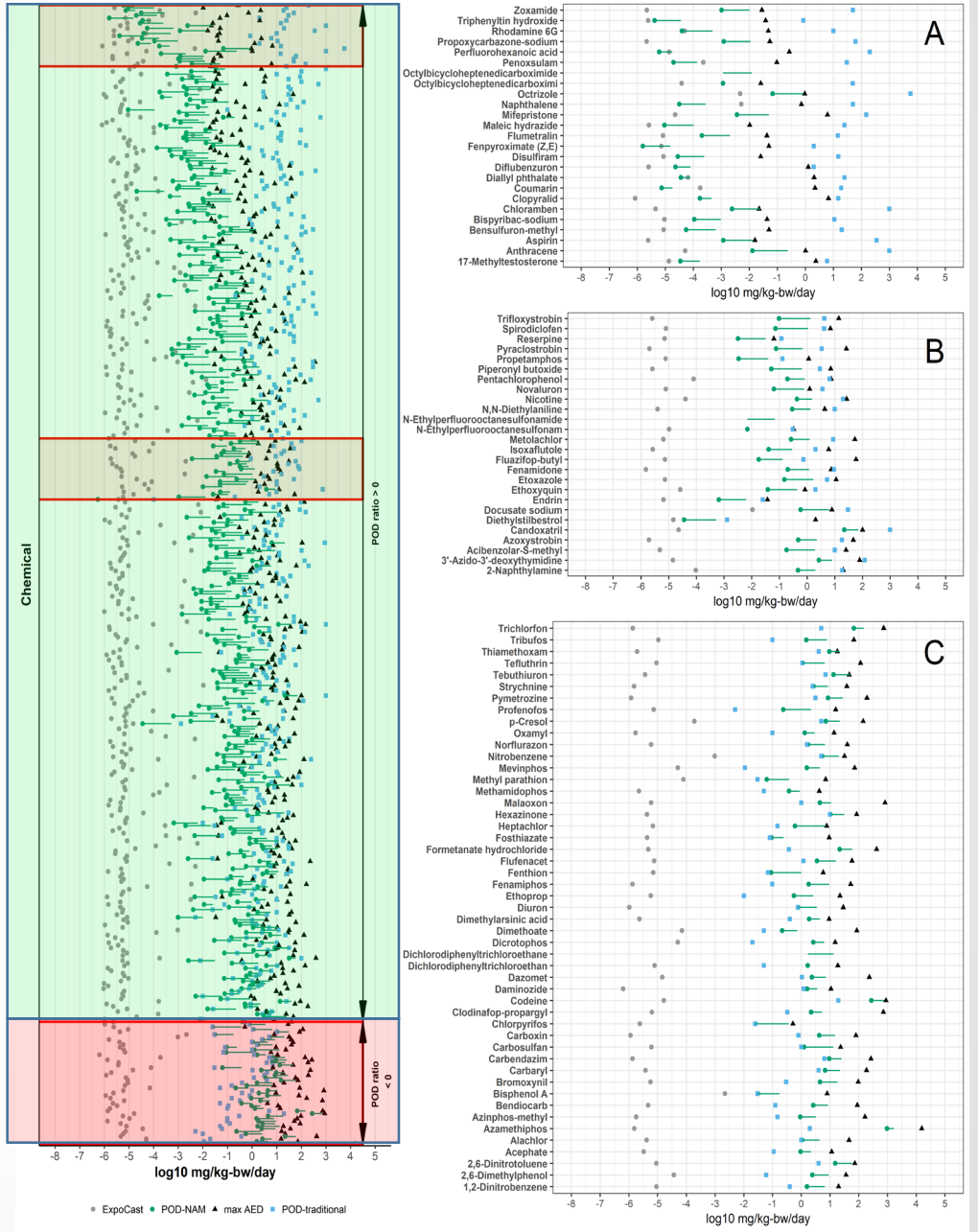
# Can *in vitro* bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and screening level risk assessment?

*Katie Paul Friedman, et al. Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization*
Toxicol Sci. 2020 Jan 1;173(1):202-225. doi: 10.1093/toxsci/kfz201

# Case study workflow

*For 400/448 chemicals (89%) this approach appears conservative*




*For 48/448 chemicals (11%)*
*$POD_{NAM}$ > $POD_{Traditional}$*

*Katie Paul Friedman, et al.*
Toxicol Sci. 2020 Jan 1;173(1):202-225. doi: 10.1093/toxsci/kfz201

# Conclusions and limitations

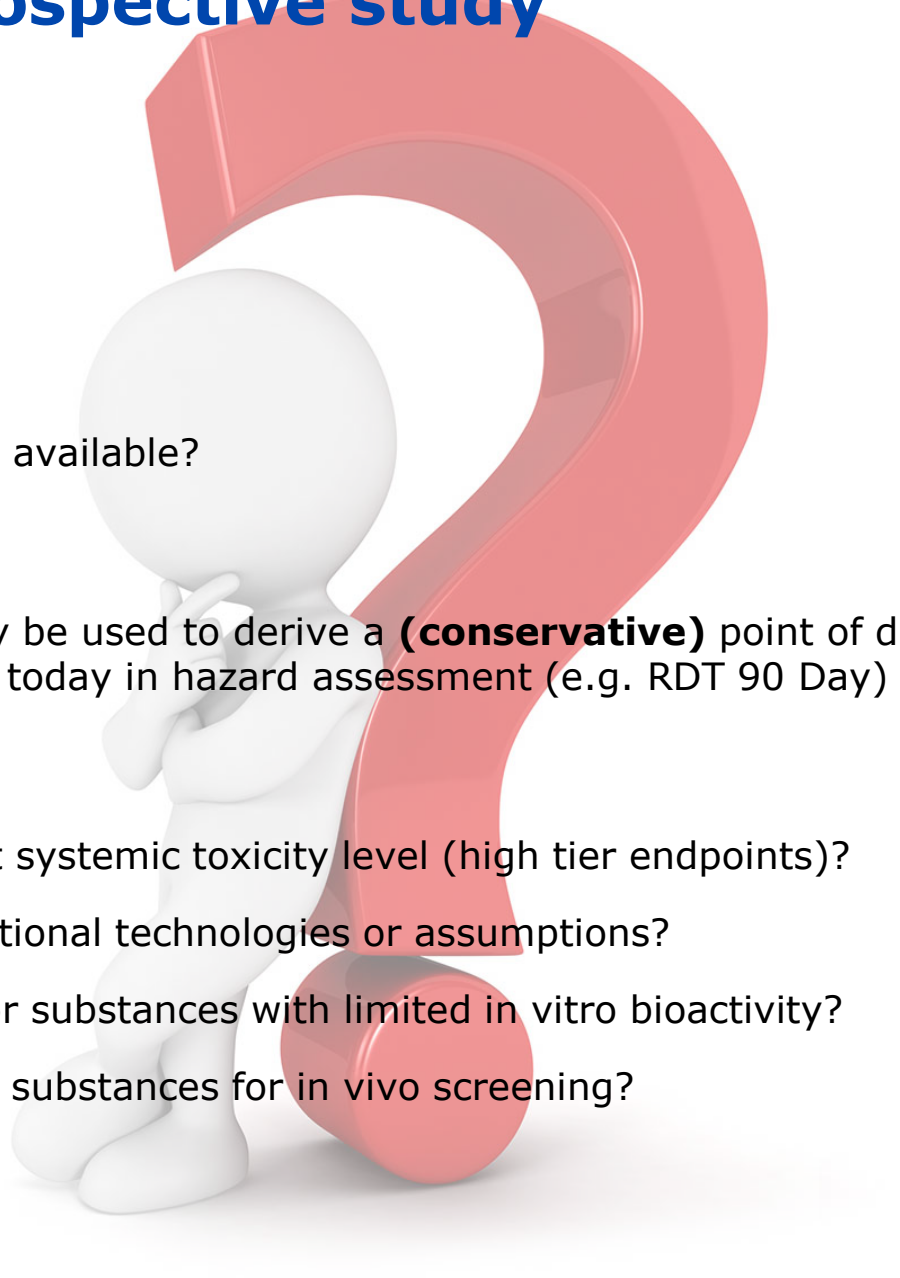
- An approach to using *in vitro* bioactivity data as a POD appears to be a conservative estimate ~ 90% of the time for 448 chemicals.
- $POD_{NAM}$ estimates appear conservative with a margin of ~100-fold.
- $POD_{NAM}$ may provide a refinement of a TTC approach.
- When combined with high-throughput 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.

# APCRA prospective study

## Main Case study questions:

- Technology usefulness
  - How far can we go with the NAM technologies currently available?
- Specific use case
  - Can the outcome from the refined *in vitro assay* battery be used to derive a **(conservative)** point of departure and qualitative hazard triggers comparable with these used today in hazard assessment (e.g. RDT 90 Day)
- Applicability in regulatory context
  - Could we consider application for hazard assessment at systemic toxicity level (high tier endpoints)?
  - 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?



# What 'comparable with RDT' means for NAMs?

To demonstrate that an outcome is comparable with RDT 90d in the context of hazard characterisation and risk management, NAM testing has to:

- Provide estimate of NOAEL and LOAEL:
  - NOAEL as potential source for systemic DNEL (if most conservative);
  - LOAEL for STOT RE classification (in the case of severity of the effect);
- Provide indications/triggers for:
  - Toxicity to reproduction;
  - Developmental toxicity;
  - Immunotoxicity;
  - Neurotoxicity;
  - Carcinogenicity;
  - ED related effects;

# APCRA prospective study: Project plan Steps 1-2

**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

# APCRA prospective study: Project plan Steps 3-5

**Step 3**

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

- Transcriptomics & metabolomics in liver
- Classical in vivo observations and toxicokinetics
- Setting up the dosing for Step 4

**Step 4**

*In vivo* studies compatible with the OECD guidelines

- Selection of study will depend on hazard profile (preferably RDT 90d, PNDT or combined screening)
- Classical endpoints combined with multi-omics

**Step 5**

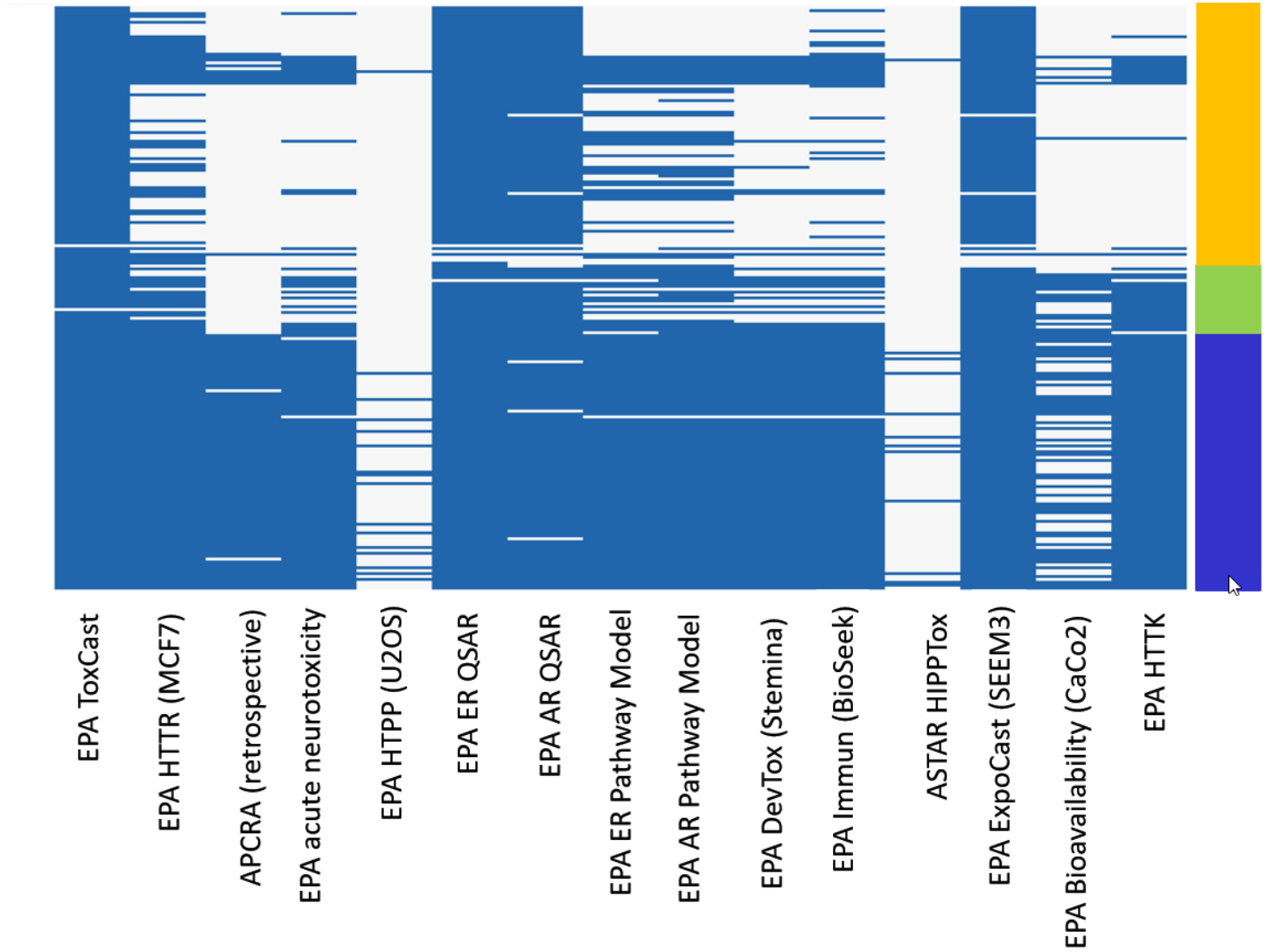
Evaluation of the results

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

**No duplication of animal studies!**

No further testing of substances with relevant *in vivo* studies available!

# APCRA prospective study: Progress on Steps 1-2




In 2020, 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 (88).

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

# Battery of *in vitro* assays / models

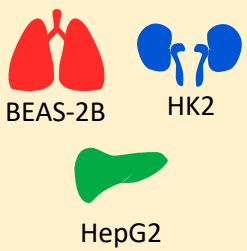
| Hazard | Toxicokinetics | Exposure |
|---|---|---|
| EPA ToxCast Assays<br>EPA HTTr Assay (2 – 3 cell types)<br>EPA HTPP Assay (2 – 3 cell types)<br>A*STAR HIPPTox Assay<br>EPA ImmunoTox Assay (Bioseek)<br>EPA Neurotox Assay (MEA acute)<br>EPA DevTox Assay (Stemina)<br>EPA ER Assays/Models<br>EPA AR Assays/Models | EPA/HC/JRC Metabolic Stability<br>EPA/HC/JRC Plasma Protein Binding<br>EPA/HC Caco-2 Bioavailability | EPA ExpoCast Exposure Model |

| NAM Endpoint | # Chemicals with Preliminary triggers* |
|---|---|
| BER < $10^4$ | 19/96<br>(7/19 with ImmunoTox flag) |
| Developmental Toxicity | 21/116 |
| ER Pathway Model | 9/136 |
| AR Pathway Model | 23/139 |

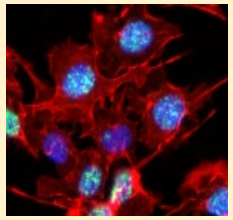
*Results based on current data and not the full suite of 200 chemicals.

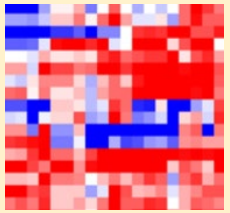
# NAM Data available from A*STAR, Singapore (2019)

**201 Selected Chemicals** (by APCRA partners) [Received 199 chemicals, 100% Done, Nov 2018]

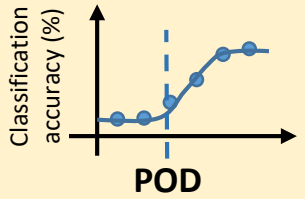



**Single-concentration Cell Viability Check** [100% Done, June 2019]





**Multi-concentration Phenotypic Profiling (HIPPTox)** [48% Done, on-going]




**Points of Departure (POD) Estimation**

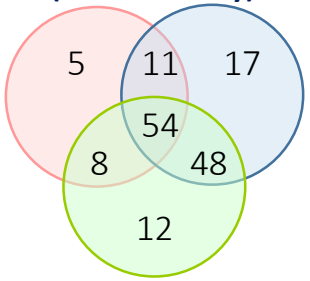
**ToxCast PODs**

**Final in vitro POD derivation**

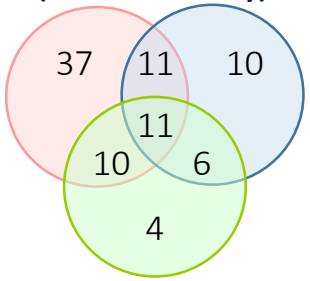
**Selection of chemicals for higher tier *in vivo* tests**

## Preliminary findings on single-concentration tests:

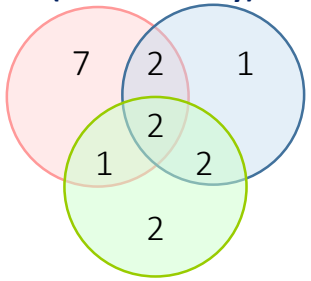
**Weakly inhibitive (>80% viability)**



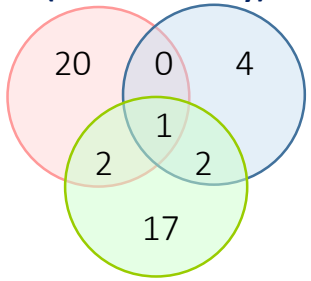

**Moderately inhibitive (21-80% viability)**




**Strongly inhibitive (<20% viability)**




**Pro-proliferative (>120% viability)**




- ~73% (145) of the compounds are moderately or strongly inhibitive in at least one cell model
- Interestingly, ~23% (46) of the chemicals are pro-proliferative in at least one cell model

# Incorporating NAMs into *in vivo* studies

**Thomas *et al.* study showed that…**

- The lowest transcriptional BMDs in specific target tissues (bladder, liver, and thyroid) correlated well with the lowest non-cancer apical BMDs in the **same target tissues** after 5 days of exposure **in rats**.

Thomas, et al., **Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment,** *Toxicological Sciences*, Volume 134, Issue 1, July 2013, Pages 180–194, https://doi.org/10.1093/toxsci/kft094

**NTP's current study…**

- Evaluates whether the lowest transcriptional BMDs in **liver and kidney (as 'sentinel' tissues)** after 5 days of exposure in **male rats** correlate with the lowest apical BMDs **in male and female rats and mice** from long-term (chronic or sub-chronic) toxicity studies.

John Bucher, Mike DeVito, et al., *in preparation*

**Recent work from Helmholtz Centre for Environmental Research showed that…**

- Transcriptomics provides rich insights into toxicity pathways, metabolomics provides a downstream molecular measurement of the functional condition of an organ, combined the multi-omics data "can considerably improve the confidence in detecting a pathway response"

Canzler, et al., **Prospects and challenges of multi-omics data integration in toxicology,** Arch Toxicol 94, 371–388 (2020). https://doi.org/10.1007/s00204-020-02656-y

# *in vivo* 5-day multi-omics assay

- NTP is currently demonstrating the use of a broad screening approaches to determine concentrations associated with pathway-level perturbation.
- Can estimate apical in vivo BMDs in sub-chronic or chronic exposures using transcriptomic and metabolomic analyses of sentinel tissues in 5-day studies.
- This approach can considerably improve the confidence in detecting a pathway response
- This assay represents a bridge between HT in vitro assays (Step 2) and traditional regulatory tests, originally relying entirely on apical endpoints (Step 4).

# Project timeline

- Substance selection
  Q3 2017 - Q3 2018 (*finished*)
- *In vitro* testing & *in silico* modelling
  Q4 2018 - Q2 2020 (*nearing completion*)
- *In vivo* testing
  Q3 2019 - Q4 2021 (*preparatory work is ongoing*)
- Analysis of the results & communication
  Q1 2019 – Q2 2022 (*on-going*)

# Desired outcome

- Provide a **conservative** estimate of *in vivo* LOAEL/POD (Systemic):

$LOAEL_{NAM}$ or $POD_{NAM}$ <= $LOAEL_{TRADITIONAL}$ or $POD_{TRADITIONAL}$

- Triggers (qualitative indicators) for: immunotoxicity, acute neurotoxicity, developmental/repro toxicity, carcinogenicity and endocrine effects

# What does this case study aim to achieve?

- Confirmation that NAM test battery can be successfully applied for screening with minimal risk of false negatives
- Verification whether/when NAM test battery can be directly used for quantitative hazard assessment
- Development of optimised assessment protocols aiming at implementation of the 'NAM type' of data in the multi-tiered hazard assessment
- Confidence building in application of NAMs for hazard characterisation
- Chemicals assessed at international level

# Conclusions

- This project will assess the usefulness of NAM technologies in a specific regulatory context
- Will provide insights on NAMs added-value (incl. comparison to classical methods)
- Will guide the design of potential future NAMs strategies
- This project will also address emerging questions (from the retrospective case study)
  - Why for small fraction of cases NAM estimates are not conservative enough;
  - Why some NAM estimates are over conservative;
  - How NAM will perform for substances with lower bioactivity/bioavaliability;
  - Can BER, and additional hazard flags, be used to reliably indicate potential hazard/risk;
  - What are the limitations (applicability domain) of NAM approach;

# Thank you

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