Examining the Utility of In Vitro Bioactivity as a Conservative Point of Departure: A Case Study

Katie Paul Friedman

With coauthors from A*STAR, ECHA, EFSA, EPA-ORD, and Health Canada

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Project collaborators

A*STAR	ECHA	EFSA	EPA	Health Canada
Lit-Hsin Loo	Mike Rasenberg	Jean-Lou Dorne	Russell Thomas (ORD-NCCT)	Tara Barton-Maclaren
Peiying Chuan	Tomasz Sobanski		Katie Paul Friedman (ORD-NCCT)	Matthew Gagne
	Tatiana Netzeva		Tina Bahadori (ORD-NCEA)	
	Panagiotis Karamertzanis		Jill Franzosa (ORD-CSS)	
	Andrea Gissi		Michelle Angrish (ORD-NCEA)	

The big question:

Can in vitro bioactivity be used to derive a conservative point-of-departure for prioritization and risk assessment?

Defined project objectives

- Compare in vitro bioactivity-derived administered dose equivalents (ADEs) and publicly available PODs from traditional chemical assessments ($POD_{traditional}$) to determine whether ADEs provide a conservative estimate of $POD_{traditional}$.

- Calculate the bioactivity-exposure ratio (BER) based on the ADE distribution for high-throughput bioactivity compared with both high-throughput exposure estimates (e.g., ExpoCast) and exposure estimates from traditional chemical assessments;

- Determine whether these BERs provide a robust means to prioritize chemicals for additional study and/or to serve as a low tier risk assessment approach; and,

- Characterize the strengths and possible areas for improvement of NAM-derived PODs (POD_{NAM}) for use in screening-level human hazard characterization and risk evaluations.

Comparison workflow

Determine chemicals with high-throughput toxicokinetics (httk) information (largest limiter)

Determine chemicals with information in ToxCast

Beginning chemical set *Currently ~400 chemicals*

EPA - ToxCast

ASTAR

Others?

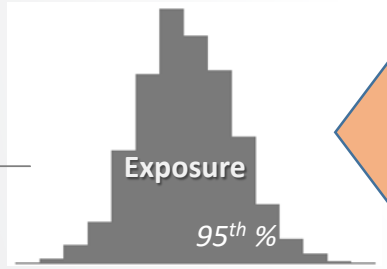
Apply httk

- *If the sum of hitcalls across the ToxCast DB > 5, then the 5th percentile on the distribution of AC50 values was used.*
- *If the sum of hitcalls across the ToxCast DB ≤ 5, the lowest AC50 was used.*
- *Beta: Flag-filtering by removing AC50 values from fits with 5 or more caution flags in invitrodb and any with flag 7 (Look for single point hits with activity not at highest conc tested 0)*

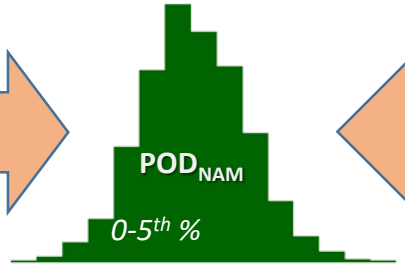
EPA - ExpoCast

Health Canada

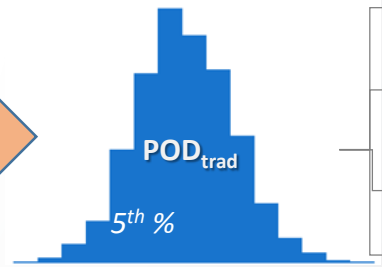
EFSA



Bioactivity-exposure ratio



PODNAM : PODtrad ratio



EPA - ToxValDB

Health Canada

EFSA

ECHA

Currently only using ExpoCast (Wambaugh et al., 2014, heuristic model)

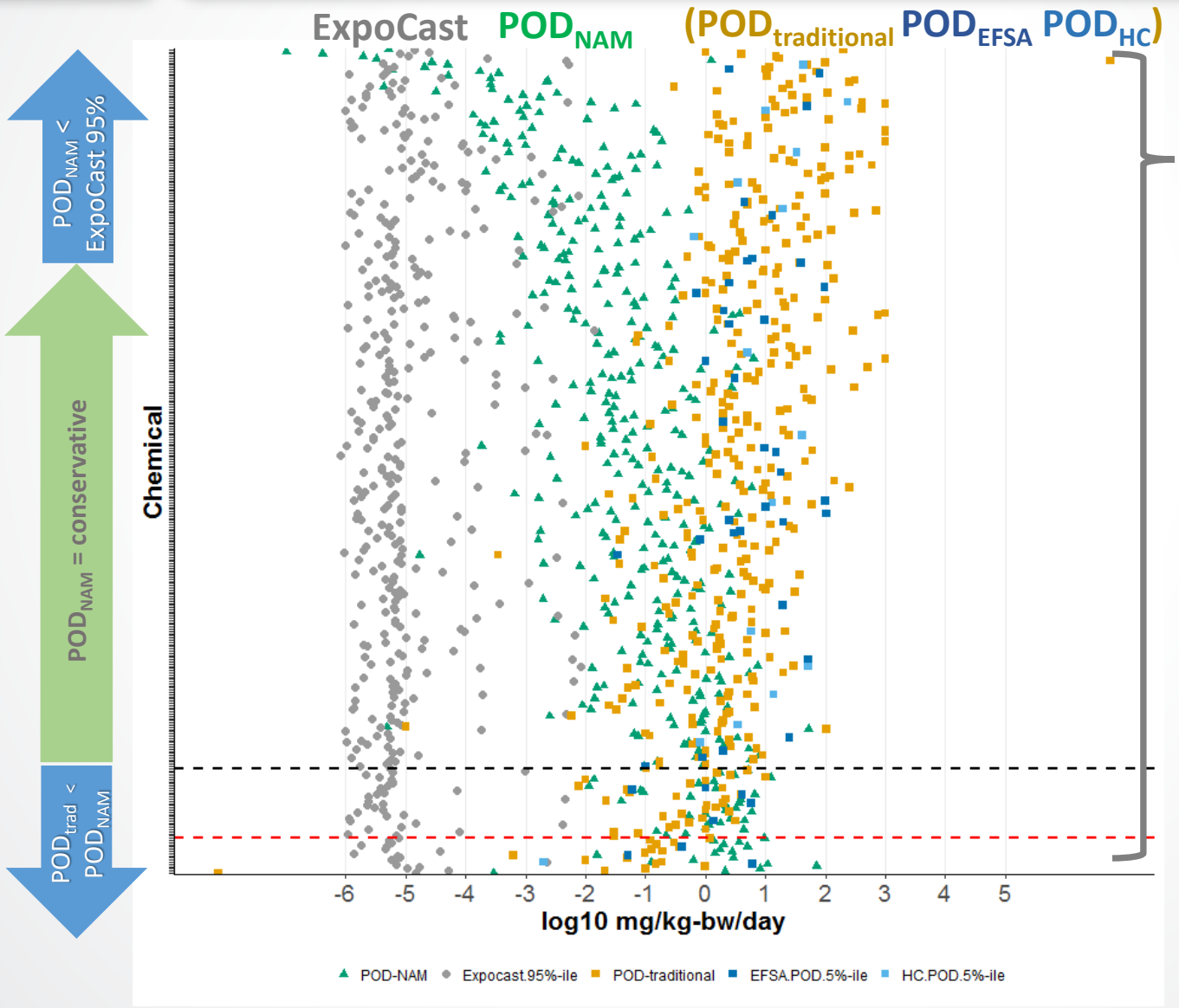
Is this log10 ratio useful for prioritizing chemicals?
Are there weaknesses here that can be addressed?

Is this log10 ratio > 0 for the majority of chemicals?
Can we learn from instances where this ratio is < 0?

- *NOEL, LOEL, NOAEL, or LOAEL*
- *Oral exposures*
- *Mg/kg-bw/day units*

A comparison of the available data highlights: general conservatism, and a need to investigate the 'extremes'

Figure 1 (draft). *Comparison of predicted exposure, POD_{NAM}, and $POD_{traditional}$.*



Total =

379 chemicals

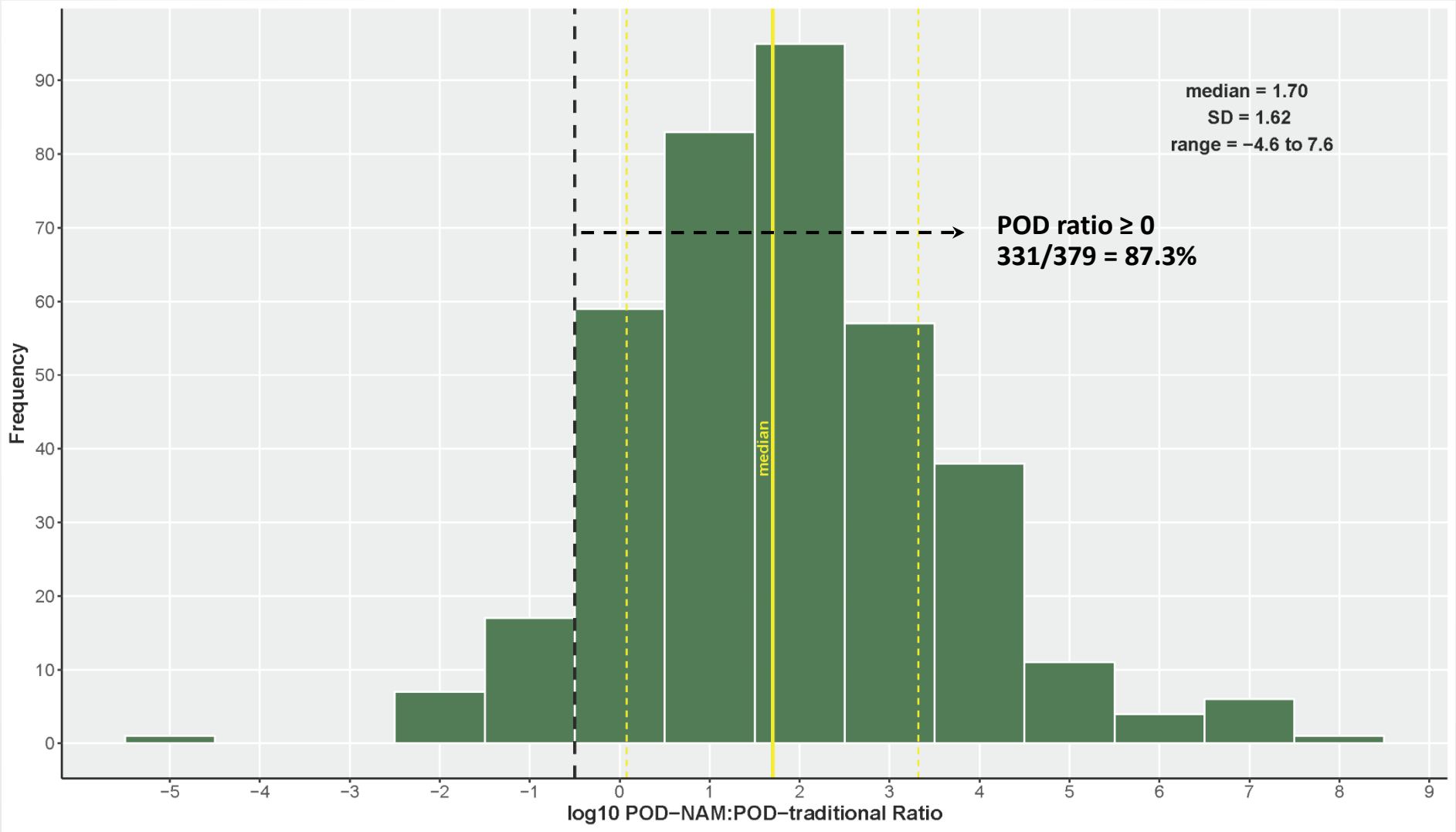
httk, ToxCast data, and POD value(s) currently available

So for ~87% of the chemicals, without modifying simplistic assumptions in the workflow, POD_{NAM} was conservative.

POD ratio ≤ 0
48/379 = 12.7%

POD ratio < -1
16/379 = 4%

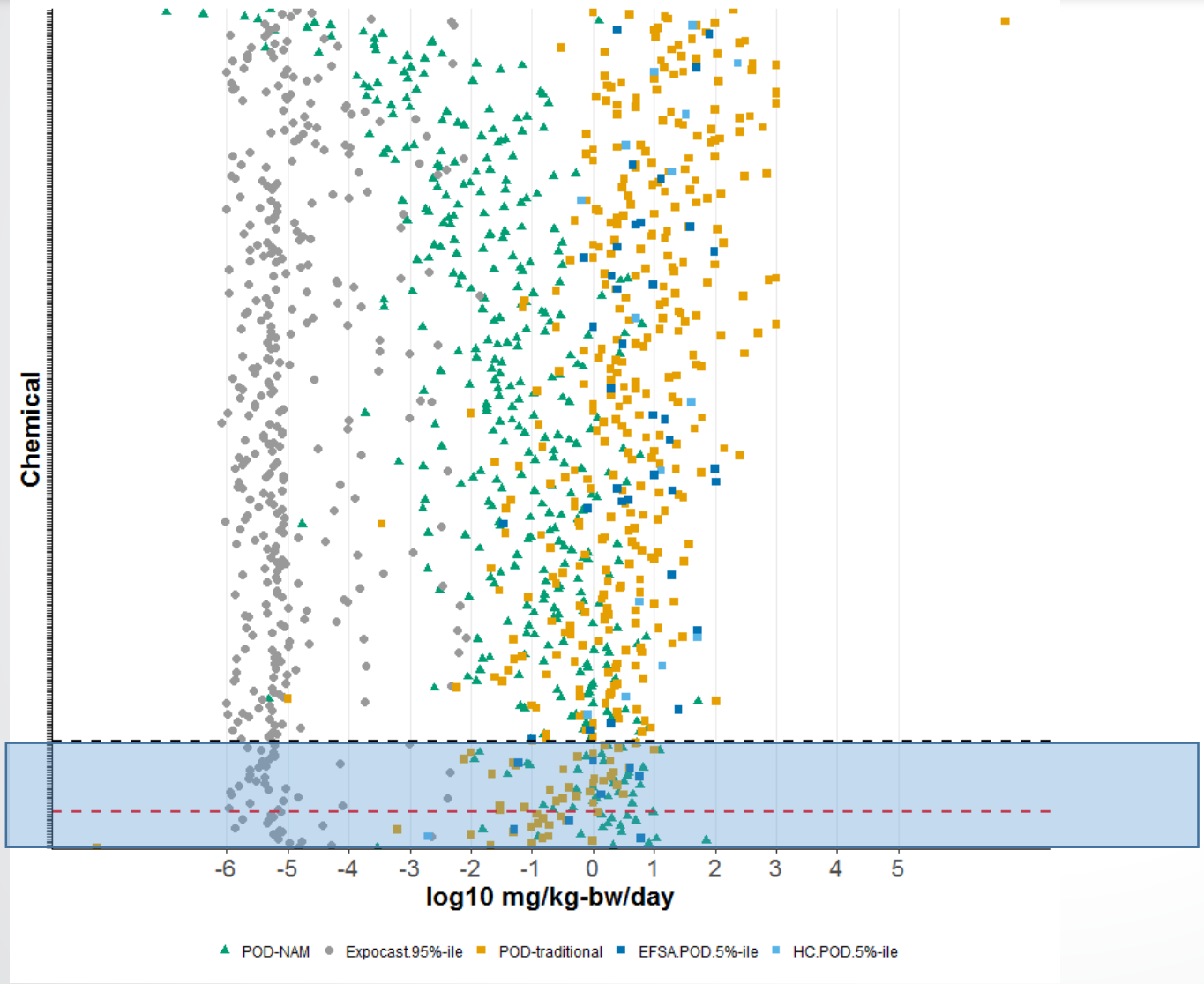
Distribution of the POD ratio demonstrates the conservatism of the current, unrefined approach



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? • Most assay data are "human" and $POD_{traditional}$ are in animals.	• 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. • Inter-individual variability not currently captured.
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 study type. • PODs were limited to NOEL/LOEL/NOAEL/LOAEL. • Have not allometrically scaled (yet) to human doses.	NA
How it is considered	• Caution flag 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 could use other developing work to indicate the variability in POD data.	• We take the **95%-ile** on the **CI for the median** for the total population.

Are there key drivers of examples where POD ratio ≤ 0?

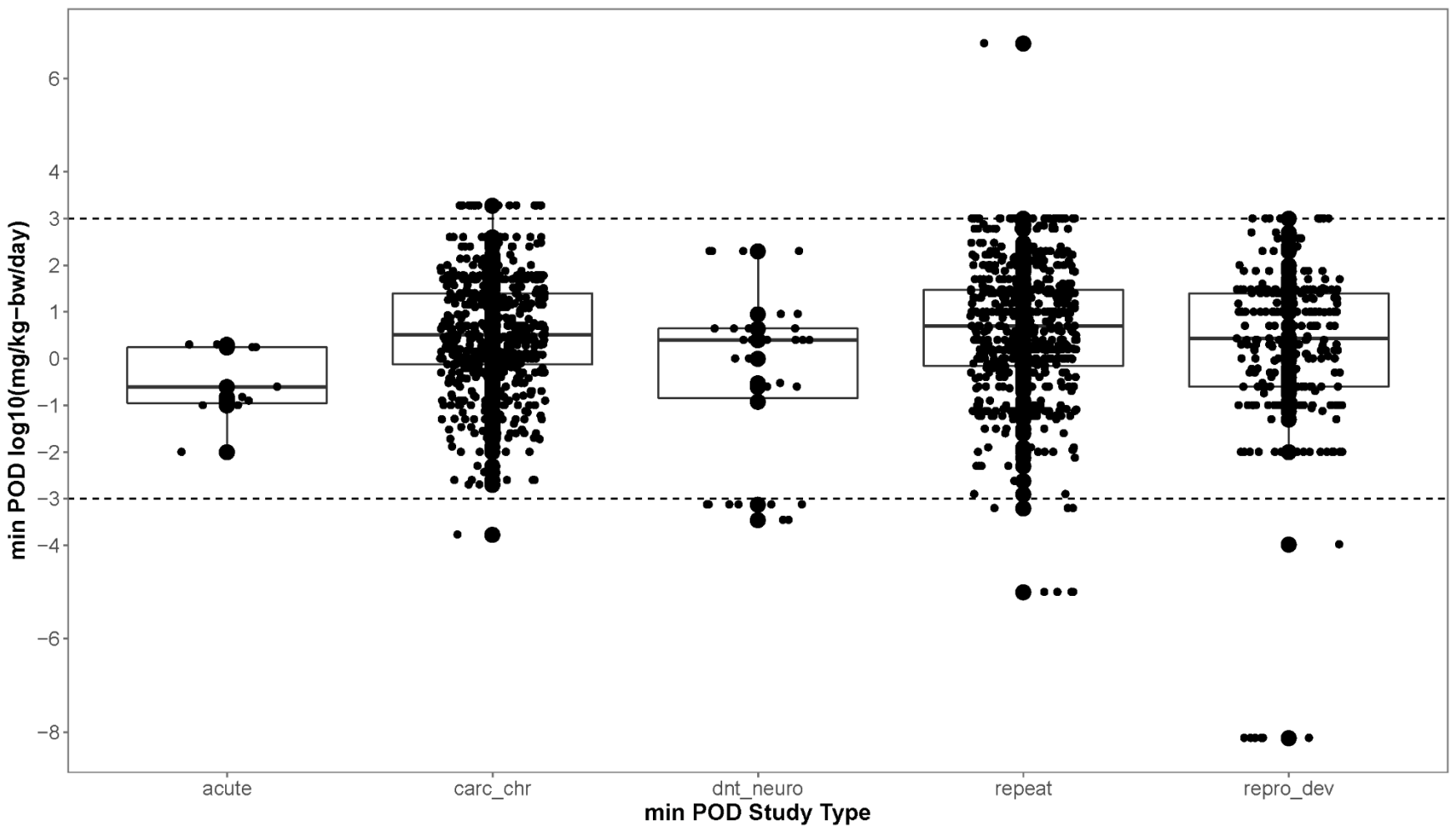


POD_{NAM} : $POD_{traditional}$ ≤ 0

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

Are minimum POD values driven by one study type more than another?

Figure 2 (draft). *Minimum log10(POD) by study type.*



- Min(POD) spanned mostly 0.001-1000 mg/kg/day with a few exceptions.
- Median(min(POD)) appeared possibly lower for DNT/neuro studies, but fewer data were available to evaluate this.
- Note that each study type was not present for every chemical (i.e., not every chemical had every study type).

Are certain study types driving the min(POD) when POD ratio ≤ 0?

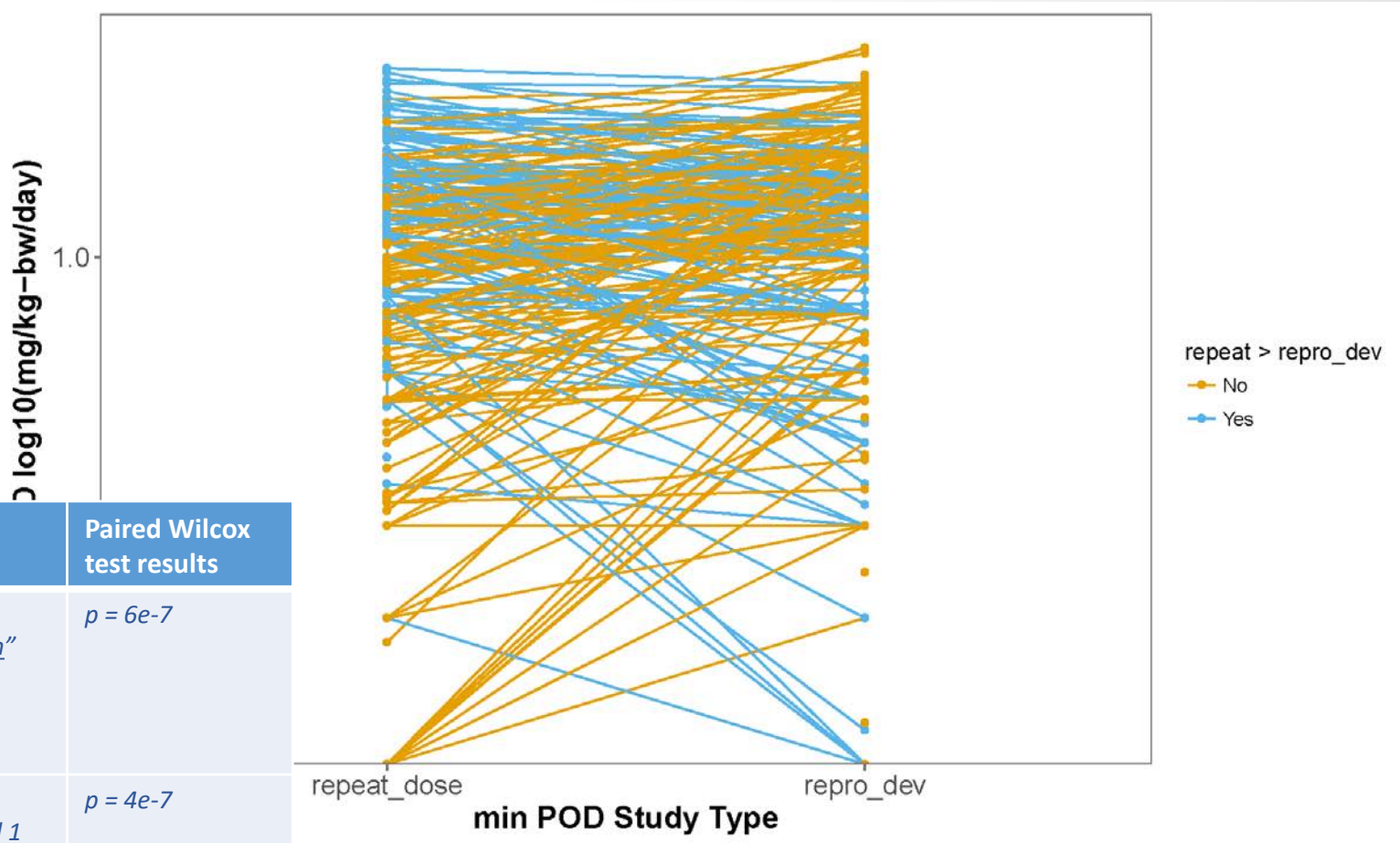
Hypothesis	Result	Fisher's exact test results	Caveats
Reproductive and/or developmental studies over-represented when POD ratio ≤ 0?	• No; • Defined the min(POD) for 4/47 with POD ratio ≤ 0 • Defined the min(POD) for 54/328 chems with POD ratio ≥ 0	• p-value = 0.9; • odds-ratio = 0.6	Fewer chemicals with these studies available?
Carcinogenicity or chronic studies over-represented when POD ratio ≤ 0?	• Yes; • Defined the min(POD) for 31/47 chems with POD ratio ≤ 0 • Defined the min(POD) for 168/328 chems with POD ratio ≥ 0	• p-value = 1.91e-5; • odds-ratio=3.77	Min(POD) study type was assigned preferentially to carcinogenicity/chronic when equivalent to repeat dose due to ambiguity in assigning these study classes.

- This highlights the potential importance of alternative models to predict the outcome of long-term repeat dose exposure.
- A *major caveat* to this type of examination of the data is a lack of a controlled vocabulary for study type and effects observed.
    - *Makes current assignment of study type subject to some amount of error.*
- Future work includes direct examination of some or all of the 48 chemicals with POD ratio ≤ 0.

Generally, do certain study types yield lower PODs?

- This is more appropriate to ask on a per chemical basis.
- Though repeat POD > repro_dev POD for 43% of the chemicals, 57% of the time the repeat POD was = or < the repro/dev POD.
- So we cannot make a universal assumption that repro_dev will yield the lowest PODs.

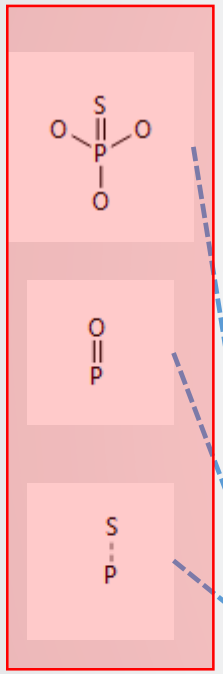
Figure 3 (draft). *Relationship between repeat dose and reproductive/developmental POD by chemical.*



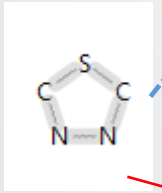
Hypothesis	Conclusion	Paired t-test results	Paired Wilcox test results
Repeat dose > Carc/Chronic?	Repeat dose > carc/chronic is statistically significant.	*p = 4.5e-7 two-sided; p=9.7e-5 for "greater than" (i.e., repeat dose > carc/chronic)*	*p = 6e-7*
Repeat dose > repro/dev?	Repeat dose < repro/dev is statistically significant.	*p = 4.5e-7 two-sided; p=2e-7 for "less than" and 1 for "greater than" (i.e., repeat dose < repro/dev)*	*p = 4e-7*

Are there chemical structure features that are enriched in the set with POD ratio ≤ 0?

13/48 chems with POD_{NAM}:POD_{trad} ≤ 0 are organophosphate pesticides.



ToxPrint ChemoType (CT)	Total # chems in the full 376 chem set with the CT	#, POD ratio < 0	# POD ratio > 0	# , chems without CT & POD ratio < 0	# chems without the CT & POD ratio > 0	Balanced Accuracy	Odds Ratio	p-value
bond:P=O_phosphate_thioate	12	4	8	42	317	0.608171	3.774	0.049
bond:P=O_phosphorus_oxo	10	5	5	41	320	0.693213	7.805	0.004
bond:P~S_generic	28	11	17	35	308	0.645408	5.694	0
ring:hetero_[5]_N_S_thiadiazole_(1_3_4-)	2	2	0	44	325	0.940379	inf	0.015
CONSENSUS ROW	36	15	21	31	304	0.662065	7.005	0



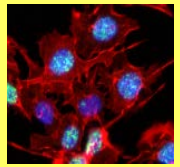
Common to methidathion (an OP) and tebuthiuron (urea pesticide; ratio was -0.08).

Preliminary work using the ChemoType Enrichment beta workflow, Ann Richard and Ryan Lougee, EPA-ORD-NCCT

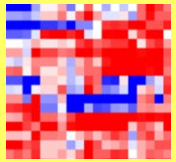
Would assays with cellular phenotypic endpoints improve the bioactivity prediction?

64 Chemicals of interest

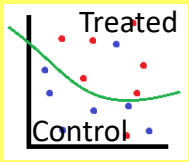
In vitro cell lines
Lung cells (BEAS-2B)
Liver cells (HepG2)
Kidney cells (HK-2)



High-dimensional Phenotypic profiles
(165 features based on DNA, actin cytoskeleton, and γH2AX stains)

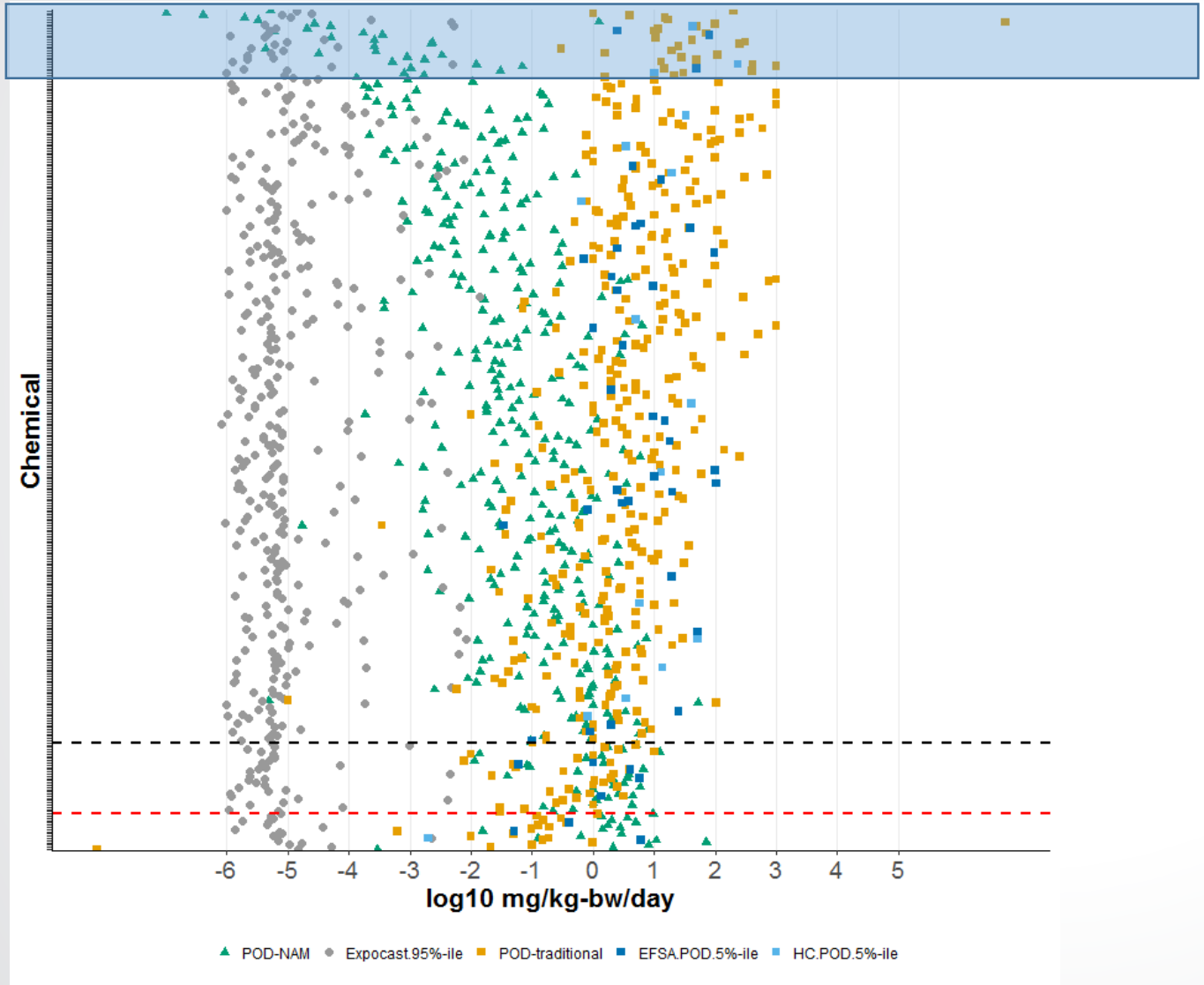


***In vitro* POD**
(EC_{50} values based on either **phenotypic change** or **cell count**)



- All experiments and analyses were performed by A*STAR without knowing the identities of the supplied chemicals (blinded study).
- Data have now been analyzed and suggest that for a number of chemicals phenotypic changes occurred at concentrations < cytotoxicity.
- A comparison of the POD_{NAM} and the POD from these experiments is part of our ongoing work.

Are there key drivers of examples where BER ≤ 0?

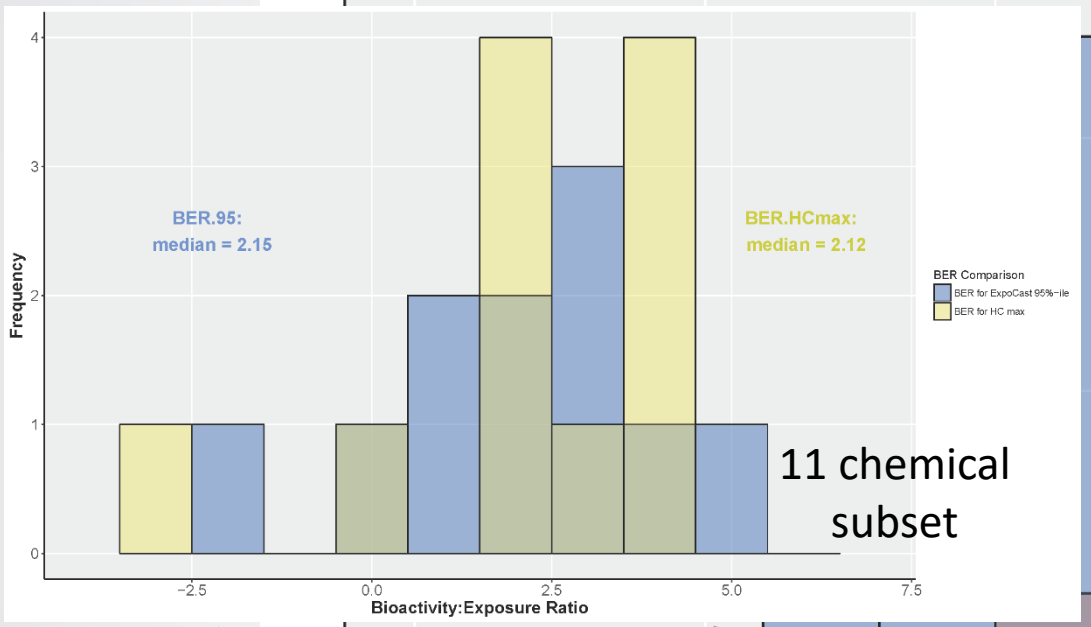


BER < 0

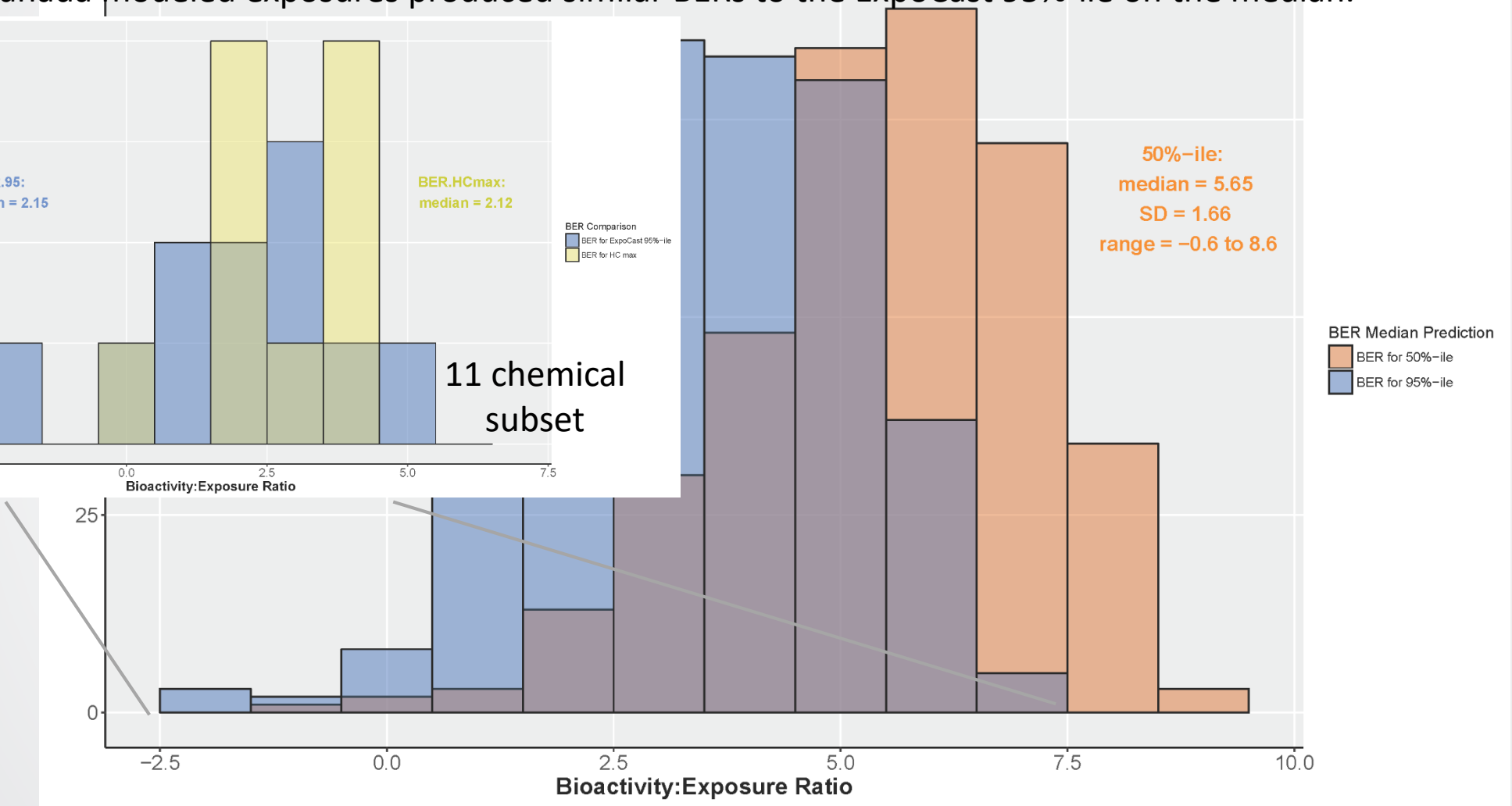
- Do some ToxCast assay AC50s drive a much lower AC50?
- Are some ExpoCast predictions overly conservative?
- The chemicals for which BER < 0 should be reviewed to understand the difference between the *in vivo* POD information and the in vitro bioactivity information [ongoing work].

A distribution of the BERs suggests that using the 95%-ile on the median exposure makes for a more conservative BER

Health Canada modeled exposures produced similar BERs to the ExpoCast 95%-ile on the median.



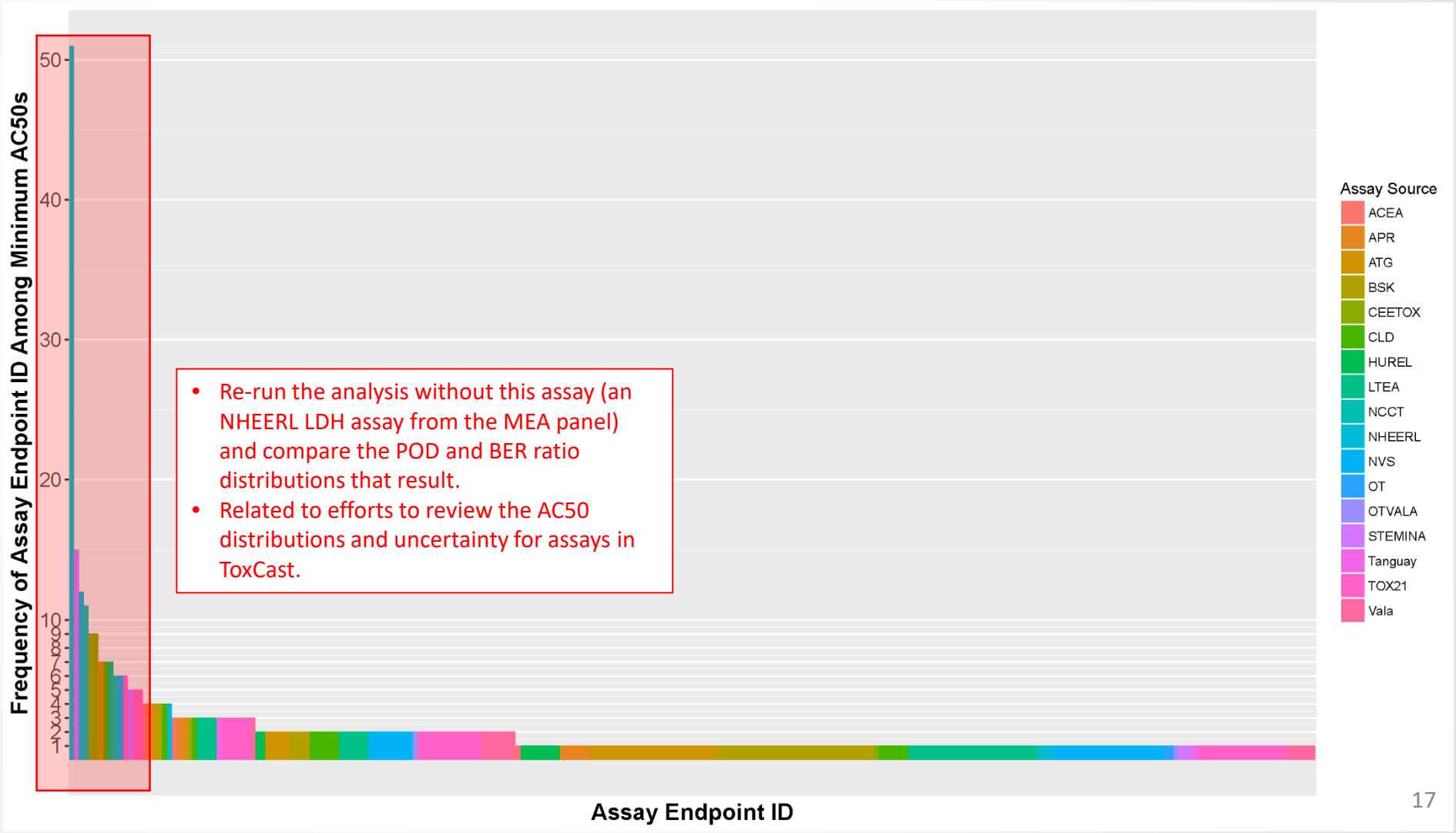
11 chemical subset



BER.95 distribution is left-shifted 2 log10 units, yielding a more conservative estimate that reflects prediction uncertainty.

Are certain assays driving the minimum ADE more often?

- Preliminary work suggests that we may want to re-examine the assay endpoint that drive 51 of the minimum ADEs.



- Re-run the analysis without this assay (an NHEERL LDH assay from the MEA panel) and compare the POD and BER ratio distributions that result.
- Related to efforts to review the AC50 distributions and uncertainty for assays in ToxCast.

Ongoing work includes…

- Continued examination of the “extremes” of the comparison
- More in-depth review of the dataset for the chemicals from these “extreme” regions of the comparison to determine underlying reasons for extreme values of the POD_{NAM}.
- Incorporation of additional information from partners including A*STAR.
- Re-run of the comparison:
  - rat high-throughput toxicokinetics and rat PODs;
  - allometric scaling to make all doses human doses (eliminate interspecies comparisons); and/or,
  - With non-steady state conditions (using C_{max}, AUC).

We are planning a manuscript for submission in 2018.

Utility of this case study

- How does the project contribute to the objectives of APCRA?
  - Prioritisation:
    - *Identification of substances with a small bioactivity:exposure ratio for prioritization.*
  - First tier assessment:
    - *Bioactivity appears to provide a conservative estimate of a point-of-departure;*
    - *Consider the uncertainties in this rapid assessment approach.*
  - Full assessment in a weight of evidence approach
    - *For substances lacking a comprehensive in vivo dataset, small POD_{NAM} : $POD_{traditional}$ ratio may indicate a need for additional in vivo study.*
  - Replacement (of animal studies) for full assessment:
    - *Substances with a bioactivity:exposure ratio > 1e6 may not need in vivo data.*
  - Classification and labelling
    - *NA*