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

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Based on collaboration with A*STAR, ECHA, EFSA, EPA-OLEM, EPA-ORD, Health Canada, and the JRC

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

Conflicts of interest

- I have no conflicts of interest to declare.

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See the forest for the trees

The big question:

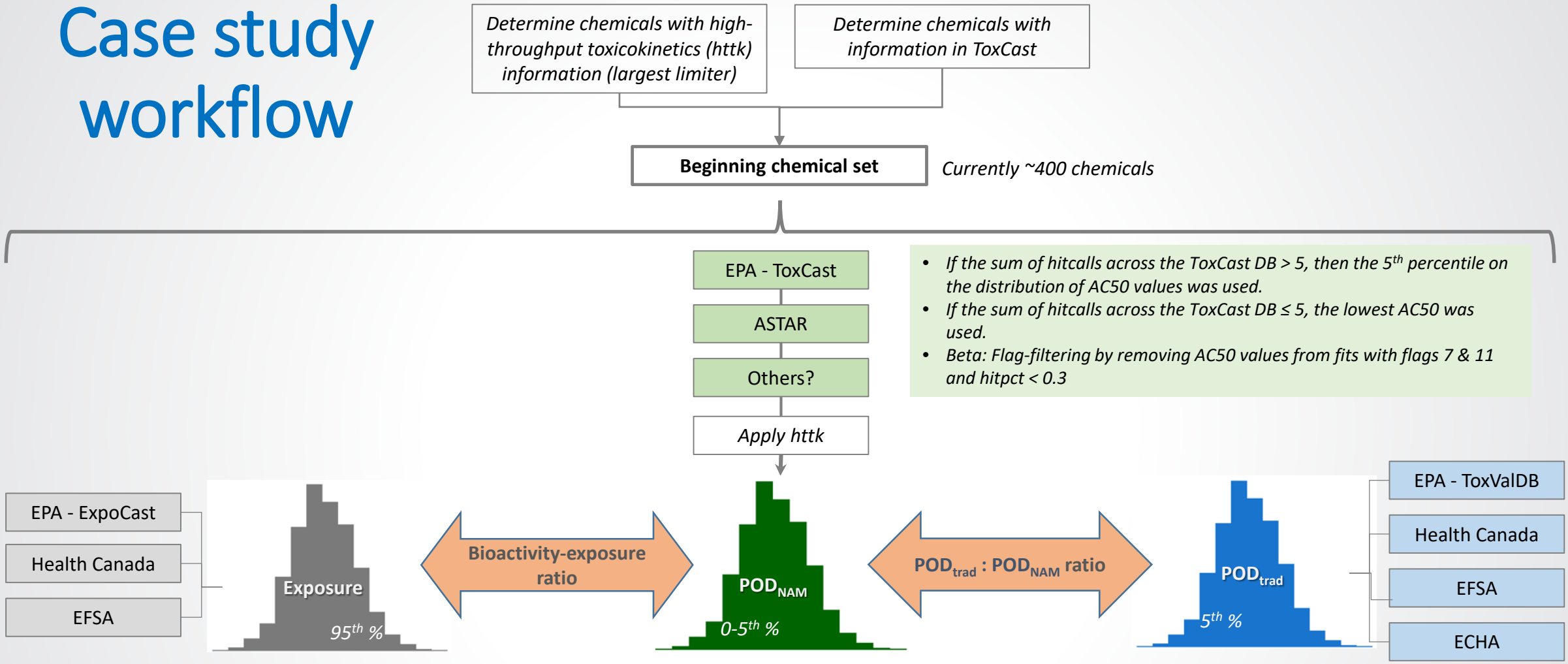
Can *in vitro* bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and screening level risk assessment?

A retrospective look at using *in vitro* bioactivity data as a POD

- POD ratio: Do new approach methods (NAMs; *in vitro* bioactivity data) provide a conservative estimate of POD?
- Bioactivity-exposure ratio (BER): Useful for risk-based prioritization of chemicals for additional study and/or to serve as a low tier risk assessment approach?

POD ratio	Compare $POD_{traditional}$ to POD_{NAM}; POD ratio > 0 means the POD_{NAM} was a conservative estimate of $POD_{traditional}$	• When was POD ratio > 0? • When POD ratio < 0, are there clear areas for improvement?
BER	Compare POD_{NAM} to ExpoCast exposure estimate; BER > 0 indicates POD_{NAM} was at a higher dose than predicted exposure	• When was BER ratio > 0? • When BER ratio < 0, where there any distinguishing factors?

Case study workflow



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

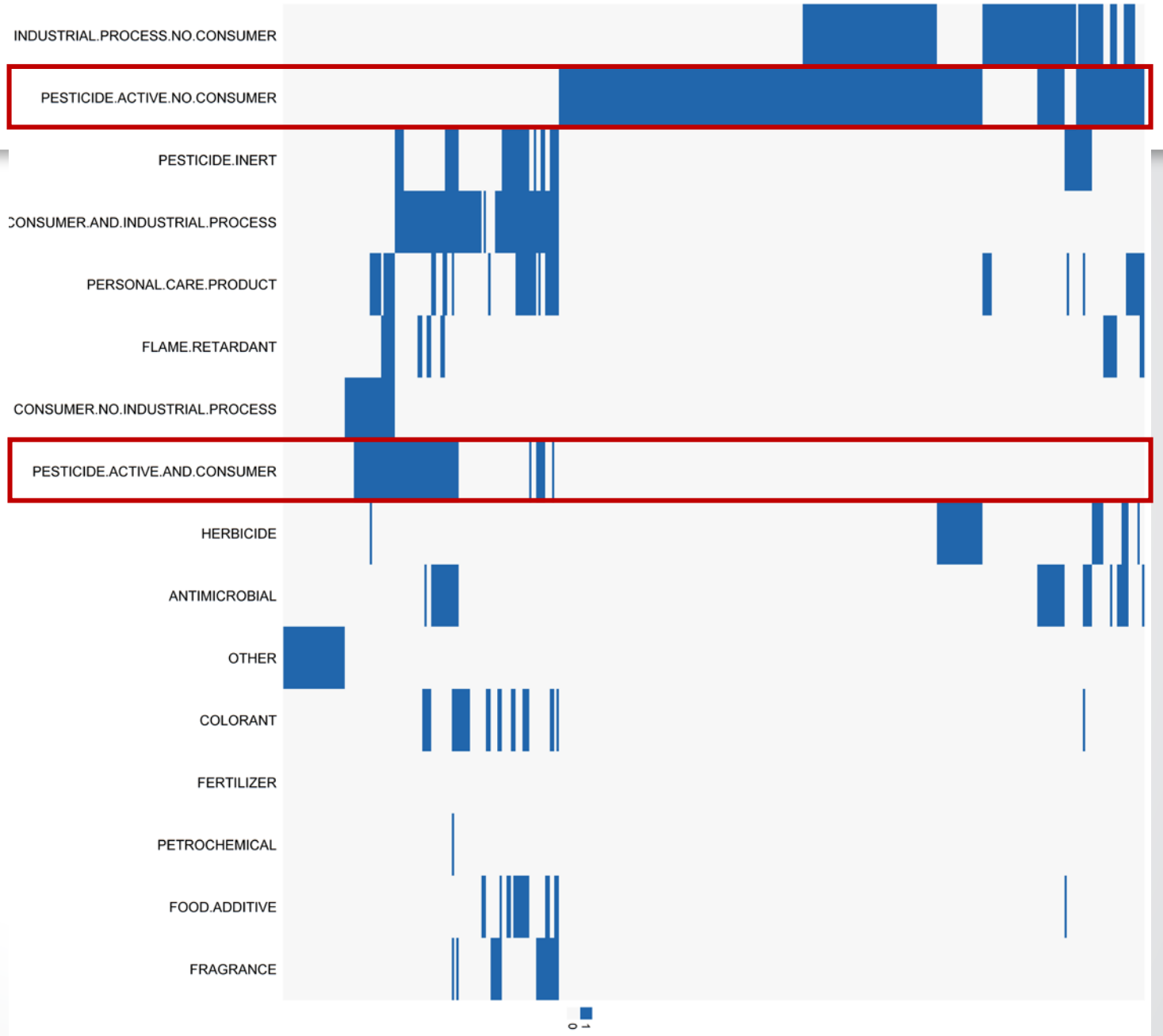
Is this log10 ratio useful for prioritizing chemicals?
Are there weaknesses here that can be addressed?

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*

The functional use space of chemicals in the study

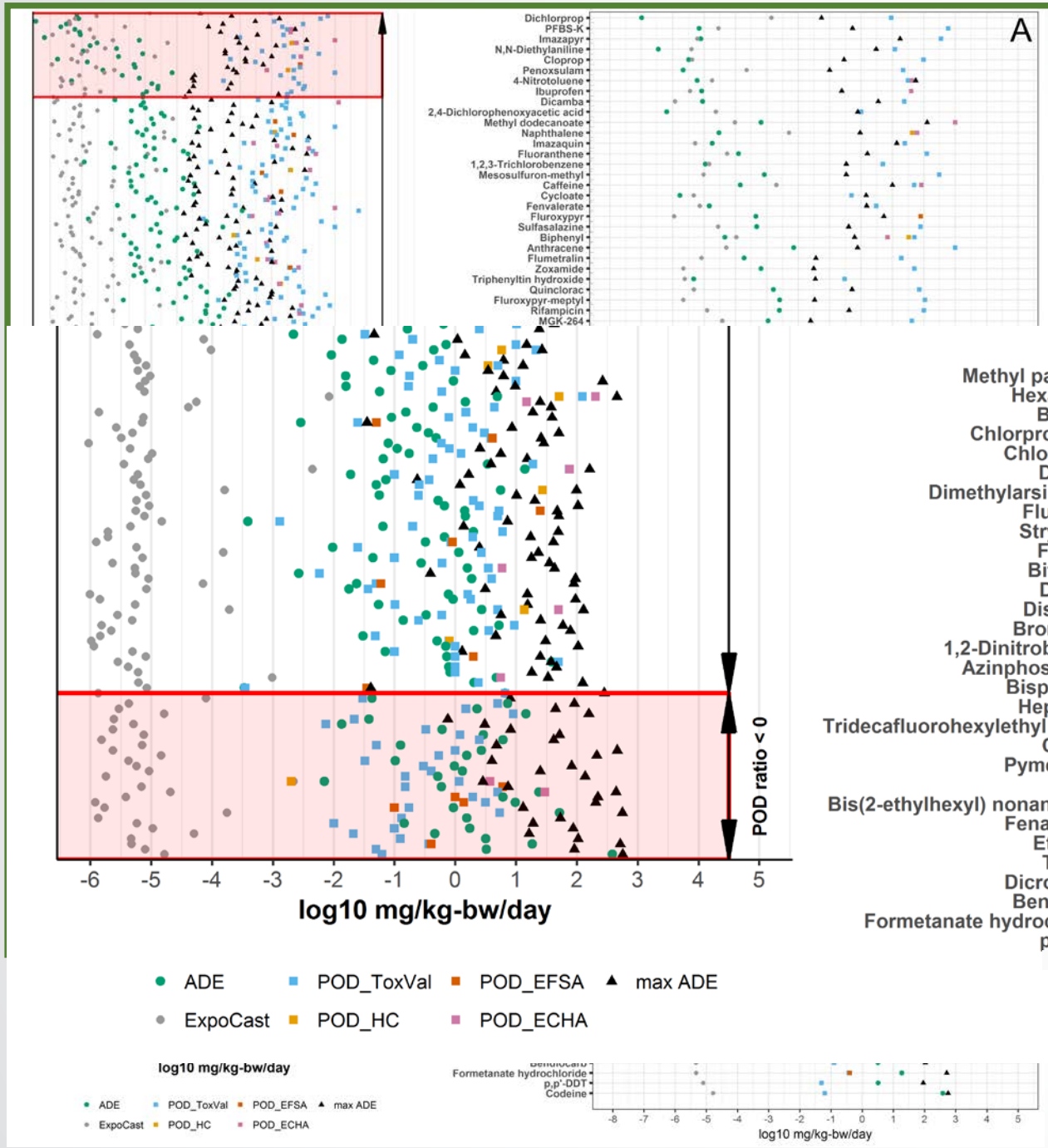
- This analysis used the simplistic use types available via AcTOR that are applied qualitatively.
- ~280/380 total have use as pesticide actives (74%).



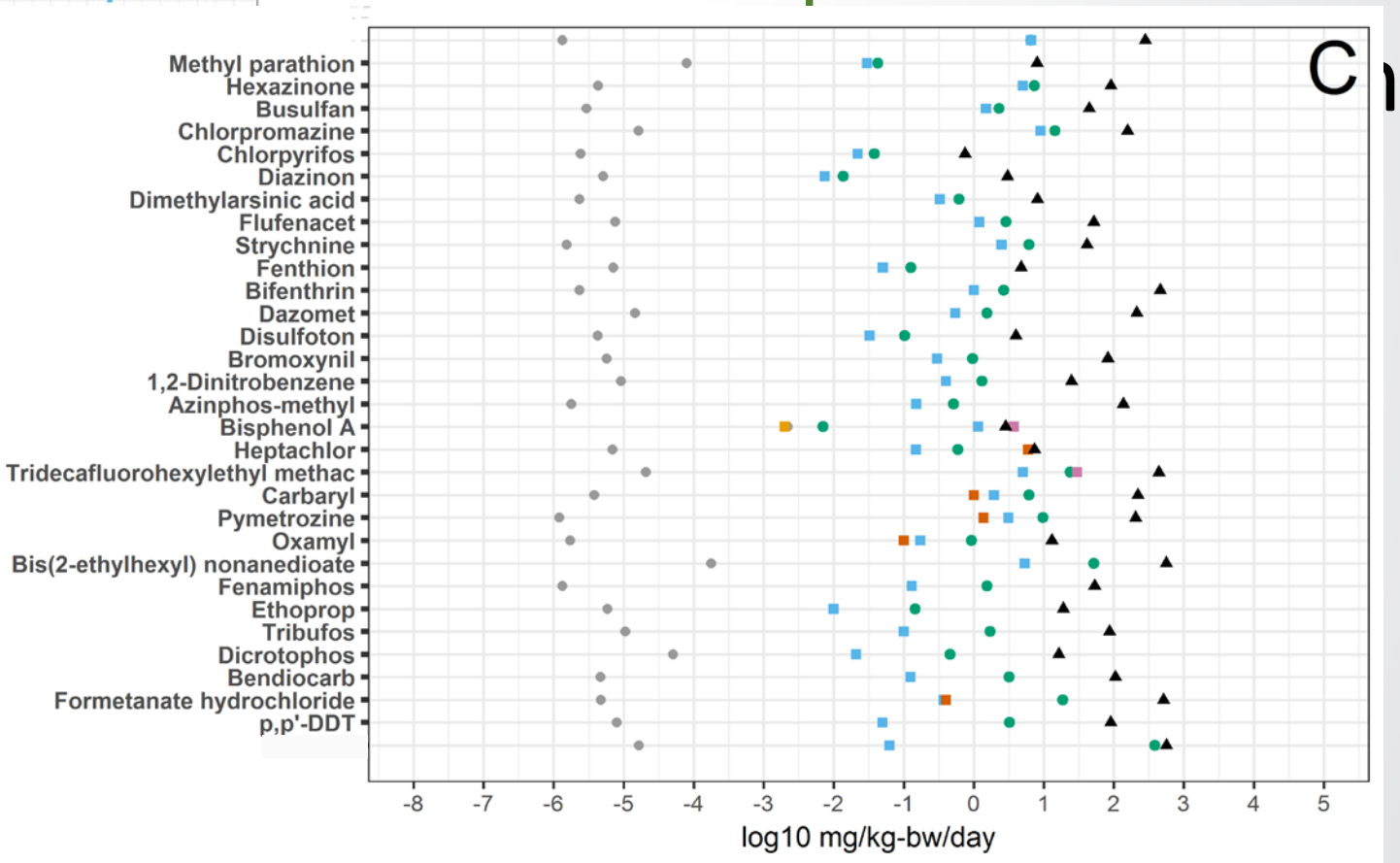
AcTOR functional use categories available from Wambaugh et al., 2014; Dionisio et al., 2015

Figure 1, Paul Friedman et al. *in prep.*

Preliminary results



349/380 chemicals =
91.8% of the time this naïve approach appears conservative

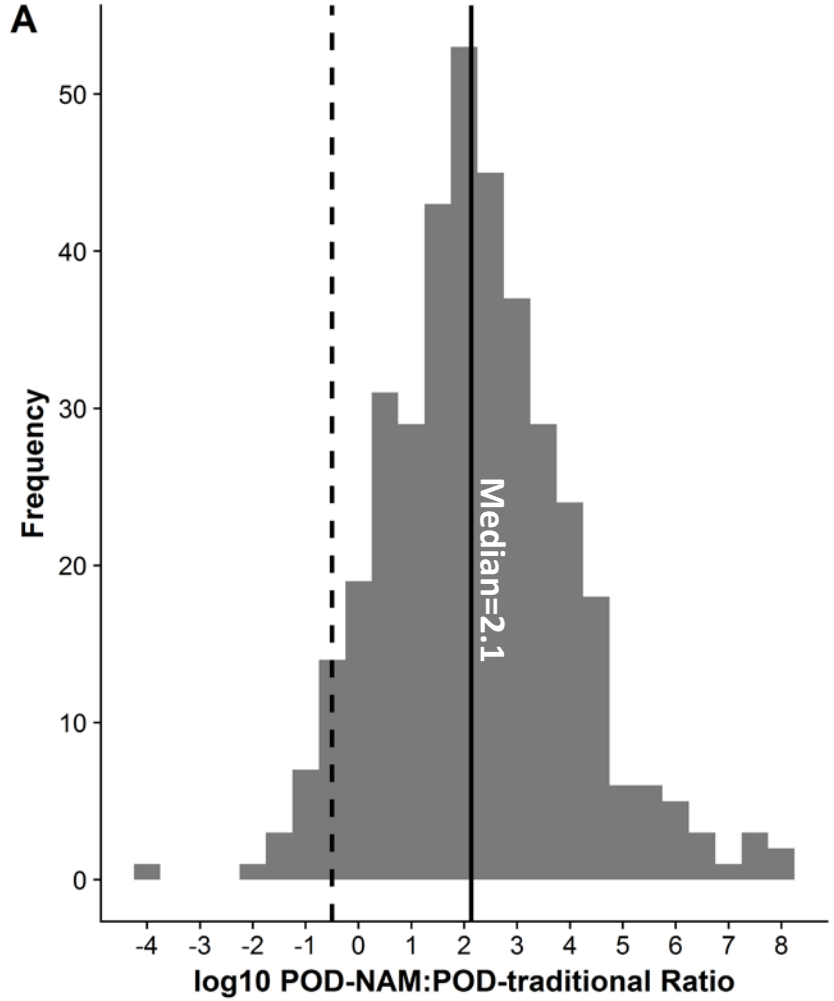


POD_{NAM} < $POD_{traditional}$ most of the time

8.2% where POD_{NAM} > $POD_{traditional}$

Figure 3, Paul Friedman et al. *in prep.*

Distribution of the POD ratio demonstrates the conservatism of the naïve approach



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Did the max ADE (ADE at 100 µM) limit overlap between POD_{NAM} and $POD_{traditional}$?

Condition	MaxADE < minPOD	MaxADE > minPOD
POD ratio > 0	196	153
POD ratio < 0	0	31

Figure 4, Paul Friedman et al. *in prep.*

The POD ratio distribution is similar when using either human or rat high-throughput toxicokinetic (httk) information

Human

Rat

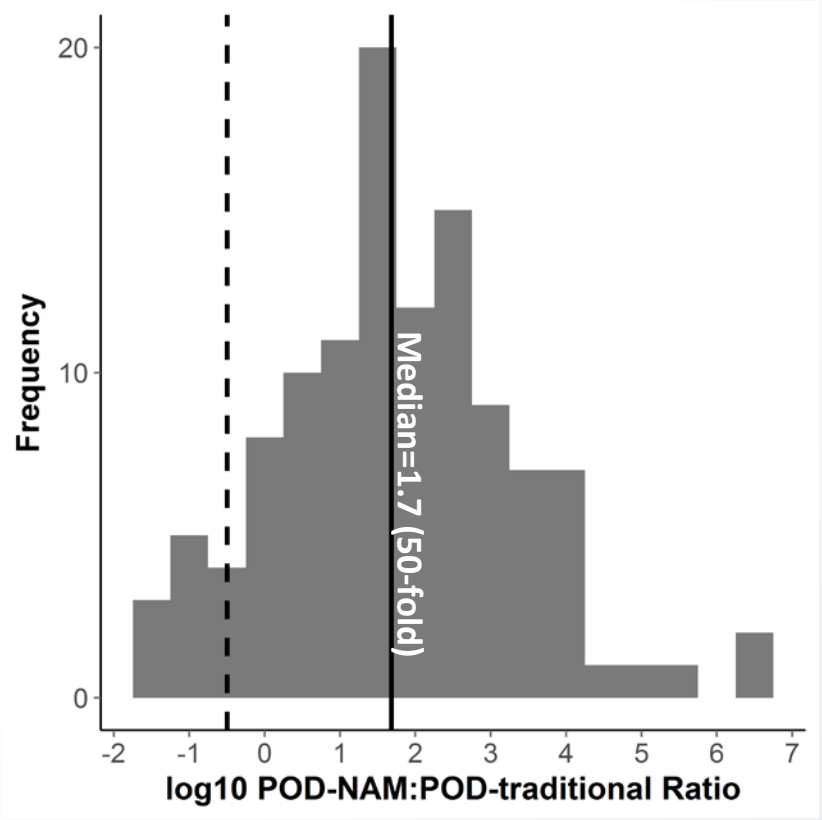
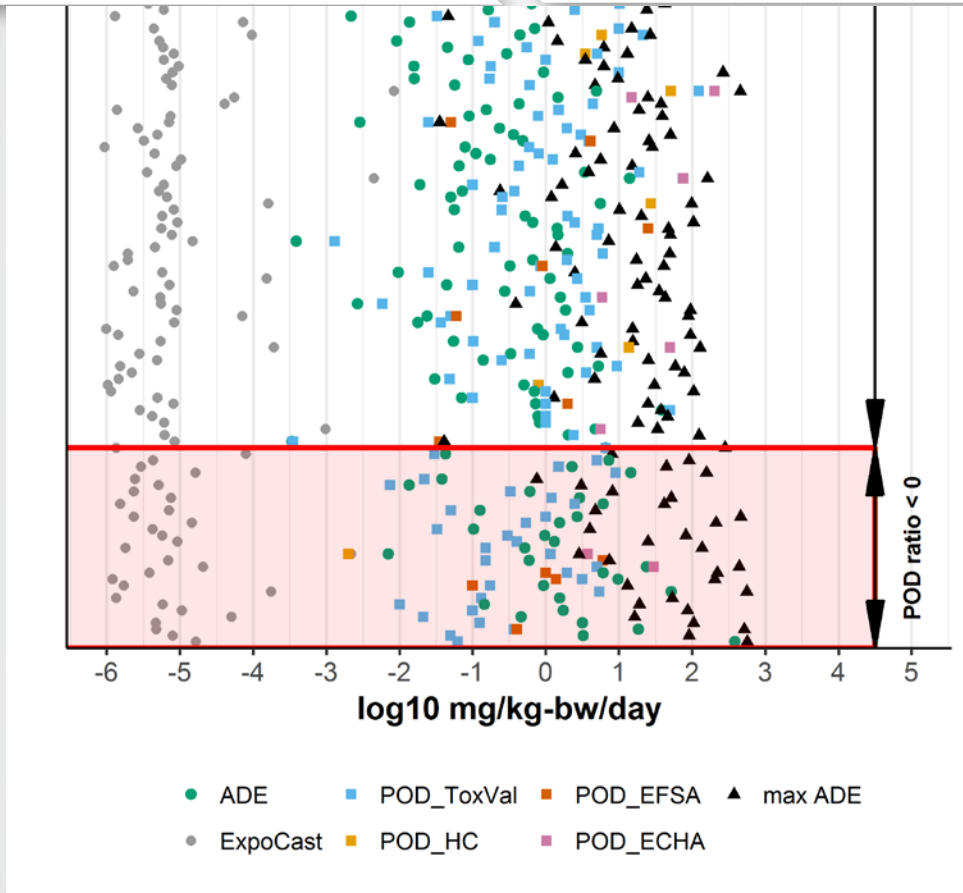


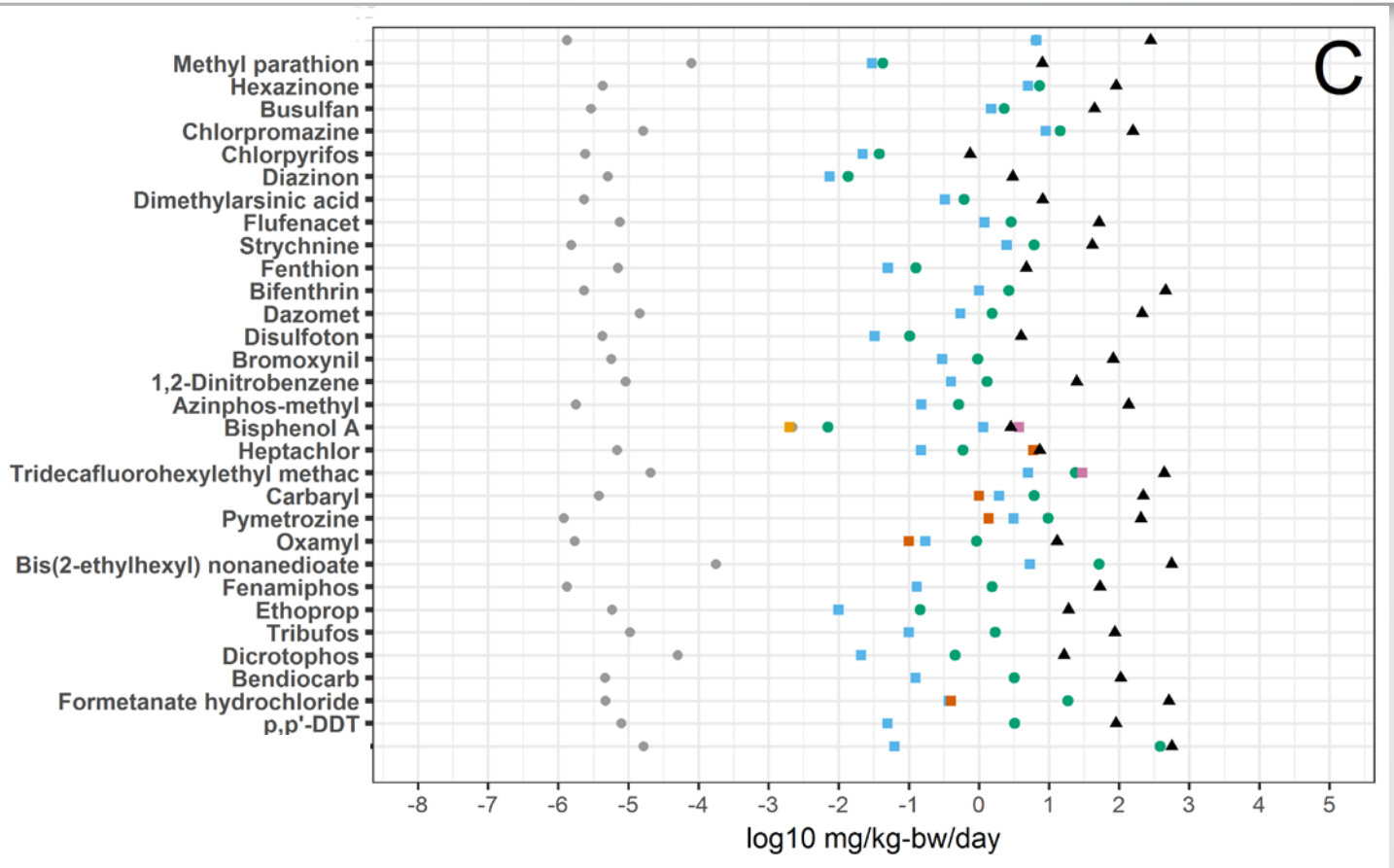
Figure 5, Paul Friedman et al. *in prep.*

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 to human doses.	NA
How it is considered	• Caution flag + hit pct filtering. • **5%-ile** of the **distribution** of all available AC50s was taken. • A rat-only example was generated with similar results in terms of % library.	• 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 (adds about 2 log's of conservatism)

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?

Minimum POD vs. chemical (color-coded study type)

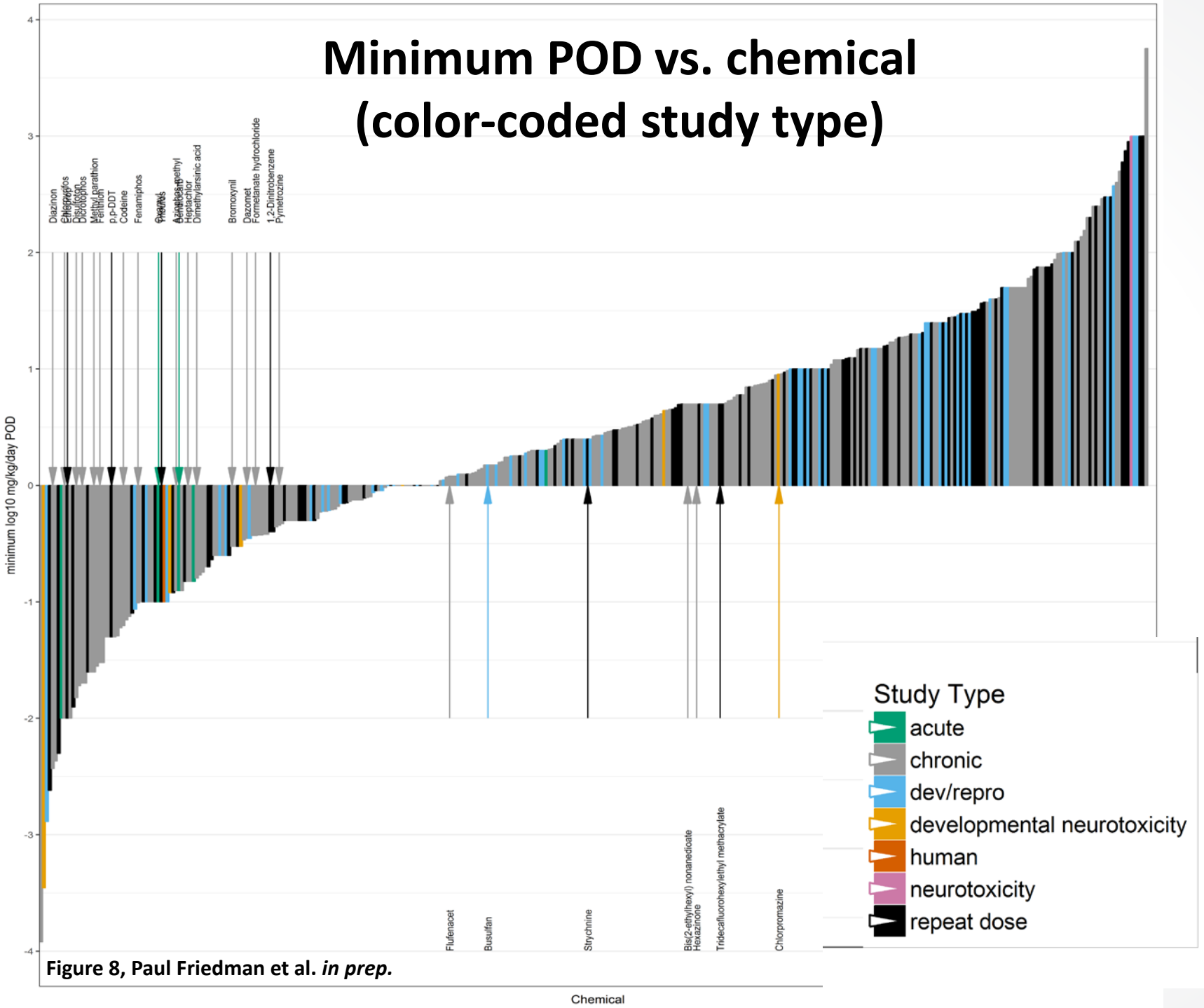


Figure 8, Paul Friedman et al. *in prep.*

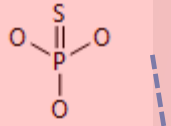
- POD ratio < 0 was not enriched for any risk_assessment_class (study type identifier)
- Greater chance that POD ratio < 0 when the min POD was lower.

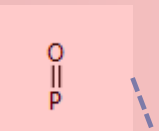
It does not seem like particular study types are driving the minimum(POD) when POD ratio ≤ 0.

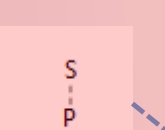
Hypothesis	Fisher's exact test results	Caveats
Reproductive and/or developmental studies over-represented when POD ratio ≤ 0?	• No • p-value = 0.79; • odds-ratio = 0.73	Some ambiguity or error expected in assigning study classes; preference given to: DNT, neuro, dev/repro, acute, repeat, chronic (in that order) in the event of a min POD tie
Carcinogenicity or chronic studies over-represented when POD ratio ≤ 0?	• No • p-value = 0.52; • odds-ratio=1.055	

Chemical structure features associated with organophosphate pesticides are enriched in the set with POD ratio ≤ 0.

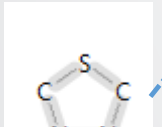
13 chems with POD ratio ≤ 0 are organophosphate pesticides.







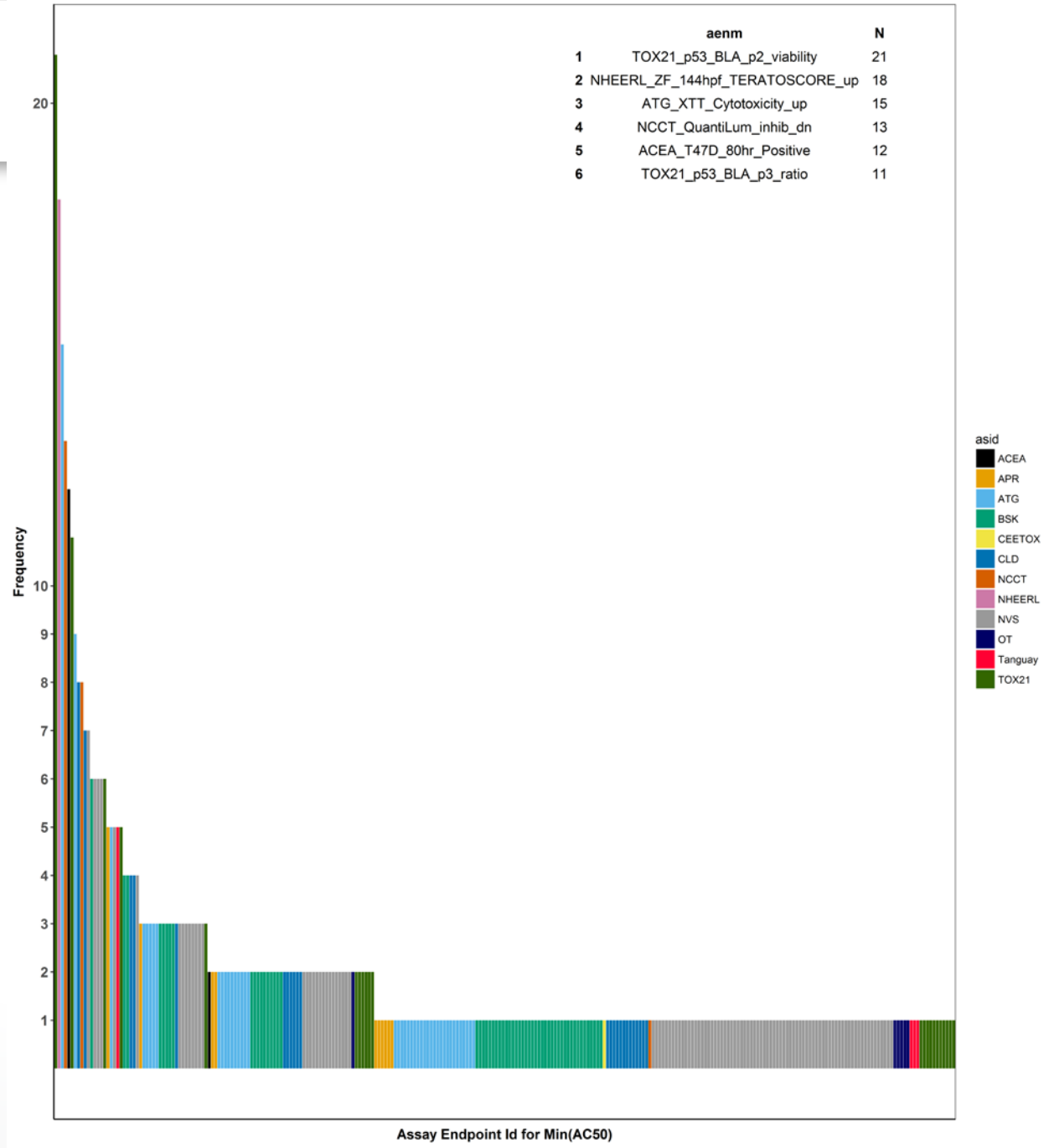
ToxPrint ChemoType (CT)	Total # chems in the full 376 chem set with the CT	#, POD ratio < 0	# POD ratio > 0	# , chems without CT & POD ratio < 0	# chems without the CT & POD ratio > 0	Balanced Accuracy	Odds Ratio	p-value
bond:P=O_phosphate_thioate	12	4	8	42	317	0.608171	3.774	0.049
bond:P=O_phosphorus_oxo	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 (#2542, Poster P904 Tue 10:45-12:15) and Ryan Lougee, EPA-ORD-NCCT

The ToxCast assay that set the minimum AC50 was investigated, with no evidence of a particular *in vitro* bioactivity causing bias.



Are there key drivers of examples where POD ratio >> 0 and 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].

Only ~4% of chemicals in the case study have BER < 0 using the more conservative estimate of exposure.

Figure 10, Paul Friedman et al. *in prep.*

Preliminary work to compare thresholds of toxicological concern (TTC) for Cramer classes

- Shown here: Cramer classes for 116 of 380 chemicals clearly free of any alerts for genotoxicity*
- General trend:

  in vitro bioactivity-derived POD > TTC

  (for 93/116 chemicals ~ 80%,
  with a median margin = 1.2log$_{10}$)

TTC values from pipeline developed by Matthew Gagne and Tara Barton-Maclaren (#2550) at Health Canada (from "Scientific Approach Document: Threshold of Toxicological Concern (TTC)-based Approach for Certain Substances," 2016)

***Rules for distinguishing genotoxicity and non-genotoxicity are part of ongoing detailed work on predicting carcinogenicity.*

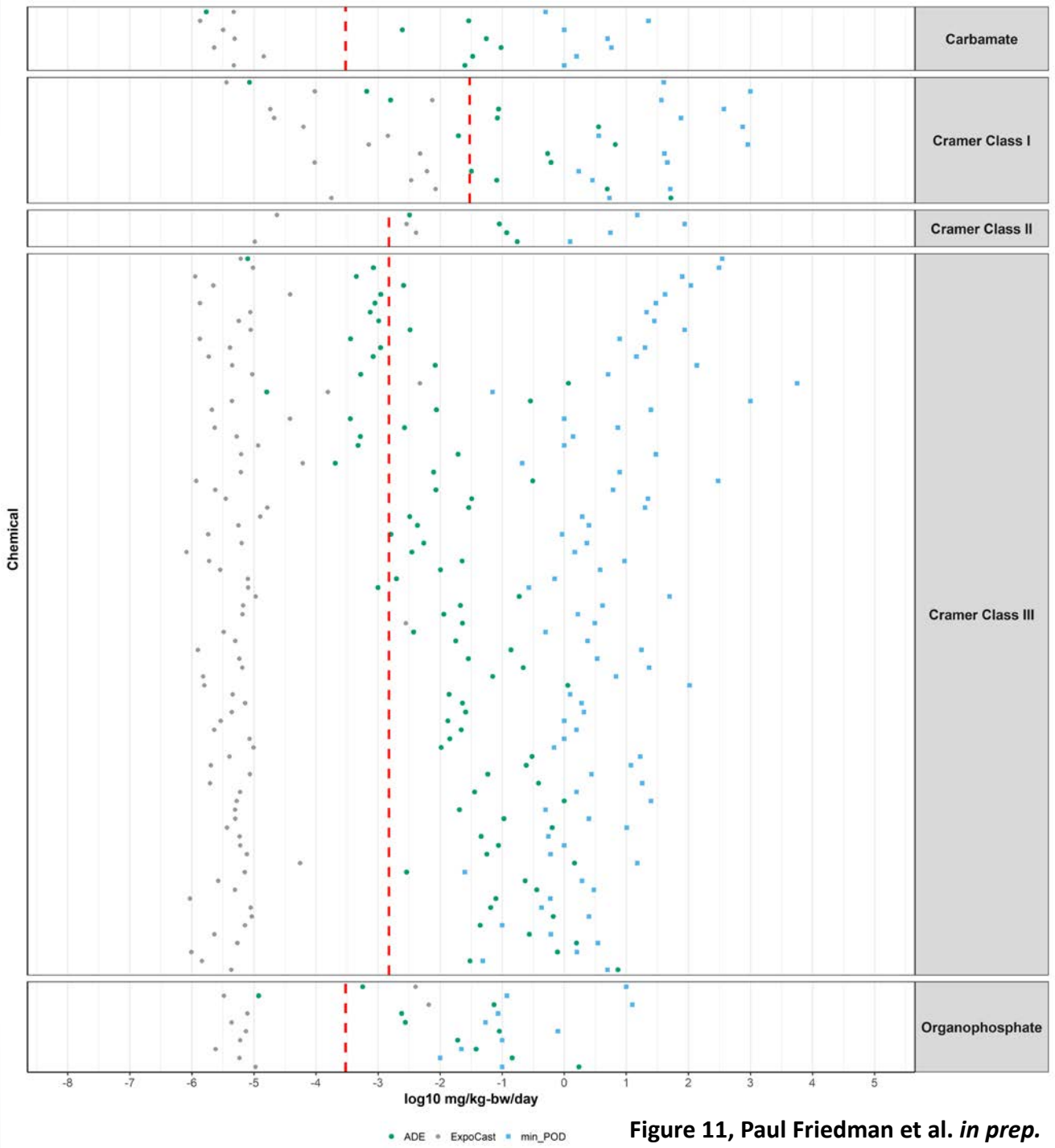


Figure 11, Paul Friedman et al. *in prep.*

Conclusions and limitations

- A simplistic approach to using *in vitro* bioactivity data as a POD appears to be a conservative estimate > 90% of the time for 380 chemicals.
- POD_{NAM} estimates appear conservative with a margin of ~100. When potential cross-species differences in toxicokinetics was considered the margin was ~50.
- 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.