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

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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

ASTAR HIPPTox EC10s (µM)

ToxCast AC50s (µM)

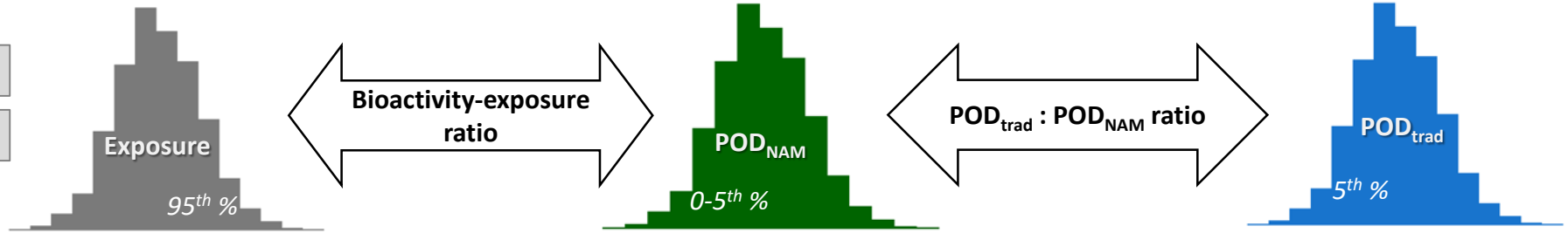
- *If the sum of hitcalls across the ToxCast DB > 5, then the 5th percentile on the distribution of AC50 values was used.*
- *If the sum of hitcalls across the ToxCast DB ≤ 5, the lowest AC50 was used.*
- *Flag-filtering by removing AC50 values from fits with 3+ caution flags and hitpct <= 0.5*

Apply high-throughput toxicokinetics (httk) to get mg/kg/day

- *Using httk v1.8 values for humans*
- *Default to a simple model with no partition coefficients and use of steady-state concentration.*
- *Assume 100% bioavailability and restrictive clearance.*

EPA - ExpoCast

Health Canada



EPA - ToxValDB

Health Canada

EFSA

ECHA

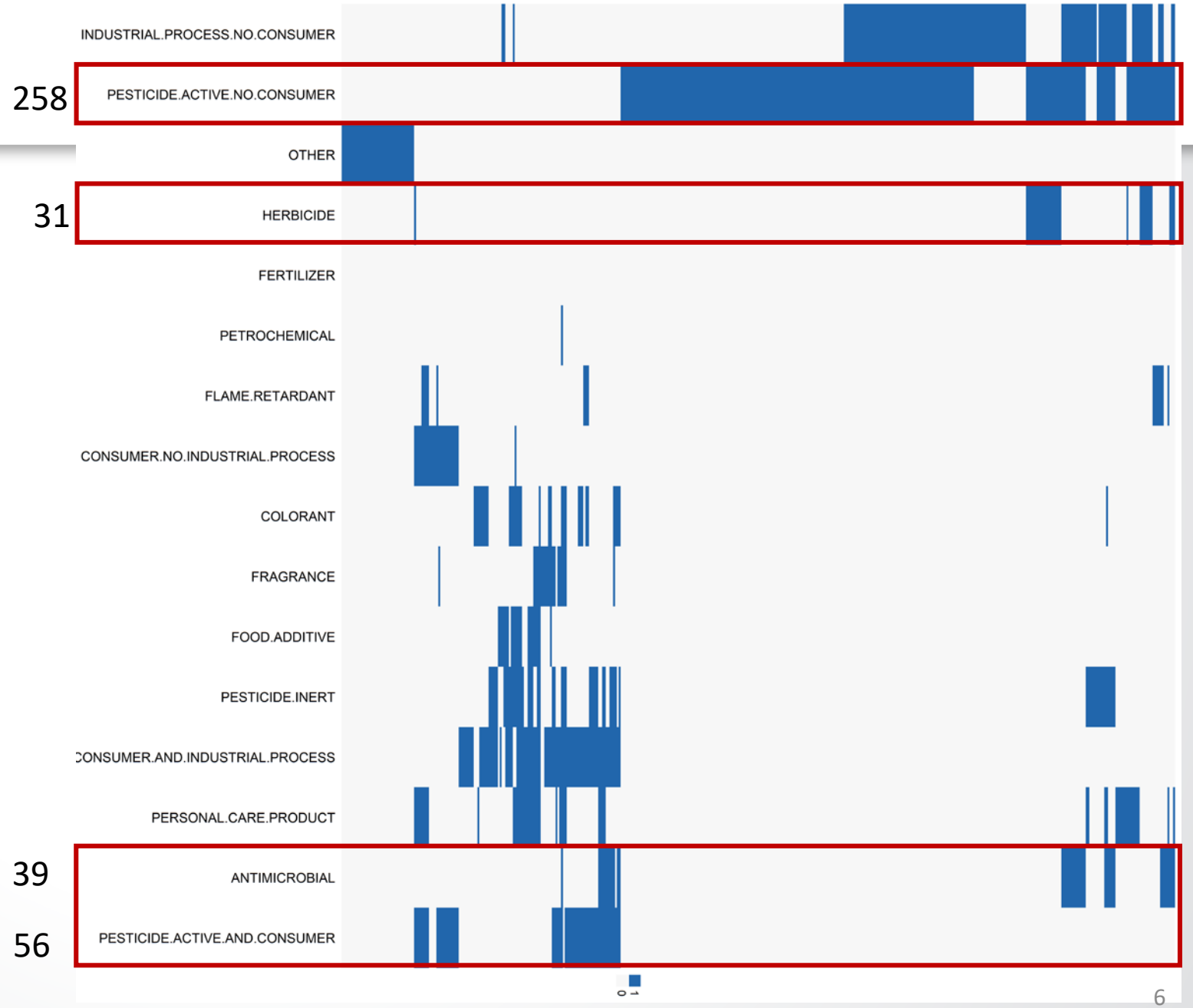
Is BER useful for prioritization?
Are there addressable weaknesses?

Is POD ratio > 0 for most chemicals?
Can we learn from POD ratio < 0?

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

The functional use space of chemicals in the study

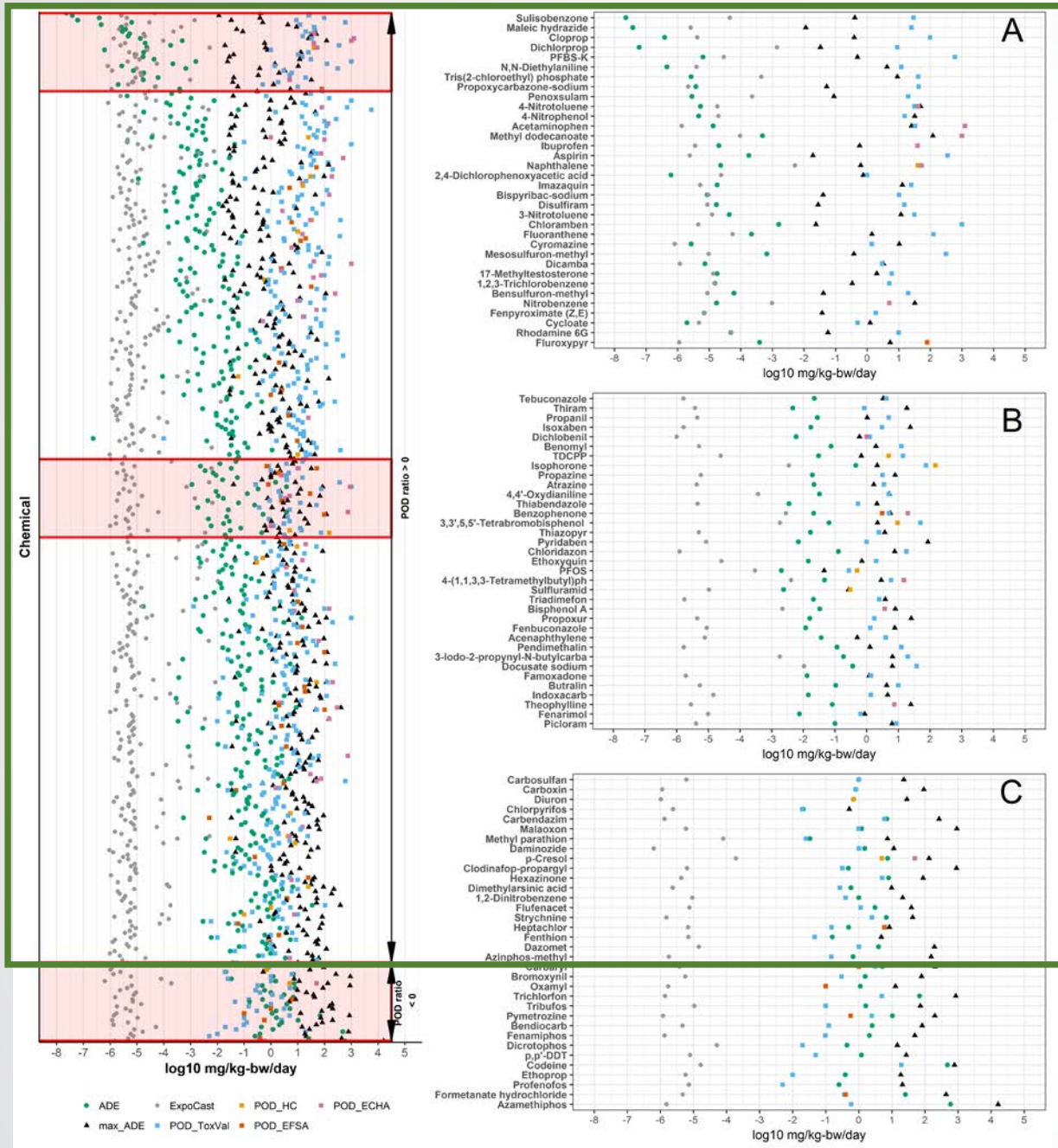
- This analysis used the simplistic use types available via AcTOR that are applied qualitatively.
- ~314/448 total have use as pesticide actives (~70%).



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

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

Preliminary results



414/448 chemicals =
92% of the time this naïve approach appears conservative

34/448 chemicals =
8% where $POD_{NAM} > POD_{traditional}$

$POD_{NAM} < POD_{traditional}$

(most of the time)

Figure 2, Paul Friedman et al. *in prep.*

Distribution of the POD ratio demonstrates conservatism

- The median POD-NAM:POD-traditional ratio is 2.2 (so approximately 100 mg/kg/day separation between values)

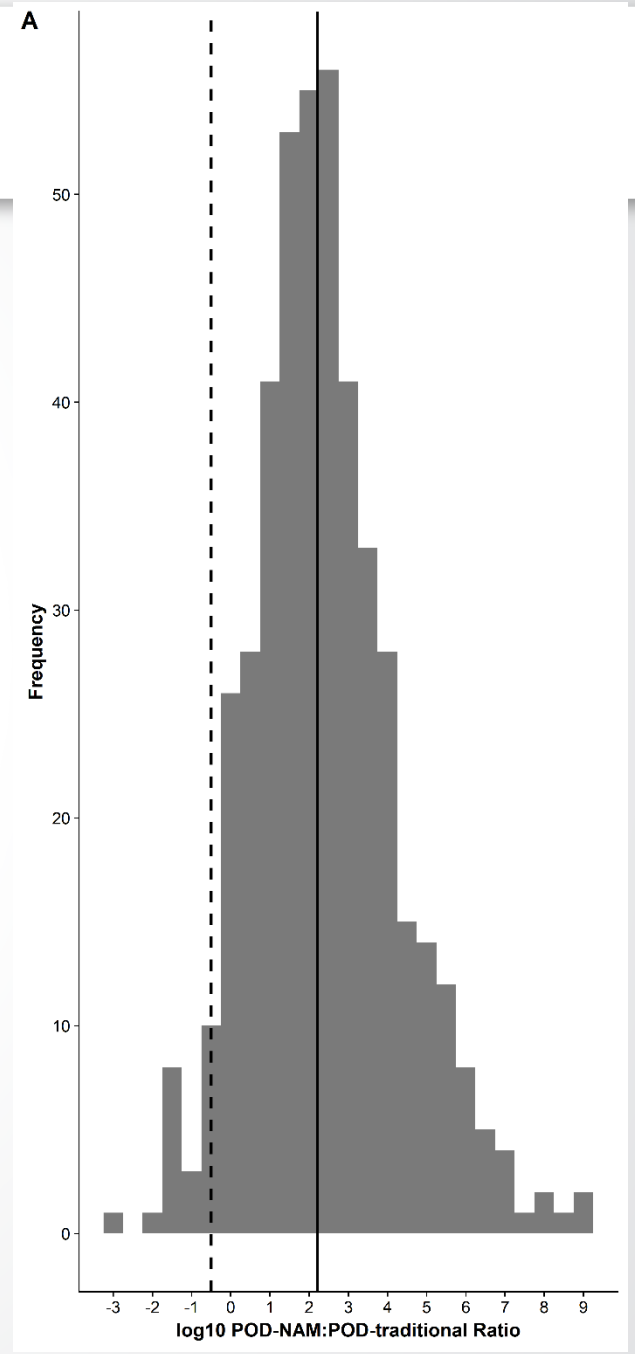
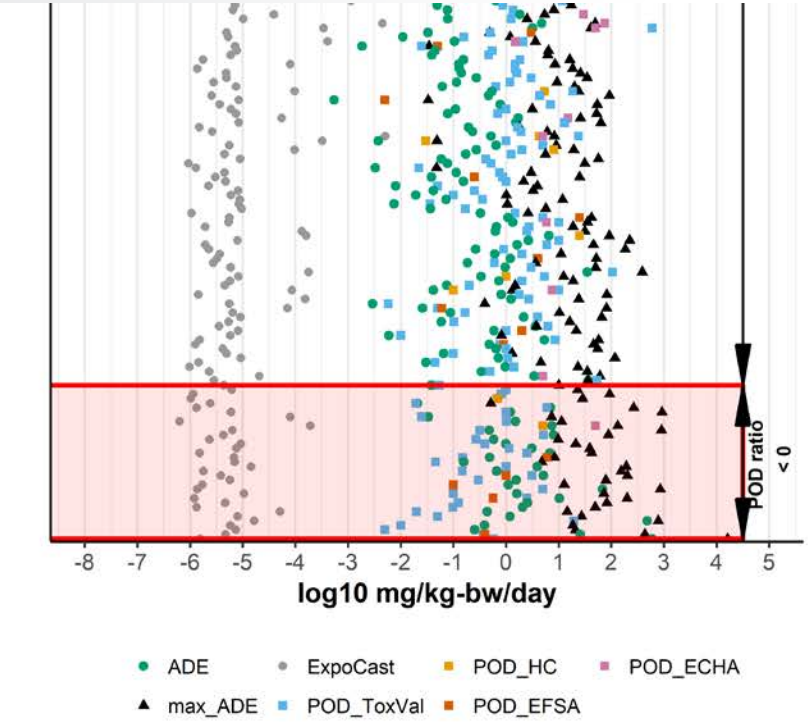


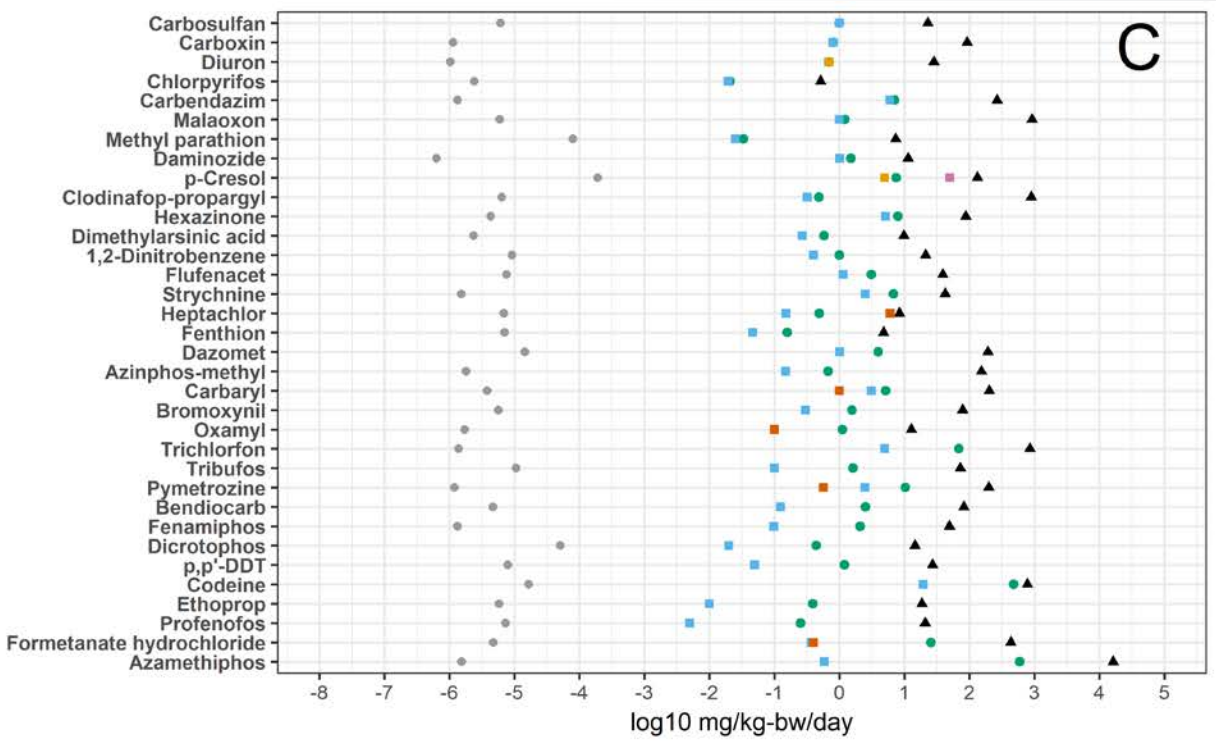
Figure 4, Paul Friedman et al. *in prep.*

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.	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 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?

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

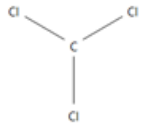
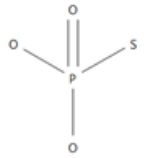
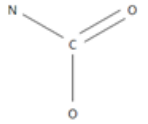
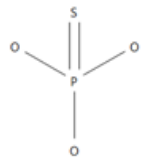
Condition	Dev/Repro is minPOD	Dev/Repro is not minPOD
POD ratio < 0	1	33
POD ratio > 0	44	370

Condition	Chronic is minPOD	Chronic is not minPOD
POD ratio < 0	23	11
POD ratio > 0	249	165

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	

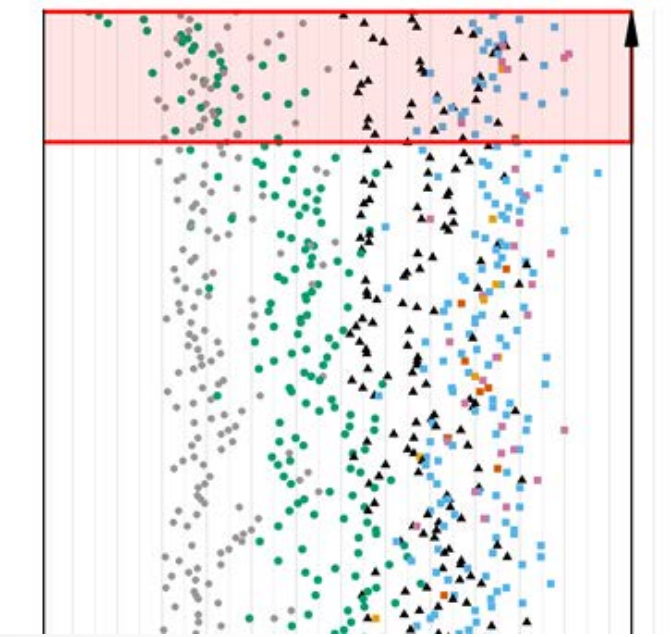
Chemical structure features associated with organophosphate pesticides are enriched in the set with POD ratio ≤ 0.

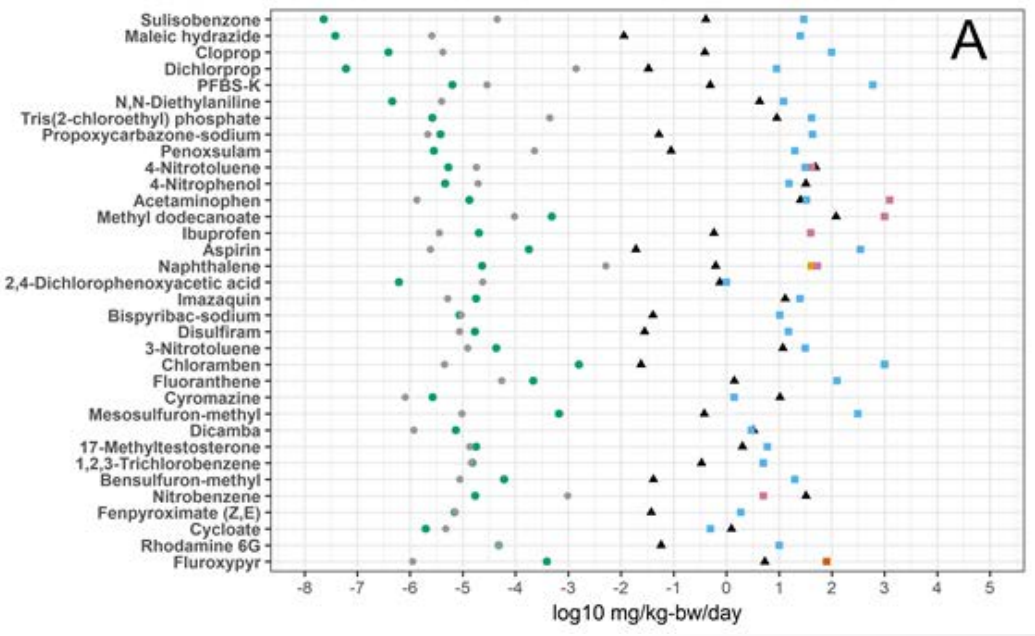
- 17 of 34 chemicals with POD ratio ≤ 0 are organophosphate pesticides.
- 20 of 34 chemicals corresponded to these chemotype enrichments.

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:CS_sulfide		53	11	42	0.57	4.2	0.000847	bond:P=O_ phosphorus_oxo		17	8	9	0.70	14	7.67E-06
bond:CX_halide_ alkyl-Cl_trichloro_ (1_1_1-)		4	2	2	0.71	13	0.031009	bond:P~S_generic		27	9	18	0.64	7.8	5.48E-05
bond:P=O_ phosphate_thio		3	3	0	0.96	NA	0.000413	bond:C(=O)N_ carbamate		20	6	14	0.62	6.1	0.002294
bond:P=O_ phosphate_thioate		9	3	6	0.63	6.5	0.025108								

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

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].

Only ~6% of chemicals in the case study have BER < 0 using the more conservative estimate of exposure.

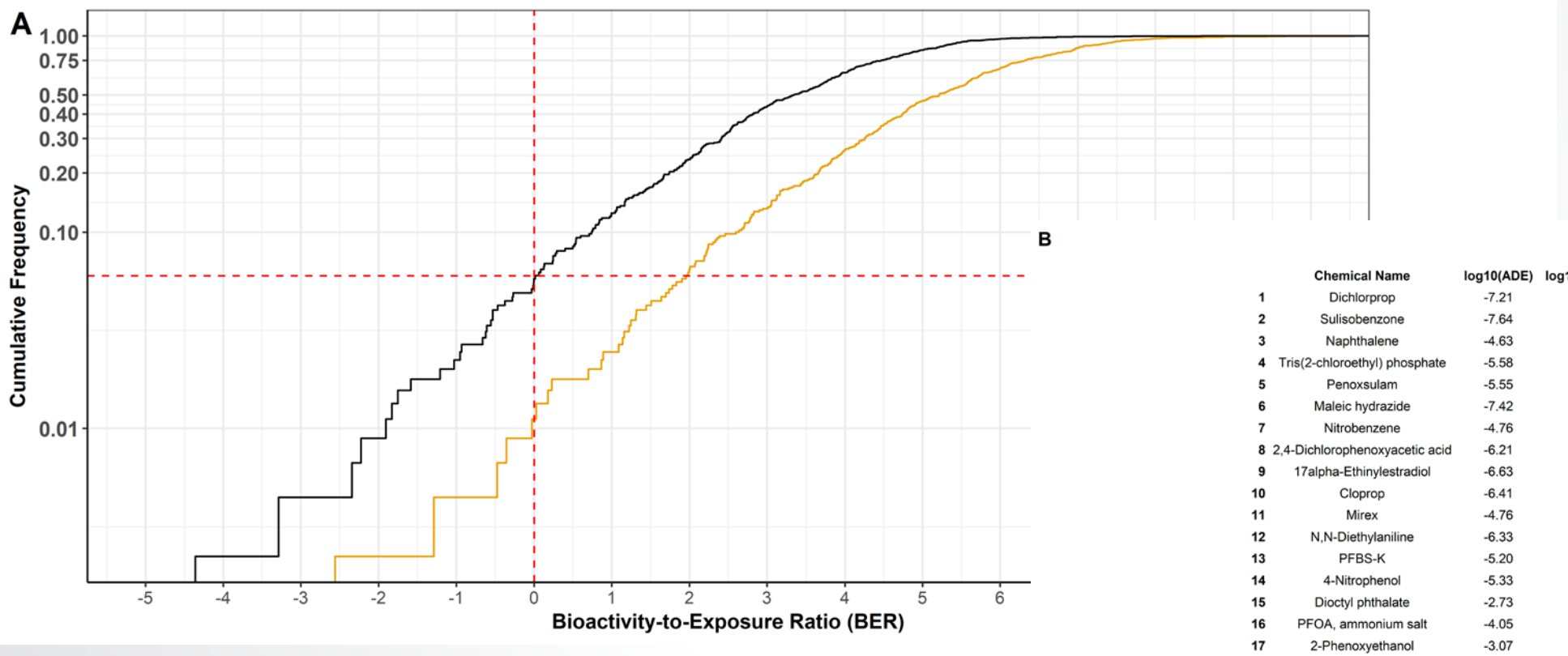


Figure 10, Paul Friedman et al. *in prep.*

B

	Chemical Name	log10(ADE)	log10(ExpoCast 95%-ile)	BER, 95%-ile
1	Dichlorprop	-7.21	-2.85	-4.36
2	Sulisobenzone	-7.64	-4.35	-3.29
3	Naphthalene	-4.63	-2.29	-2.34
4	Tris(2-chloroethyl) phosphate	-5.58	-3.35	-2.23
5	Penoxsulam	-5.55	-3.64	-1.91
6	Maleic hydrazide	-7.42	-5.59	-1.83
7	Nitrobenzene	-4.76	-3.01	-1.75
8	2,4-Dichlorophenoxyacetic acid	-6.21	-4.62	-1.59
9	17alpha-Ethinylestradiol	-6.63	-5.42	-1.21
10	Cloprop	-6.41	-5.38	-1.03
11	Mirex	-4.76	-3.81	-0.95
12	N,N-Diethylaniline	-6.33	-5.40	-0.93
13	PFBS-K	-5.20	-4.54	-0.66
14	4-Nitrophenol	-5.33	-4.71	-0.62
15	Dioctyl phthalate	-2.73	-2.13	-0.61
16	PFOA, ammonium salt	-4.05	-3.49	-0.56
17	2-Phenoxyethanol	-3.07	-2.54	-0.53
18	4-Nitrotoluene	-5.27	-4.74	-0.53
19	Biphenyl	-4.44	-3.97	-0.47
20	Cycloate	-5.70	-5.33	-0.38
21	Resorcinol	-2.68	-2.40	-0.28
22	Tributyl phosphate	-2.66	-2.39	-0.27
23	Bispyribac-sodium	-5.06	-5.03	-0.03
24	Fenpyroximate (Z,E)	-5.15	-5.14	-0.01
25	17beta-Estradiol	-5.36	-5.35	-0.01
26	Rhodamine 6G	-4.31	-4.31	0.00

Were the ToxCast AC50 values just much lower for the chemicals with BER <0? No, not uniformly.

- Top distribution shows all AC50s for chemicals in the case study.
- For some chemicals, they did appear more potent (lower AC50 values).
- Others seemed to fall squarely along the aggregate distribution.
- We've taken a conservative approach with high-throughput toxicokinetics that favors lower POD-NAM values.
- In practice there are opportunities to refine the lowest AC50 used (particularly for smaller groups of chemicals) beyond the automated refinements in place.

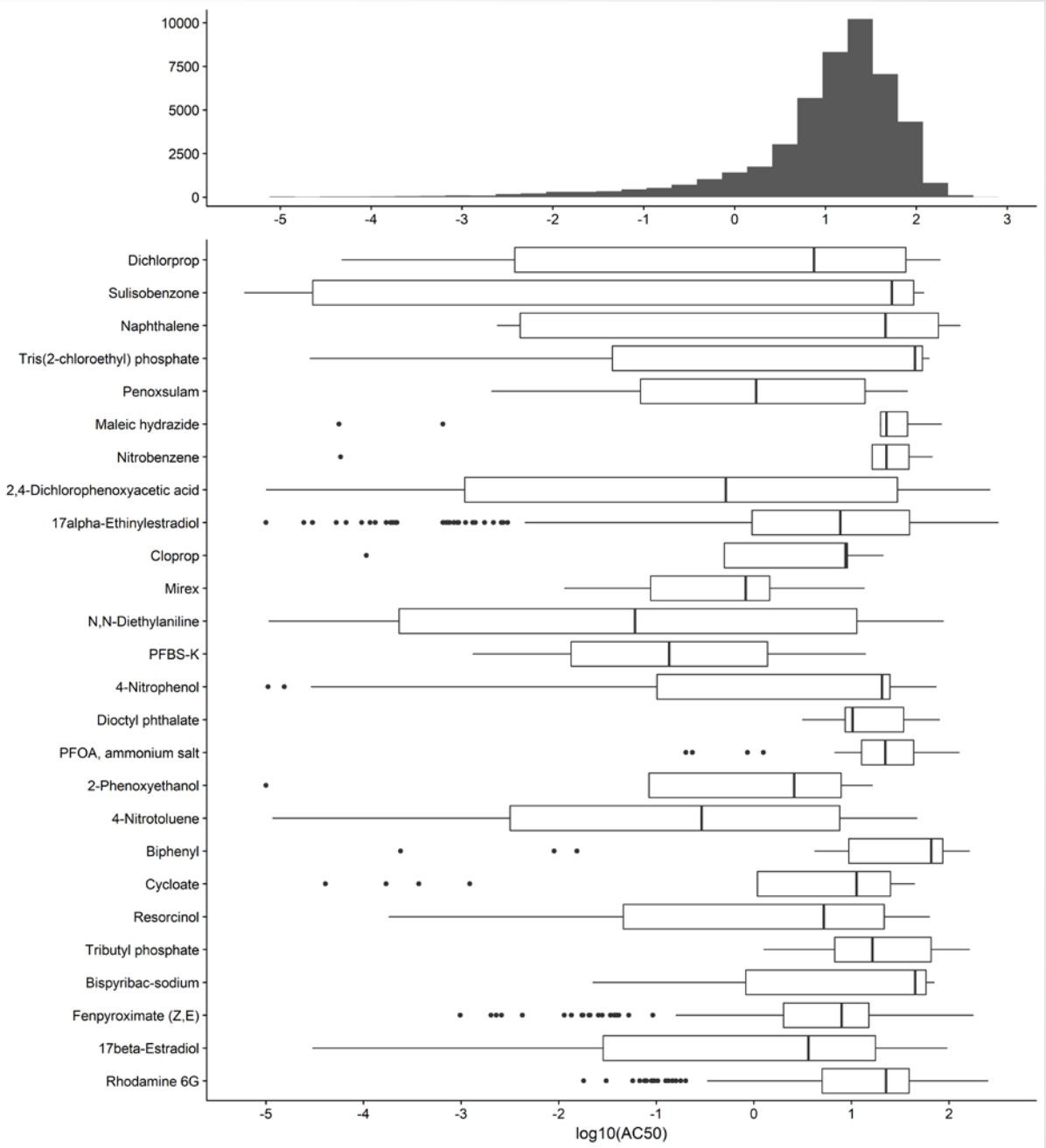


Figure 11, Paul Friedman et al. *in prep.*

Does using bioactivity as a conservative POD differ from using a TTC approach?

- Threshold of toxicological concern (TTC) = conservative
- Human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health.
- Relies on past accumulated knowledge regarding the distribution of NOELs of relevant classes of chemicals for which good toxicity data do exist.
- Useful substitute for substance-specific hazard information when human exposure is very low and there is limited or no information on the toxicity.

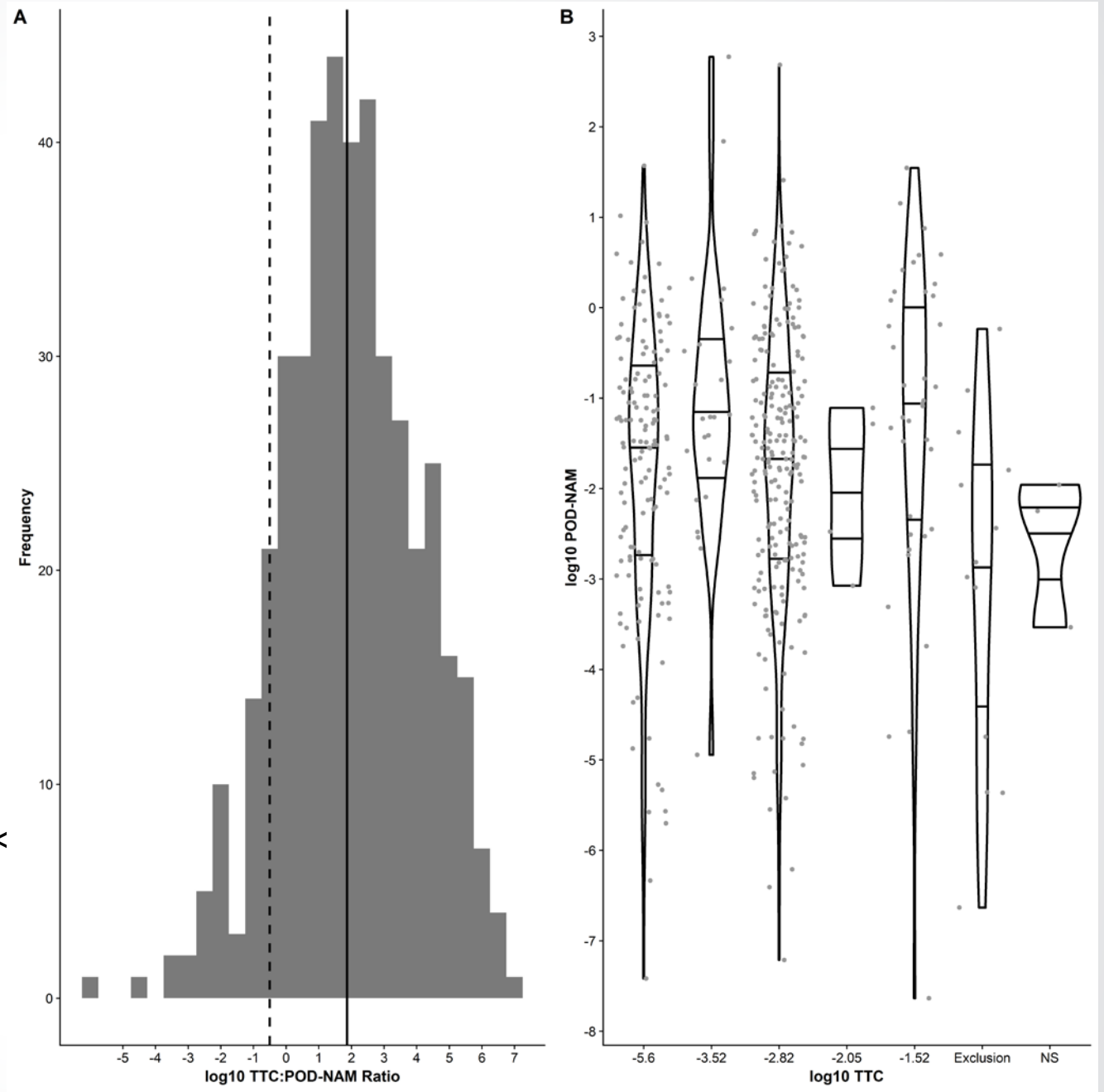
Cramer (1978) structural classes from non-cancer data

Structural Class	# of chem	5th percentile NOEL (µg/kg-bw/day)	Human Exposure threshold (µg/kg-bw/day)
I Easily metabolized; low toxicity	137	2993	30
II Intermediate structures	28	906	9
III Complex structures; Metabolism to reactive products suggestive of toxicity	447	147	1.5

Adapted from slides by Grace Patlewicz (EPA-ORD-NCCT)

TTC vs. POD-NAM

- The TTC:POD-NAM ratio distribution demonstrated median ratio of 1.88 on the log10 scale, suggesting that on average the TTC was more conservative by about 75 mg/kg/day
- Indeed 84% of the time, TTC was more conservative than POD-NAM.
- POD-NAM was possible in some cases for exclusions or “no structure” compounds in ToxTree.
- A combined approach, using the data available, might work for screening (e.g., one possibility might be to default to TTC if it is all that is available or if POD-NAM < TTC).



TTC values from ToxTree provided by Matthew Gagne and Tara Barton-Maclaren at Health Canada

Figure 12, 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 448 chemicals.
- POD_{NAM} estimates appear conservative with a margin of ~100.
- 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.