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

Katie Paul Friedman and Russell Thomas

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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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| A*STAR | ECHA | EFSA | EPA-OLEM | EPA-ORD | Health Canada | 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?<br>• 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?<br>• When BER ratio < 0, where there any distinguishing factors? |

# Case study workflow

**ASTAR HIPPTox EC10s (µM)**

**ToxCast AC50s (µM)**

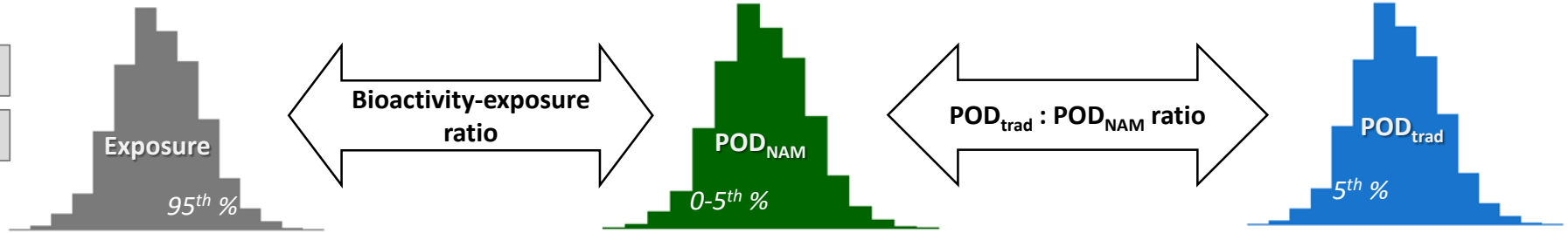
- *If the sum of hitcalls across the ToxCast DB > 5, then the 5th percentile on the distribution of AC50 values was used.*
- *If the sum of hitcalls across the ToxCast DB ≤ 5, the lowest AC50 was used.*
- *Flag-filtering by removing AC50 values from fits with 3+ caution flags and hitpct <= 0.5*

**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**

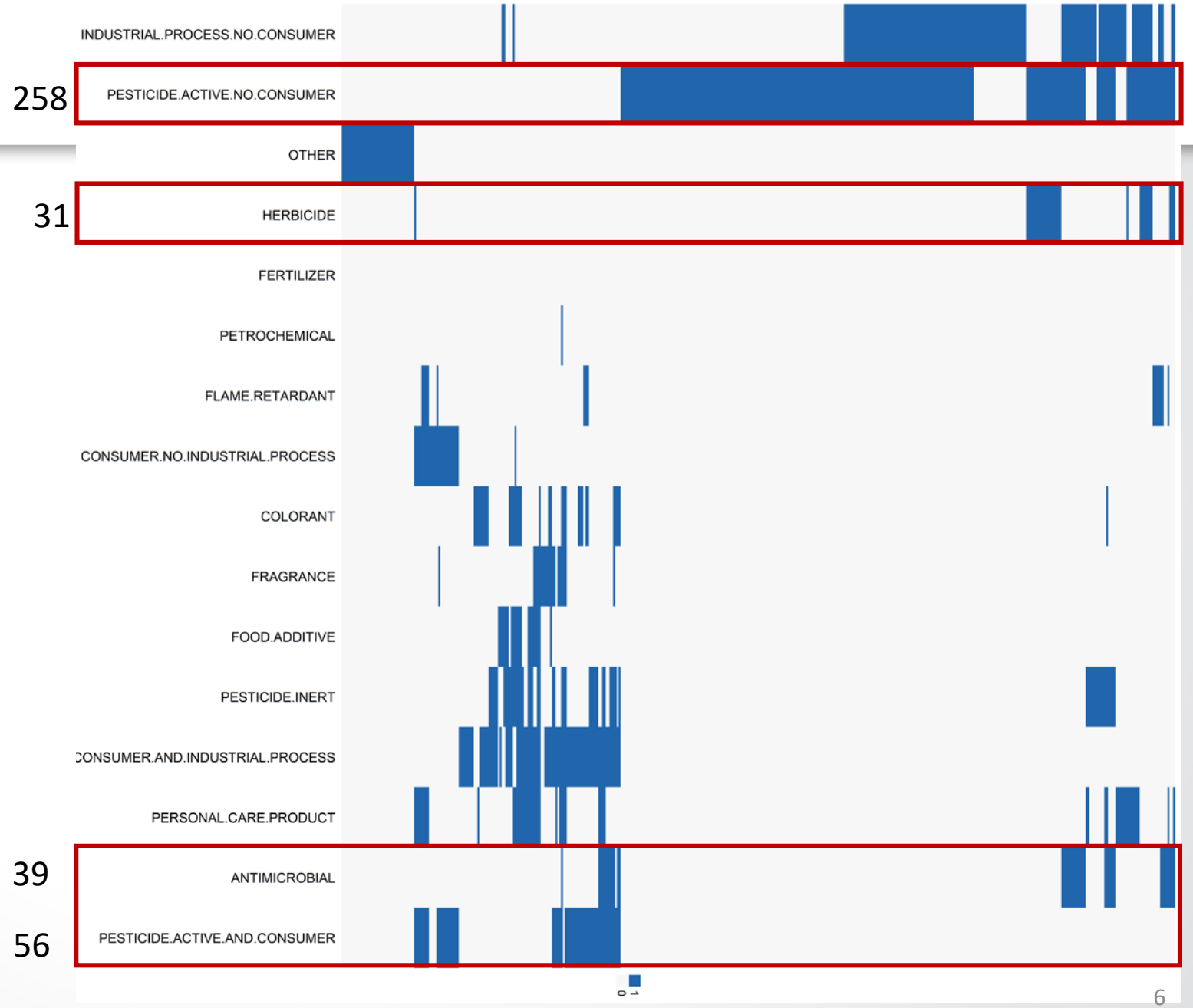
*Is BER useful for prioritization?*
*Are there addressable weaknesses?*

*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

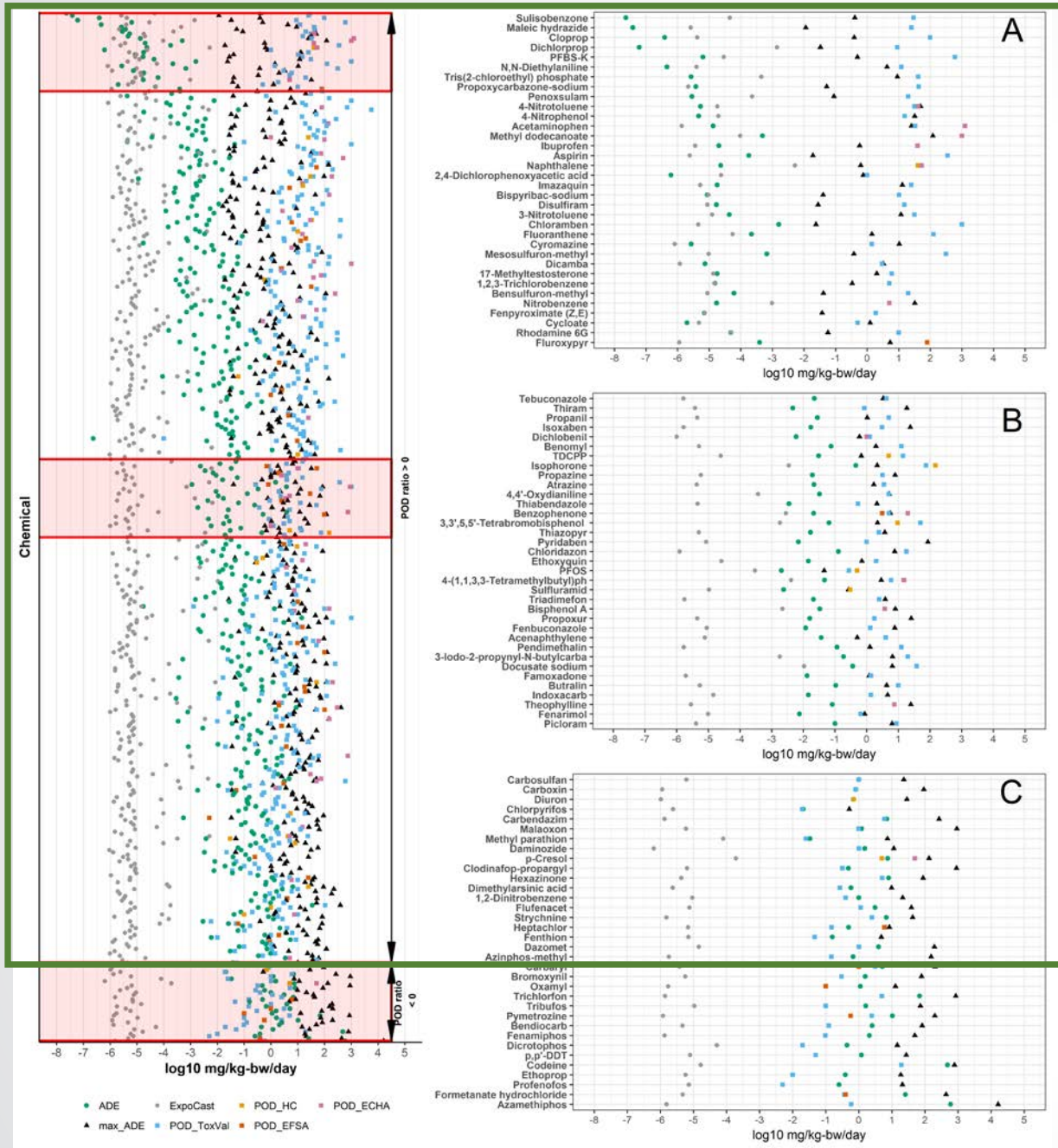
- This analysis used the simplistic use types available via AcTOR that are applied qualitatively.
- ~314/448 total have use as pesticide actives (~70%).




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)

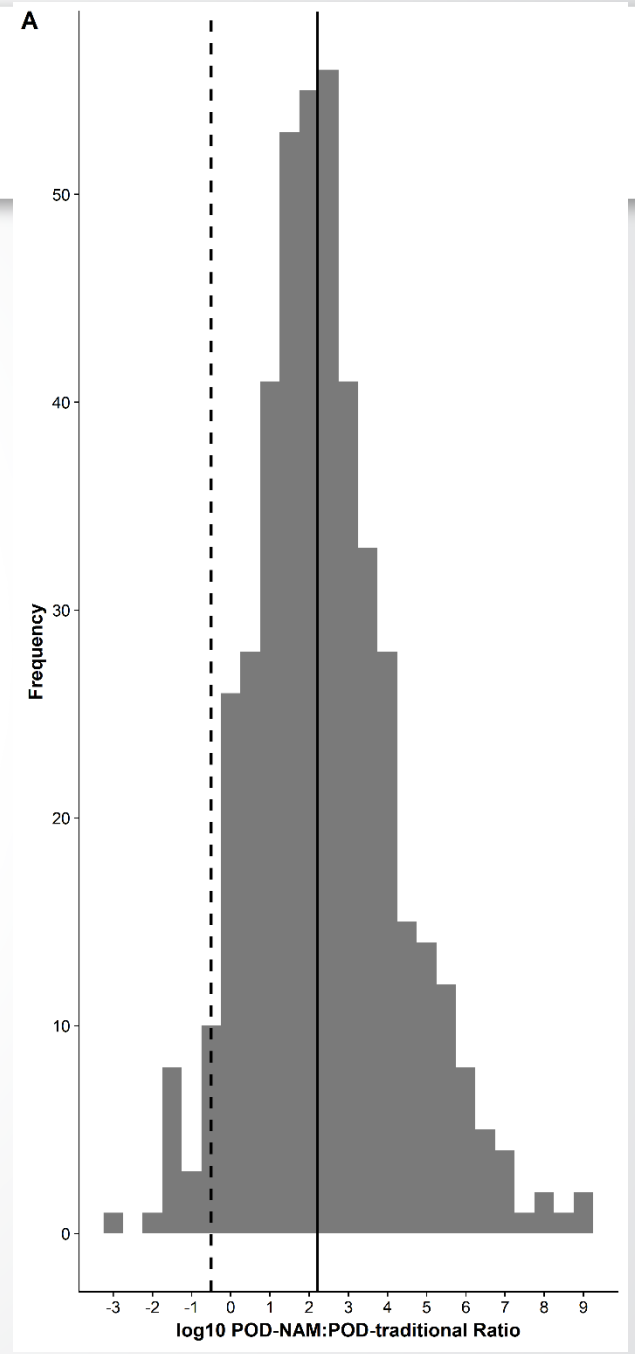
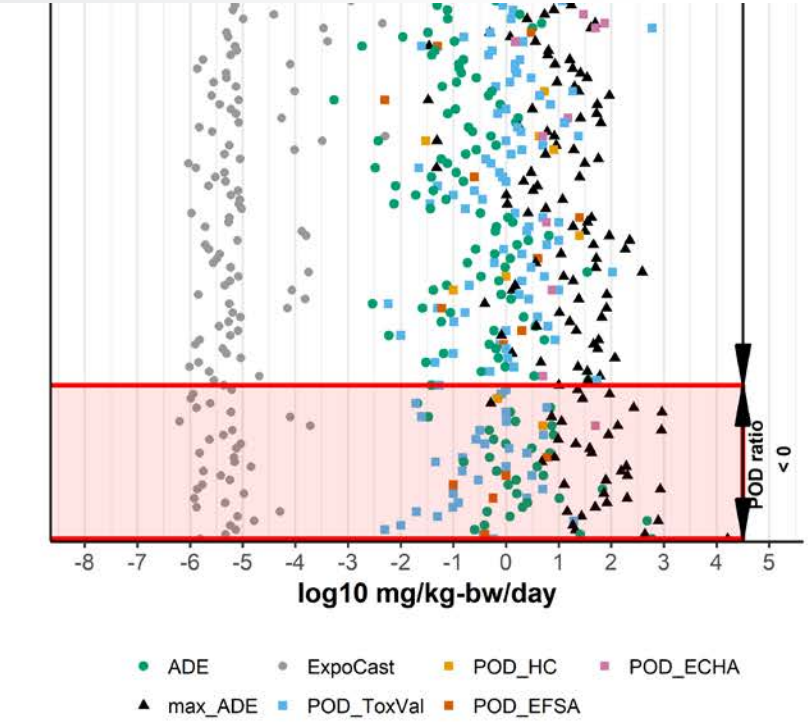


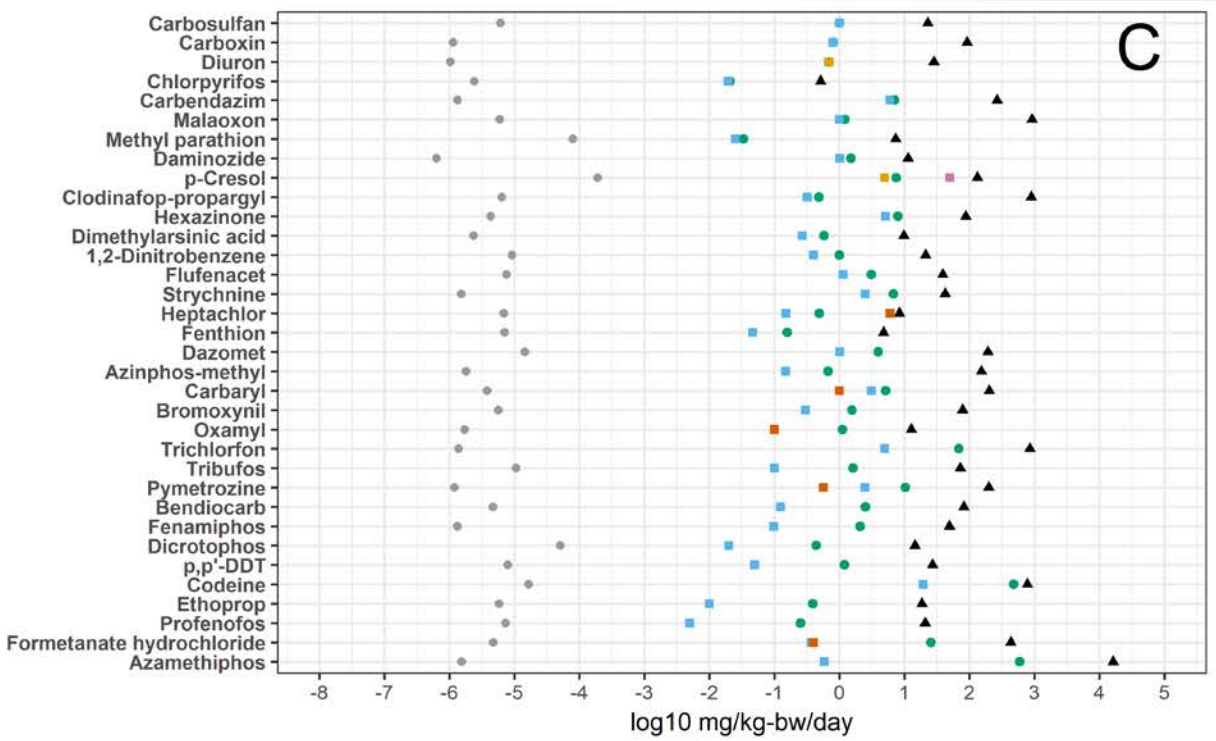

**Figure 4, Paul Friedman et al. *in prep.***

# Conceptual consideration of uncertainties

| Uncertainty sources | ToxCast AC50 values | httk model | In vivo PODs | ExpoCast predictions |
|---|---|---|---|---|
| **Biological and Systematic** | • Incomplete biological coverage<br>• Assay and curve modeling limitations.<br>• In vitro disposition and/or chemical purity<br>• Is the assay response "adverse," compensatory, or of unknown importance?<br>• 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.<br>• Currently assume 100% bioavailability.<br>• Inter-individual variability.<br>• IVIVE concordance. | • The reproducibility of the PODs, and the inherent variance in POD derivation, is not described here.<br>• Human relevance of the animal data. | • Heuristic model, trained using assumptions and limitations of NHANES data.<br>• Specific use scenarios are not defined.<br>• 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.<br>• Evaluation of AUC and $C_{max}$ could be added at a later date. | • Lack of a controlled vocabulary for study type.<br>• PODs were limited to NOEL/LOEL/NOAEL/LOAEL. | NA |
| **How it is considered** | • Caution flag + hit pct filtering.<br>• 5%-ile of the distribution of all available AC50s was taken.<br>• 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.

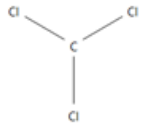
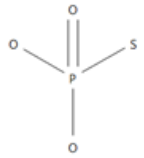
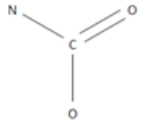
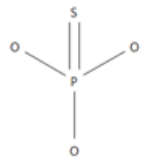
| Condition | Dev/Repro is minPOD | Dev/Repro is not minPOD |
|---|---|---|
| POD ratio < 0 | 1 | 33 |
| POD ratio > 0 | 44 | 370 |

| 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<br>• p-value = 0.98;<br>• 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<br>• p-value = 0.25;<br>• odds-ratio=1.4 | |

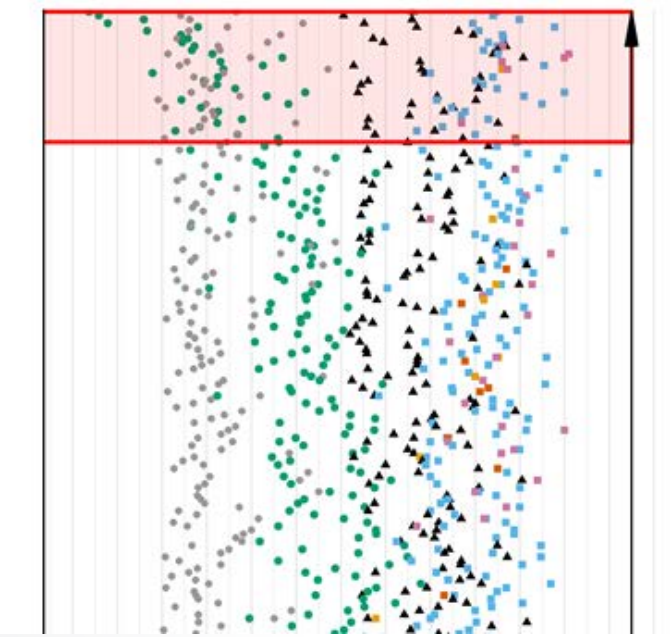
# Chemical structure features associated with organophosphate pesticides are enriched in the set with POD ratio ≤ 0.

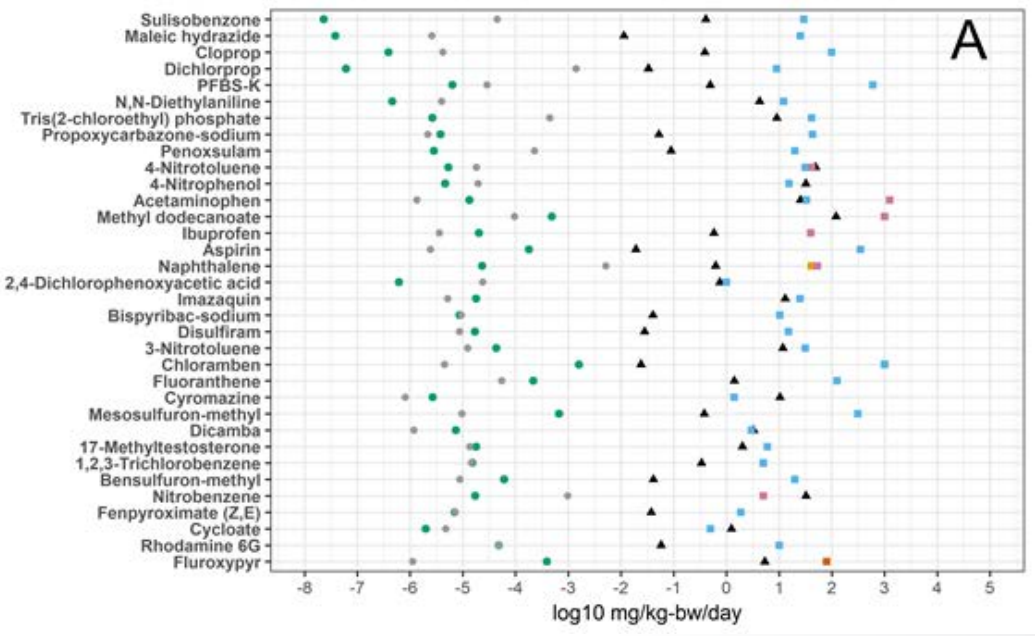
- 17 of 34 chemicals with POD ratio ≤ 0 are organophosphate pesticides.
- 20 of 34 chemicals corresponded to these chemotype enrichments.

| 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.

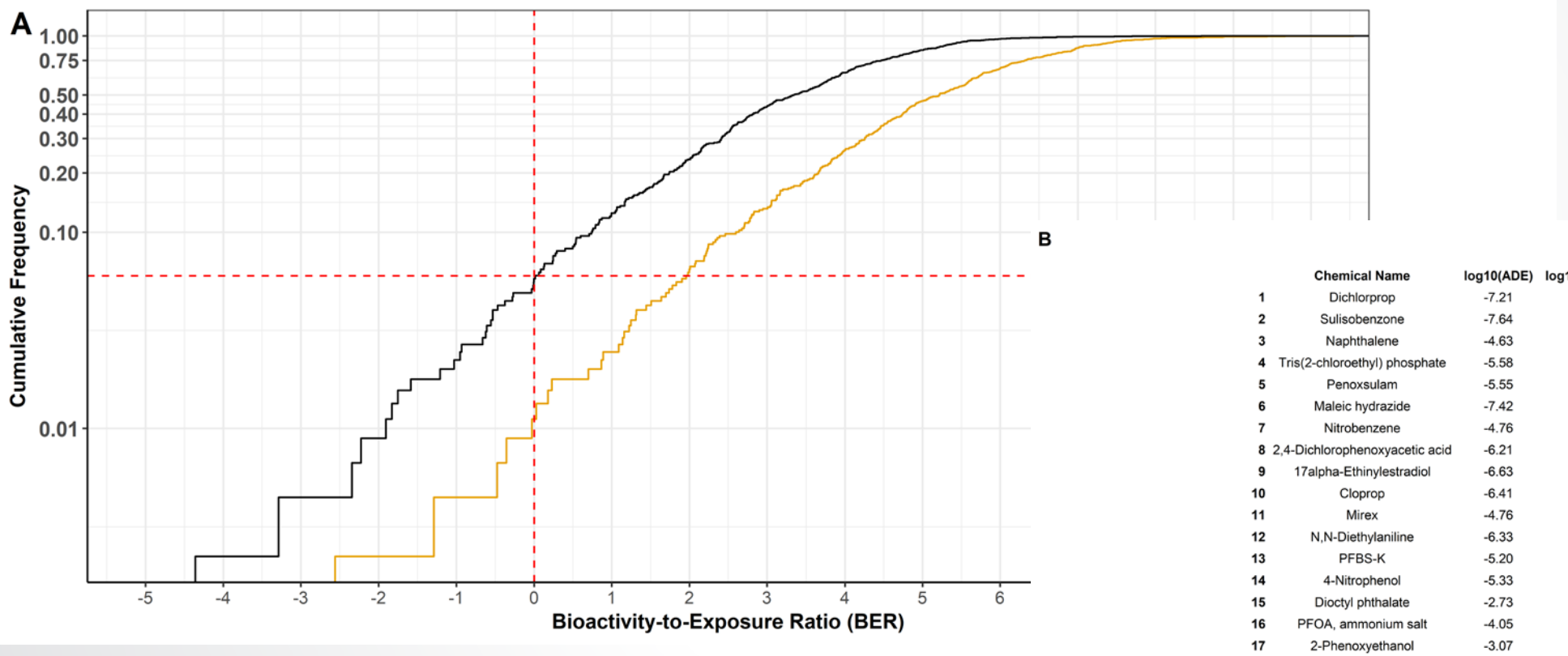



**Figure 10, Paul Friedman et al. *in prep.***

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.

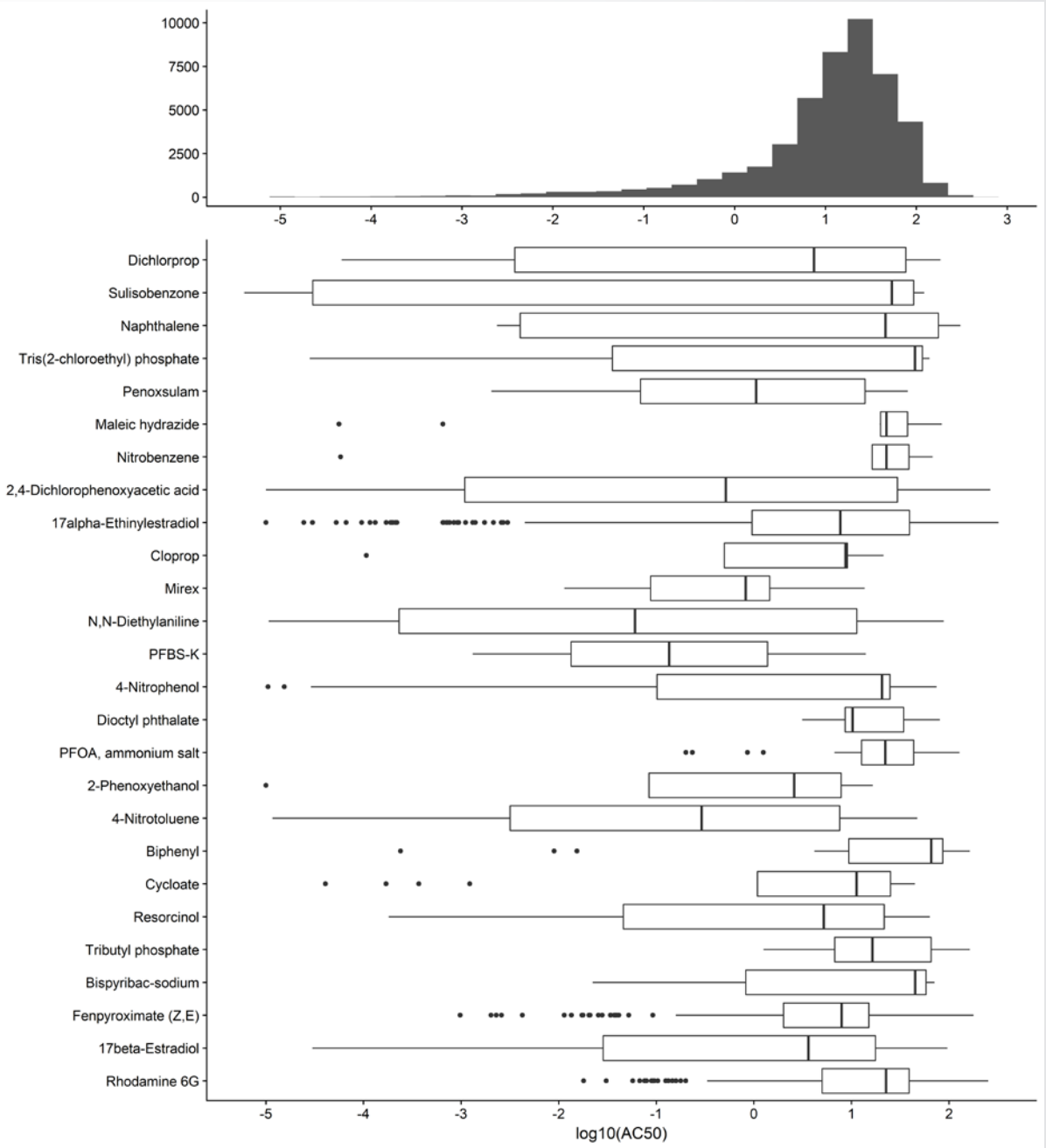



Figure 11, Paul Friedman et al. *in prep.*

# 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.

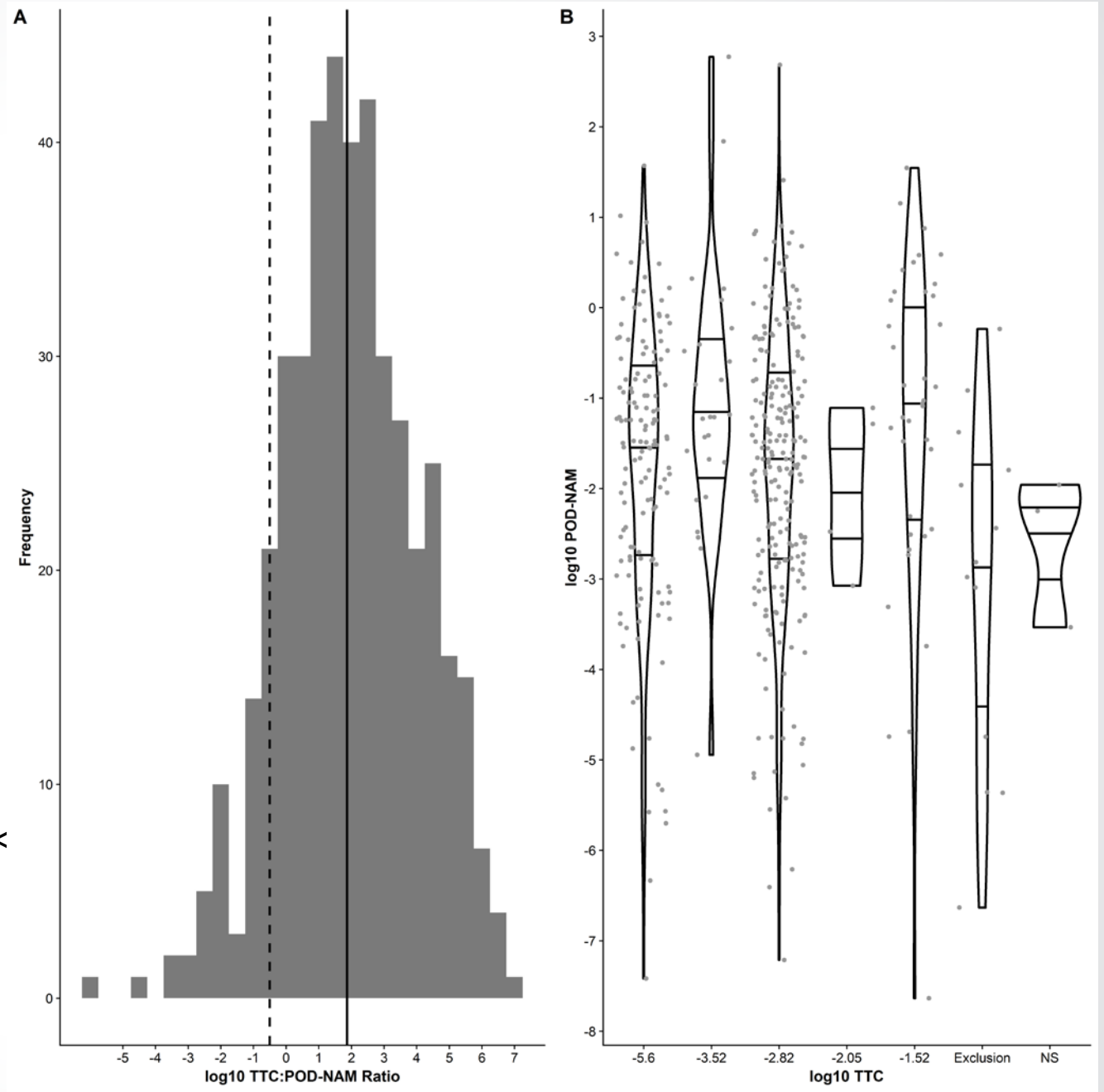
***Cramer (1978) structural classes from non-cancer data***

| Structural Class | # of chem | 5th percentile NOEL (µg/kg-bw/day) | Human Exposure threshold (µg/kg-bw/day) |
|---|---|---|---|
| I<br>Easily metabolized; low toxicity | 137 | 2993 | 30 |
| II<br>Intermediate structures | 28 | 906 | 9 |
| III<br>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.