A New Approach Method for Characterizing Inter-Species Toxicodynamic Variability

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Presentation Outline

- Introduction:
  - Problem I: Too many chemicals (too little toxicity data)
  - Problem II: Toxicodynamic variability
  - Motivation to shift from *in vivo* approaches to new approach methodologies (NAM) – *in vitro*
- Objective: Demonstrate the utility of an *in vitro* model for hazard and toxicodynamic variability assessments
  - Characterization of both inter-individual and inter-species variability
- Significance, limitations, and future directions of this work

Problem I: Too Many Chemicals




90% of the 75,000 chemicals approved for use in the United States remain **inadequately tested** for potential toxicity[1]

Traditional safety evaluation: ***in vivo***

- Expensive, time-consuming, low-throughput
- Inter-species and inter-individual differences
- Unfeasible for characterizing safety of thousands of compounds

[1]Natural Resources Defense Council
Images from https://www.niehs.nih.gov/health/topics/agents/index.cfm and https://now.tufts.edu/articles/consequences-spraying-fire-retardants-wildfires

Problem I: Too Many Chemicals

90% of the 75,000 chemicals approved for use in the United States remain **inadequately tested** for potential toxicity[1]

Alternative approach: ***in vitro***

- Cheaper, faster, higher-throughput
- Biologically relevant – can recapitulate characteristics of species/individuals
- Opportunity to evaluate variability
- Attractive alternative method for chemical safety evaluation

[1]Natural Resources Defense Council

Images from https://www.niehs.nih.gov/health/topics/agents/index.cfm and https://now.tufts.edu/articles/consequences-spraying-fire-retardants-wildfires

Problem II: Toxicodynamic Variability

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Inter-species variability

- Testing in rodents, dogs, and/or non-human primates ≠ humans
- Often 1 isogenic animal strain

Inter-individual variability

- Genetic heterogeneity in the human population = sensitive subpopulations[1]

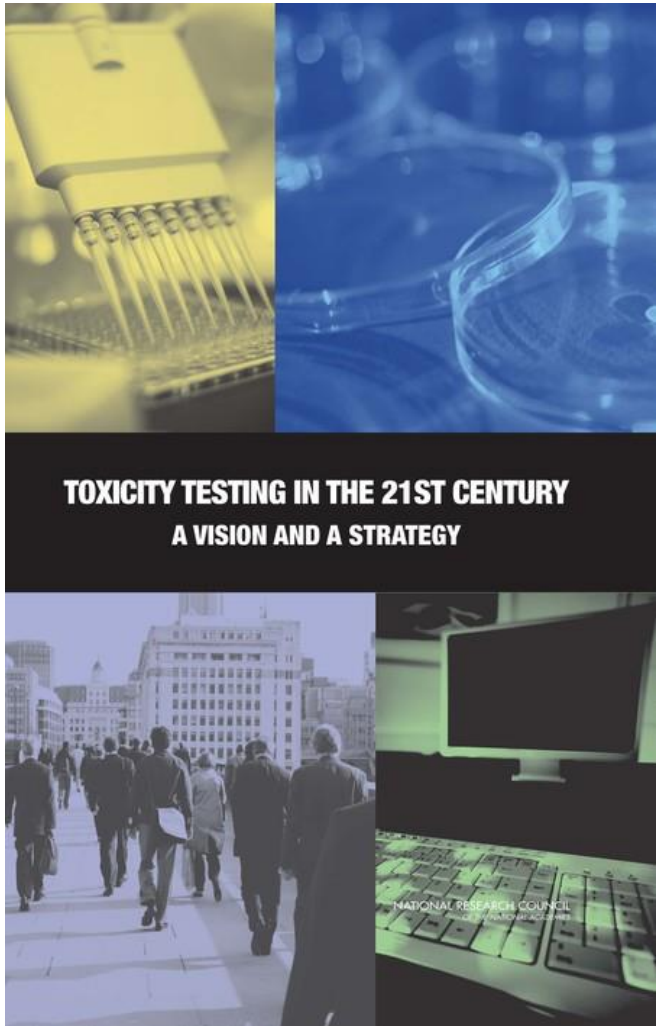
$$\mathrm{RfD} = \frac{\mathrm{NOAEL/LOAEL}}{\mathrm{UFs}}$$

- Toxicokinetics (UF = 10)
- **Toxicodynamics (UF = 10)**
  - **Inter-species (UF = 3.16 EPA, 2.5 WHO)**
  - **Inter-individual (UF = 3.16)**

- Default UFs can be replaced with chemical-specific estimates (CSAFs) to improve risk assessment[2]

[1]Zeise *et al.*, 2013 (PMID: 23086705), [2]Bhat *et al.*, 2017 (PMID: 28681680)

Toxicity Testing in the 21st Century



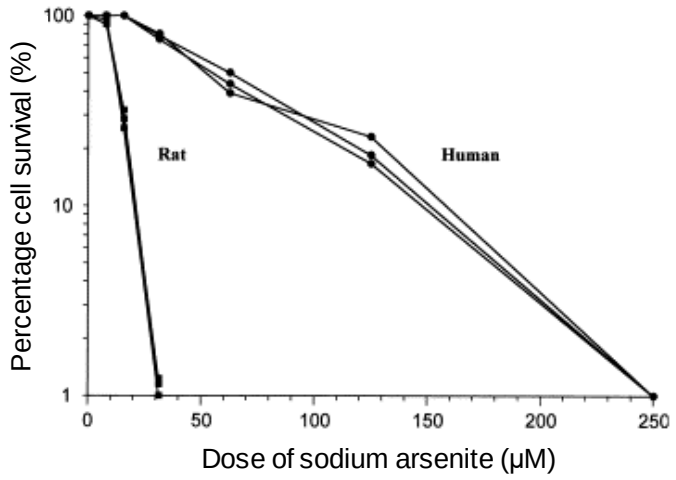
- Due to these challenges, there is strong motivation to decrease reliance on animal testing
- A paradigm shift toward the advancement and integration of alternative approaches for safety evaluation
- New approach methodologies (NAM) – *in vitro*
- Increased efficiency in toxicity testing – enables high-throughput, biology-driven evaluation

[1]Krewski *et al.*, 2010 (PMID: 20574894)

Assessing Inter-Species Variability *In Vitro*

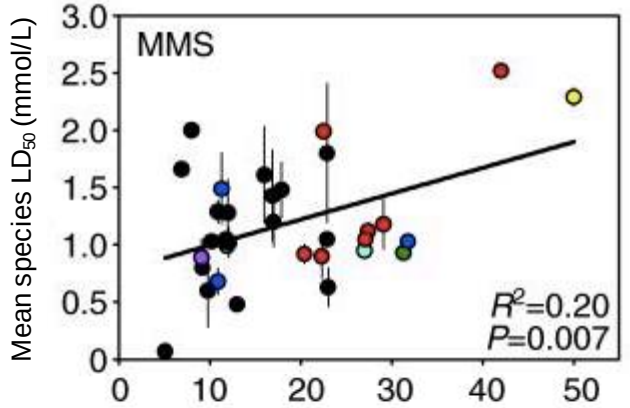
- Inter-species toxicodynamic variability is poorly understood[1]
- Risk assessment relies on allometric scaling[2] to extrapolate dose equivalencies between species ($BW^{¾}$)
  - Thought to account for metabolic differences in body size[3]
  - Does not account for all observed toxicodynamic variability

Inter-species differences in cytotoxic response



Kapahi *et al.*, 1999 (PMID: 10218637)

Positive correlation between **lifespan** and cellular resistance to stress



Harper *et al.*, 2011 (PMID: 21562178)

[1]Price *et al.*, 2008 (PMID: 18514247), [2]Schneider *et al.*, 2004 (PMID: 15135212), [3]Ungvari *et al.*, 2011 (PMID: 21059837)

Research Objective

Long-term goal:

- Improve *in vitro* approaches for chemical safety evaluation
- Better characterize both chemical hazard and variability
- Ultimately, **advance hazard and risk assessment**

Objective:

- To characterize inter-species and inter-individual variability in toxicodynamic response and investigate factors driving *in vitro* relationships

Hypothesis:

An *in vitro* model using fibroblast cells from many species and individuals can be used to assess inter-species and inter-individual variability.

Approach and Study Design

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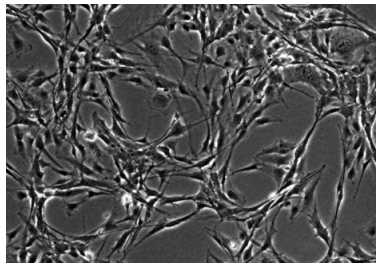
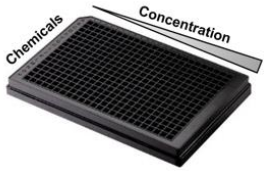
Fibroblasts from 68 individuals of 54 diverse species



Concentration-response screening of 40 chemicals

Anti-neoplastic drugs

Other drugs

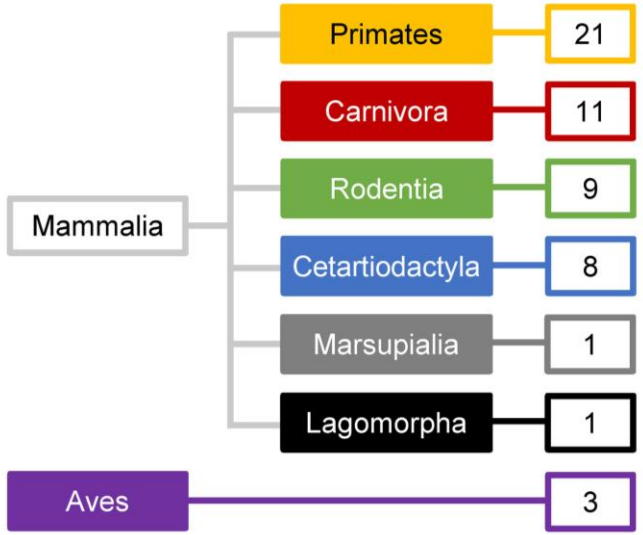


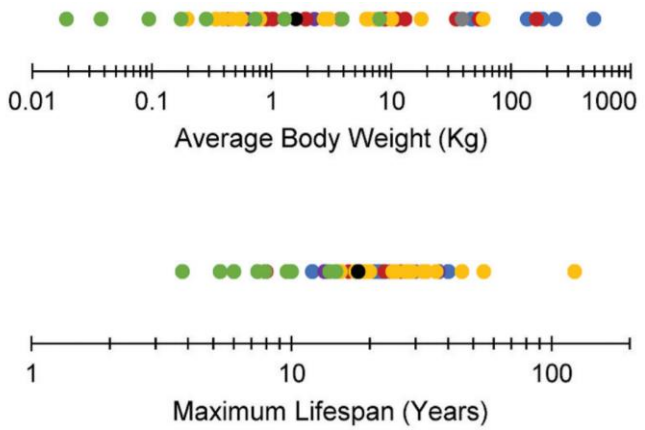
Flame retardants

Pesticides

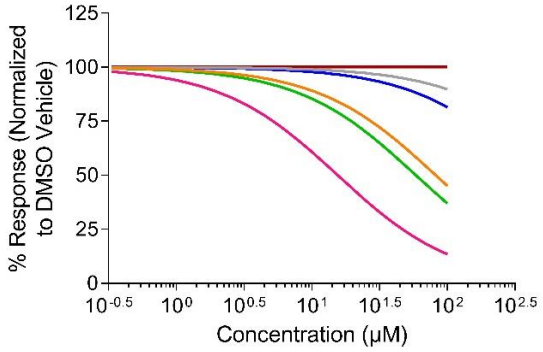
Food/flavor/fragrance agents

Cell viability evaluation [Cell Titer-Glo® assay]





Dose-Response Profiling
[Quantitative POD values]



- Characterize inter-individual and inter-species toxicodynamic variability
- Derive quantitative estimates directly comparable to UFs
- Investigate the contribution of allometric scaling to toxicodynamic sensitivity

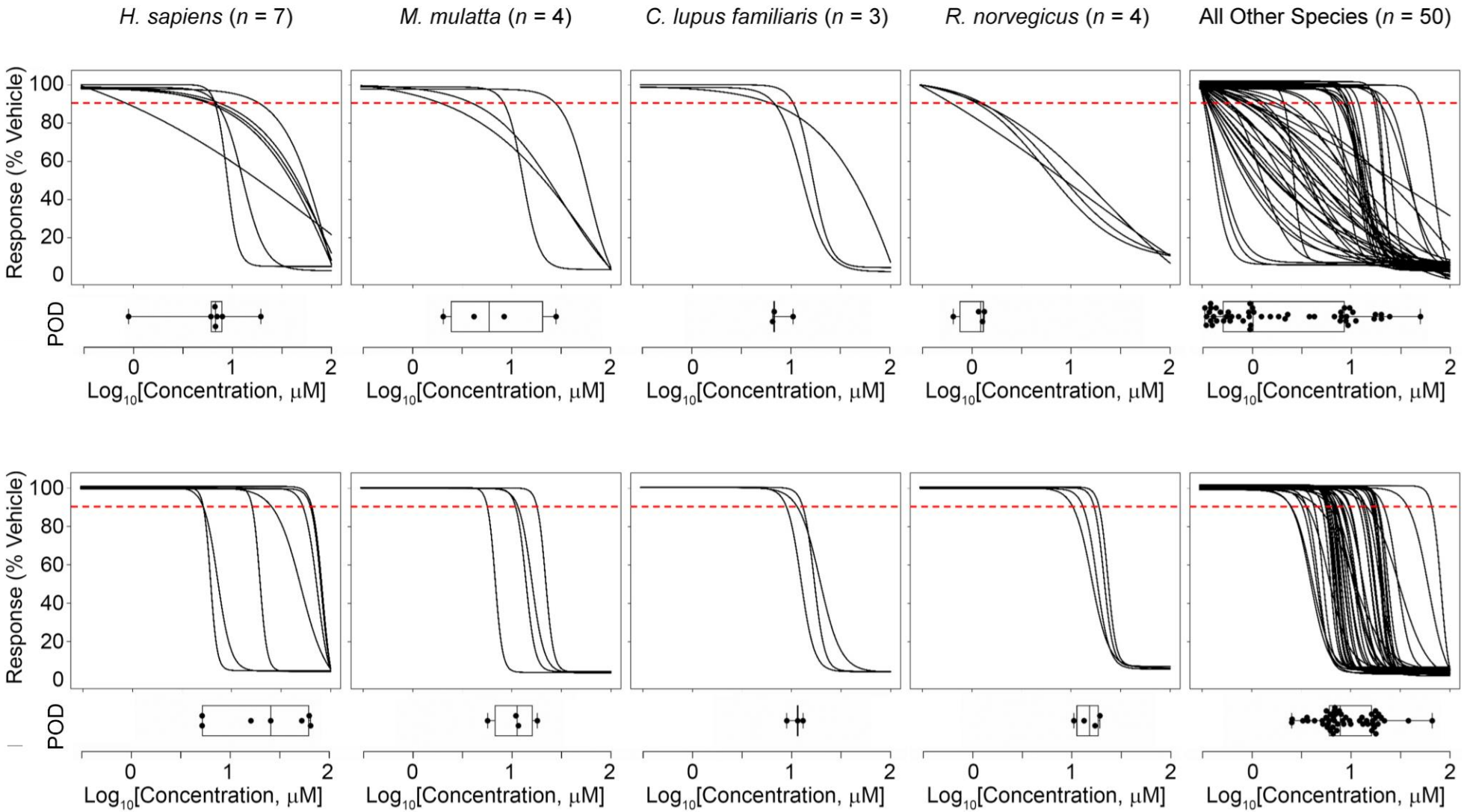
Chemical-Specific Differences in Variability

Higher variability:

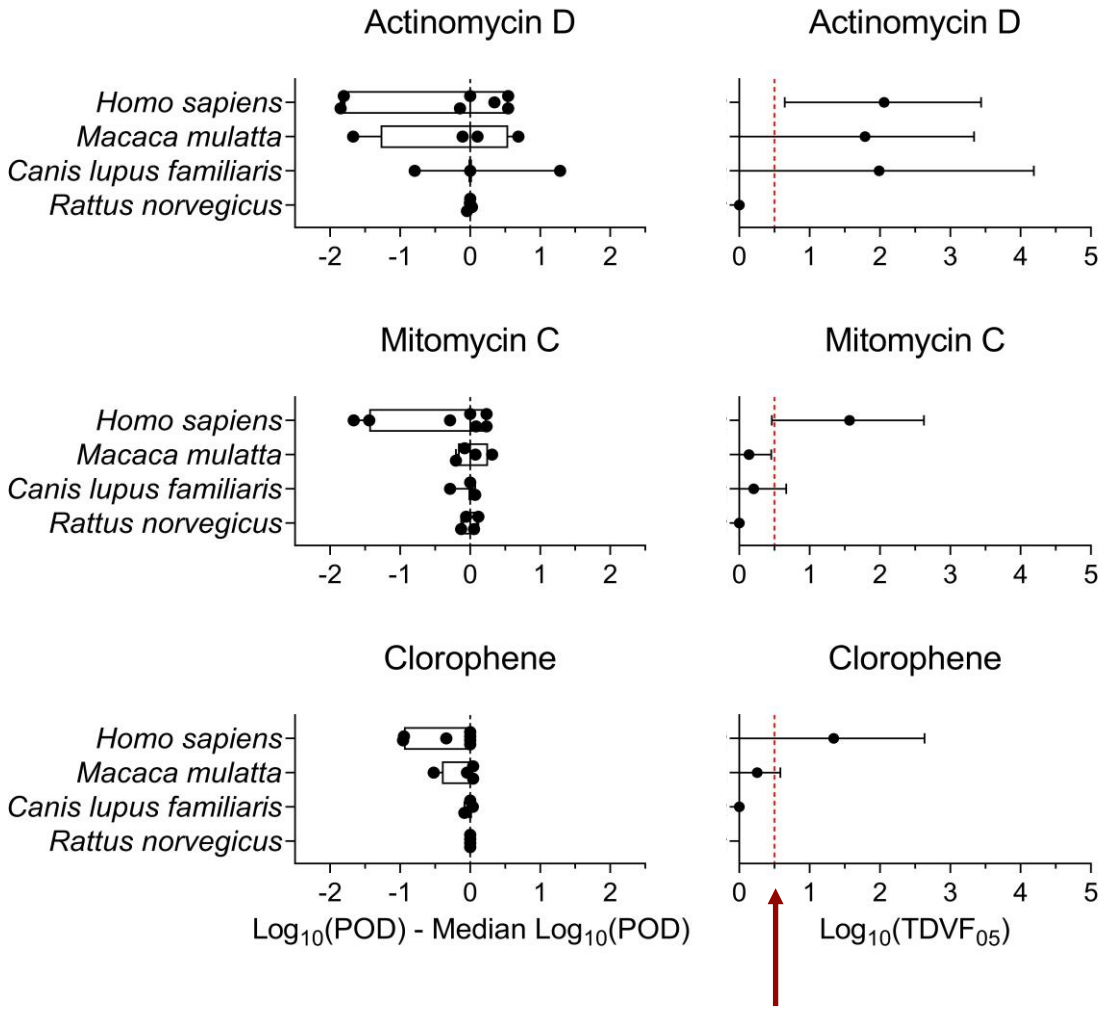
Daunomycin hydrochloride

Lower variability:

4,4-Thiobis(6-tert-butyl-m-cresol)



Species Differences in Extent of Inter-Individual Variability



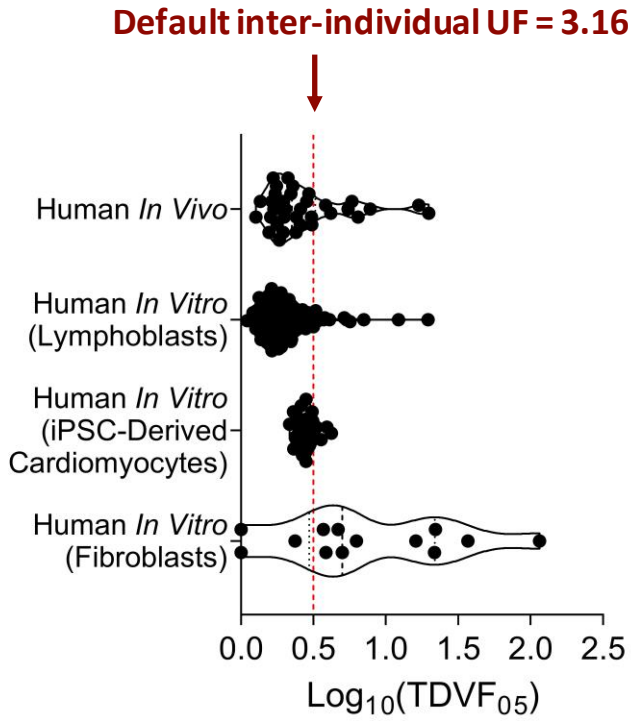
Default inter-individual UF = 3.16

POD in a 5th percentile individual ⟷ **$TDVF_{05}$** ⟷ POD in a median individual

Larger $TDVF_{05}$ = greater degree of variability across the population

- General trend for inter-individual variability: human > monkey > dog > rat
  - Median human $TDVF_{05}$ ~2.5x greater than monkey, ~5x greater than rat
- 71% of chemicals had $TDVF_{05}$ higher than the default UF for inter-individual variation

Human Inter-Individual Variability



N of Chemicals	N of Individuals	Source
34	5 - 3,115	WHO/IPCS (2018)
119	1,086	Chiu et al. (2017)
24	43	Blanchette et al (2020)
13	7	This study

- Across studies, $TDVF_{05}$ estimates generally close to the default UF of 3.16 (1.87 – 5.01)
- Observable chemical-specific differences, with some chemicals exceeding the default UF

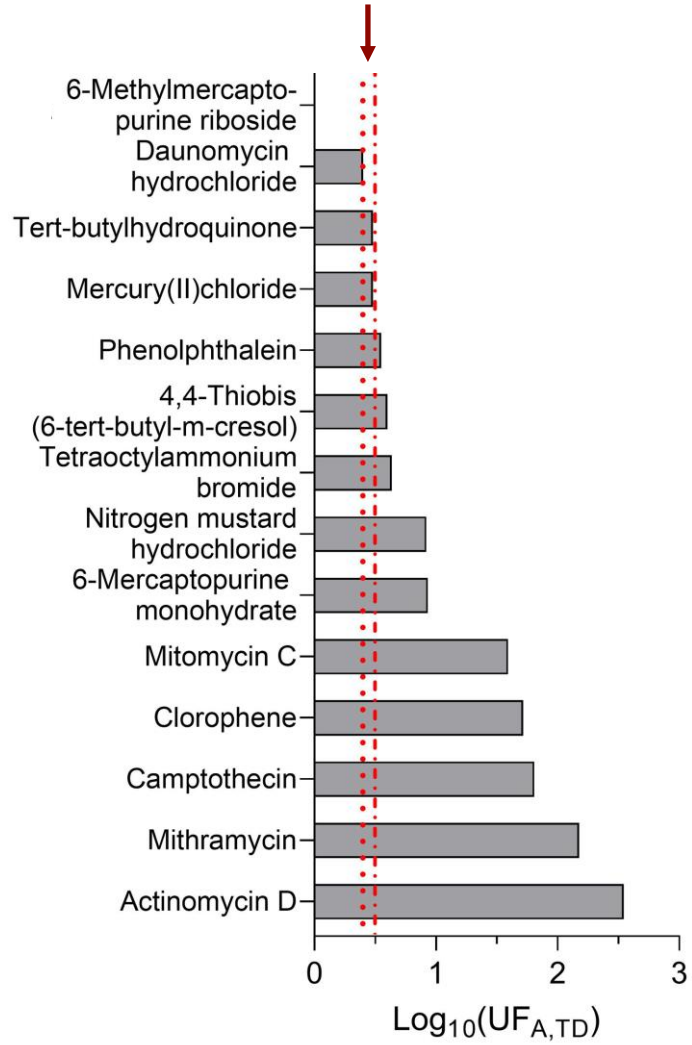
Inter-Species Variability

5th percentile animal-to-human extrapolation ← **$UF_{A,TD}$** → Median animal-to-human extrapolation

Larger $UF_{A,TD}$ = greater degree of variability across species

- Overall, median $UF_{A,TD}$ estimate is close to the default UF
- Observable chemical-specific differences, with over half of chemicals exceeding the default UF
- Little to no relationship between body weight or lifespan and toxicodynamic sensitivity → toxicokinetics primarily drives allometric scaling *in vivo*
  - But overall allometric power may be chemical-specific

Default inter-species UF = 3.16 (U.S. EPA), 2.5 (WHO)



Summary and Conclusions

- Both the inter-individual and inter-species components of variability contribute to inter-species toxicodynamic differences
- On average, *in vitro*-derived estimates of inter-individual and inter-species variability are similar to the default UFs
- Chemical-specific differences → supports incorporation of CSAFs into risk assessment

This research demonstrates the utility of using an *in vitro* model to characterize both inter-individual and inter-species variability.

Significance, Limitations, and Future Directions

Significance of This Work

- This research demonstrates the utility of an *in vitro* model for hazard and toxicodynamic variability assessments
  - Both inter-individual and inter-species variability

- This approach is feasible for chemical screening in a manner unfeasible using traditional methods

This work contributes to the paradigm shift from traditional animal models of toxicity toward alternative approaches to inform hazard and risk assessment.

Limitations

- Sample size considerations – balancing estimates of potency and variability[1]
  - Need to optimize sample size for accurate hazard and variability assessments
  - For cardiotoxicity, central estimate of population-wide potency *in vitro* is feasible with 5 donors, but ~20 donors are needed for accurate variability estimates

- Clinical relevance of some *in vitro* phenotypes is unclear
  - Careful selection and biological understanding of phenotype of interest

- Differences in *in vitro* and *in vivo* dose metrics
  - *In vitro*-to-*in vivo* extrapolation to inform risk assessment[2,3]
  - Need to understand chemical free fraction, clearance, and *in vivo* exposure levels

[1]Blanchette *et al.*, 2021 (in press), [2]Wetmore *et al.*, 2012 (PMID: 21948869), [3]Blanchette *et al.*, 2019 (PMID: 30346629)

Future Directions

- Evaluating chemical safety (*i.e.*, drugs, environmental chemicals)
  - Automated, high-throughput screening pipeline[1]
  - Mass production of cells[2]
  - Characterized chemical library[3]

- Addressing metabolism in *in vitro* models[4]
  - Bioactivation vs. bioinactivation
  - Incorporating hepatic S9 fraction (individual- or species-specific)
  - Retrofitting *in vitro*-derived data with metabolism → more relevant effect predictions

[1]Attene-Ramos *et al.*, 2013 (PMID: 23732176), [2]Halloin *et al.*, 2019 (PMID: 31353227), [3]Richard *et al.*, 2021 (PMID: 33140634), [4]Deisenroth *et al.*, 2021 (PMID: 32991717)

Thank you!

Questions?

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