Quantitative Integration of NAMs into Regulatory Decision-Making: Current State of the Science

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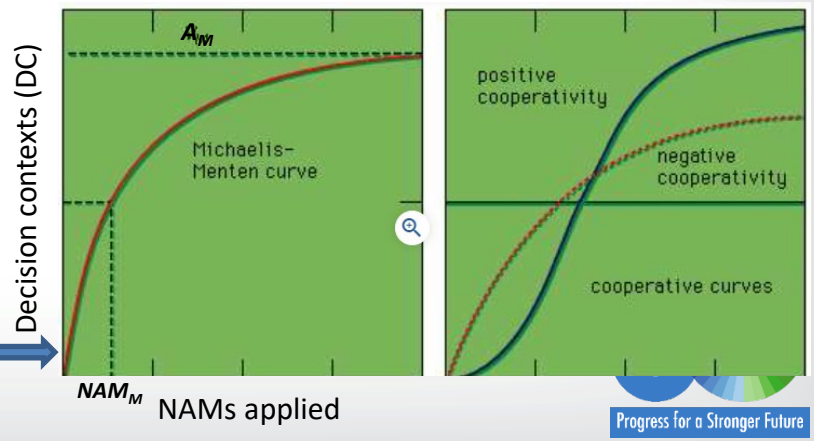
Outline

- Traditional human health risk assessment practice and transition to New Approach Methodologies (NAMs)
- Examples of NAM data application
  - Expert-driven read-across (including bioactivity data)
  - Transcriptomic-based PODs
- Practical quantitative applications of transcriptomic data to risk assessment
  - Bioactivity-exposure ratio
  - Transcriptomic reference dose
  - Mixtures assessment

If application of NAMs were a kinetic process

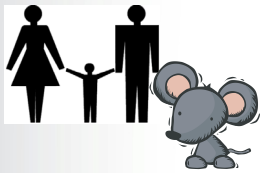
According to Michaelis-Menten kinetics, if the rate of acceptance of NAMs is represented graphically as a function of the decision contexts applied (*DC*), the curve obtained in most cases is a hyperbola. The shape of the curve is a logical consequence of the fit-for-purpose concept; i.e., as NAMs are applied with greater confidence over time, the curve flattens at the maximum applicability (A_M), which occurs when all decision-contexts integrate NAMs. (NAM_M is the Michaelis constant.)

Where we are currently as a regulatory scientific community

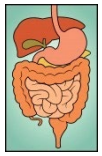


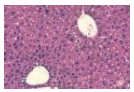
Human health risk assessment practice and a transition to new data types

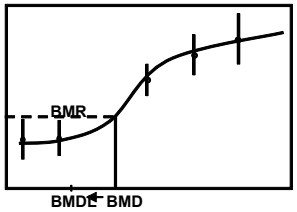
Apical responses



Apical Responses





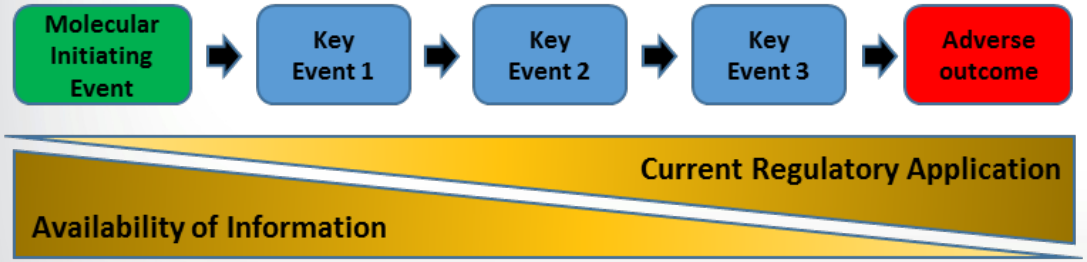


Apical POD

	BMR_1	BMR_2
$Effect_A$	POD_{1A}	POD_{2A}
$Effect_B$	POD_{1B}	POD_{2B}

- The vast majority of chemicals found in commerce and the environment are data-poor
- Commonly unaccounted for in formal quantitative evaluations of health risks to human populations
- Lack of available points-of-departure (POD) for use in derivation of non-cancer or cancer values
- POD = Dose-response point that marks the beginning of a low-dose extrapolation (e.g., BMDL; NOAEL)

Non-apical responses



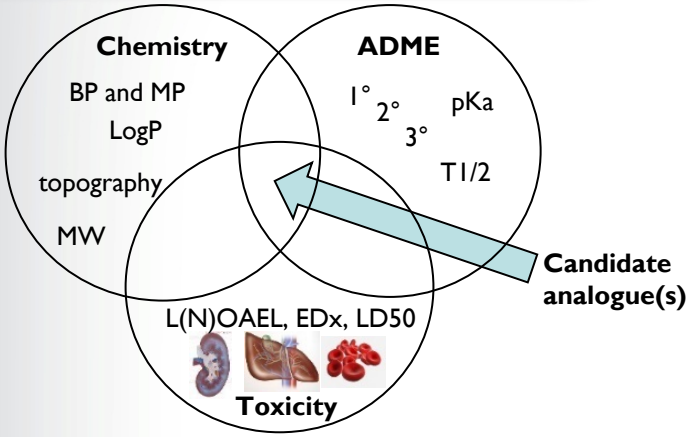
- Application ranging from data-gap filling to primary basis for qualitative and quantitative organ or tissue-based toxicity

New Approach Methodologies

- NAM is a broadly descriptive term for any non-animal technology, methodology or approach or combination thereof that can be used to provide information on chemical hazard and risk assessment https://ntp.niehs.nih.gov/iccvam/docs/roadmap/iccvam_strategicroadmap_january2018_document_508.pdf
- NAM include, for example:
  - Cheminformatics – structure-activity/read-across; QSAR; predicted physchem properties
  - Biological NAMs – in vitro cell bioactivity; high-throughput toxicogenomics (e.g., transcriptomics; cell painting/phenotypic profiling)
  - Toxicity Pathway annotation (e.g., Adverse Outcome Pathway development and application)
  - High-throughput toxicokinetics; in vitro to in vivo extrapolation (IVIVE)/reverse dosimetry
  - Exposure modeling; environmental fate and transport modeling

4

Expert-driven read-across



Data-poor chemicals

- Inferred/interpolated hazard
- Surrogate based POD and subsequent derivation of non-cancer reference values (e.g., oral RfD)

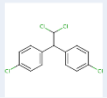
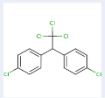
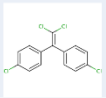
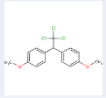
Data-rich chemicals

- Data-gap filling
- Augment WOE
- Potential for reducing uncertainties

Expert-driven read-across of p,p'-DDD

Identification of structural analogues

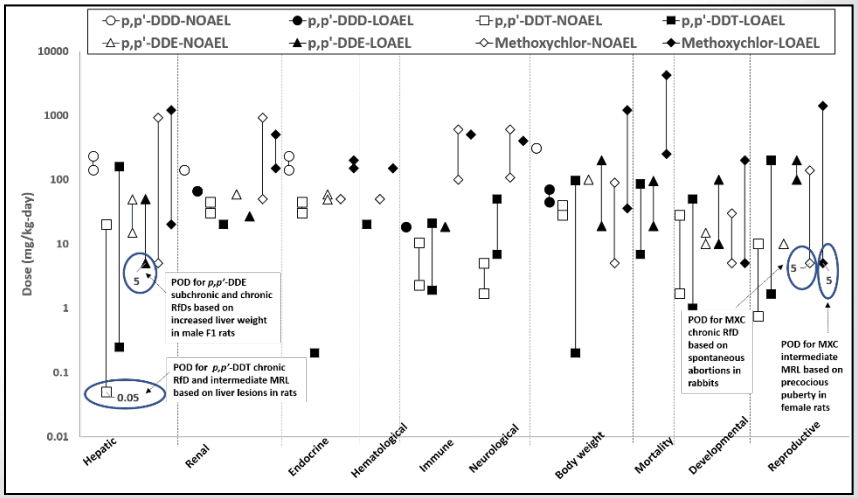
Table 1. Structural Analogues of p,p'-DDD

	Target Chemical	Analogues[a]		
Name	p,p'-Dichlorodiphenyl dichloroethane (p,p'-DDD)	p,p'-Dichlorodiphenyl trichloroethane (p,p'-DDT)	p,p'-Dichlorodiphenyl dichloroethylene (p,p'-DDE)	p,p'-Dimethoxydiphenyl trichloroethane (Methoxychlor)
CASRN	72-54-8	50-29-3	72-55-9	72-43-5
Structure				
ChemIDplus similarity score (%)	100	77	67	65
DSSTox similarity score (%)	100	96	61	52

[a]Analogues represent a set of structurally similar chemicals identified using two publicly available similarity databases (ChemIDplus and DSSTOX) prefiltered on the basis of availability of health reference values for non-cancer oral toxicity from regulatory agencies, including ATSDR (2002a, b) and U.S. EPA (2017 b, c).

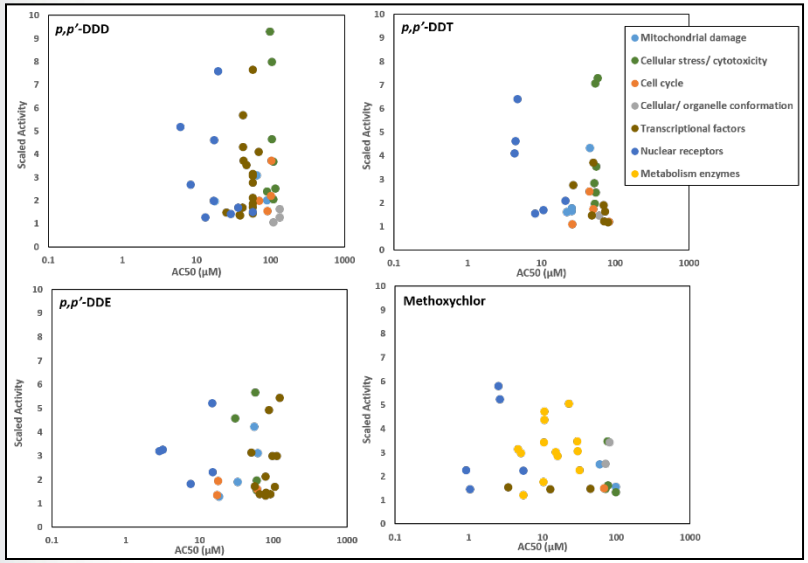
Lizarraga et al. (2019). Regul Toxicol Pharmacol 103:301-313
https://pubmed.ncbi.nlm.nih.gov/30794837/

Putative toxicity targets for *p,p'*-DDD and analogues include the liver and reproductive system in animals

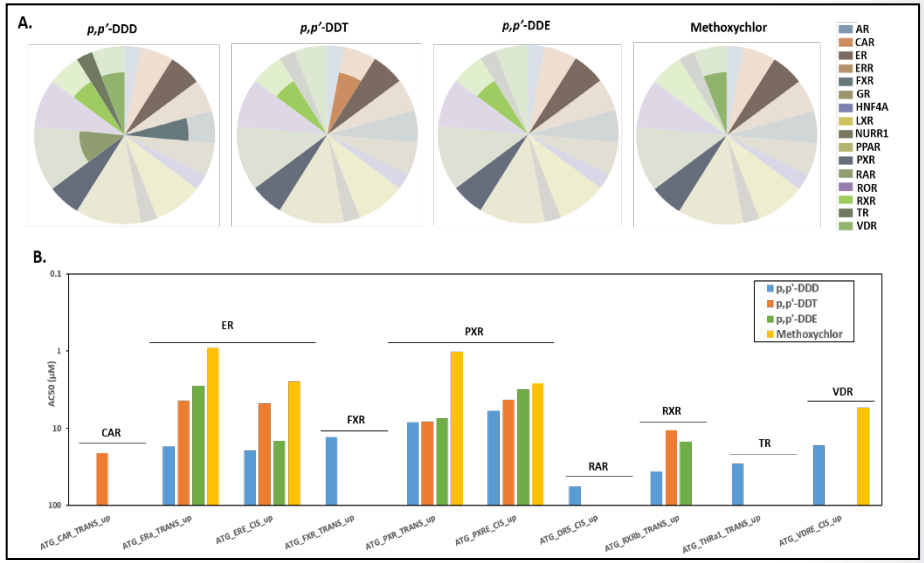


Expert-driven read-across

p,p'-DDD and analogues exhibit similarities in cell-specific responses and target gene pathways in *in vitro* ToxCast assays conducted in human liver cells

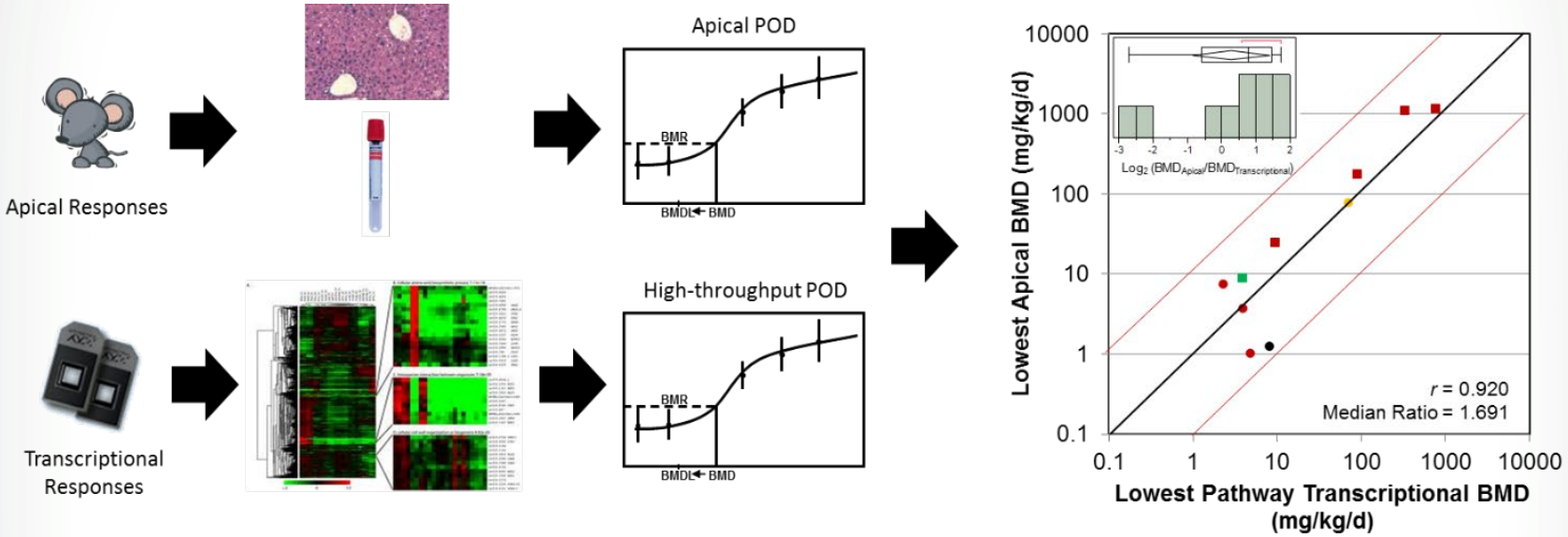


p,p'-DDD and analogues exhibit similar upregulation of steroid/xenobiotic-sensing nuclear receptors in *in vitro* ToxCast assays conducted in HepG2 Cells



- ToxCast assays and model predictions suggest that *p,p'*-DDD and analogues may act as ER agonists and AR antagonists coinciding with the estrogenic and anti-androgenic reproductive effects observed *in vivo*
- Coherence across *in vivo* toxicity and *in vitro* bioactivity similarity comparisons help reduce uncertainties associated with toxicity data gaps for the data-poor target chemical
- These findings demonstrate the utility of integrating evidence from HTS data platforms to support mechanistic conclusions and increase confidence in the application of read-across in quantitate risk assessment

Transcriptomic Pathway-Based PODs



- Concordance between genotype/phenotype across two different routes of exposure, rodent species, sexes, and, multiple target tissues

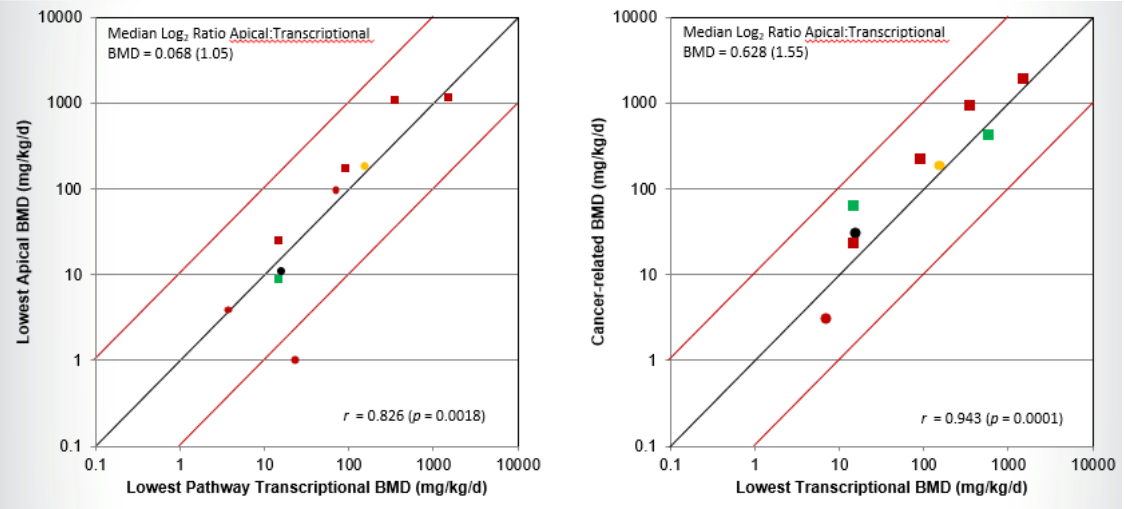
<u>Data-poor chemicals</u>

- Evidence base for hazard
- Dose-response based on pathway perturbations
- Reduce need for longer-term animal studies

<u>Data-rich chemicals</u>

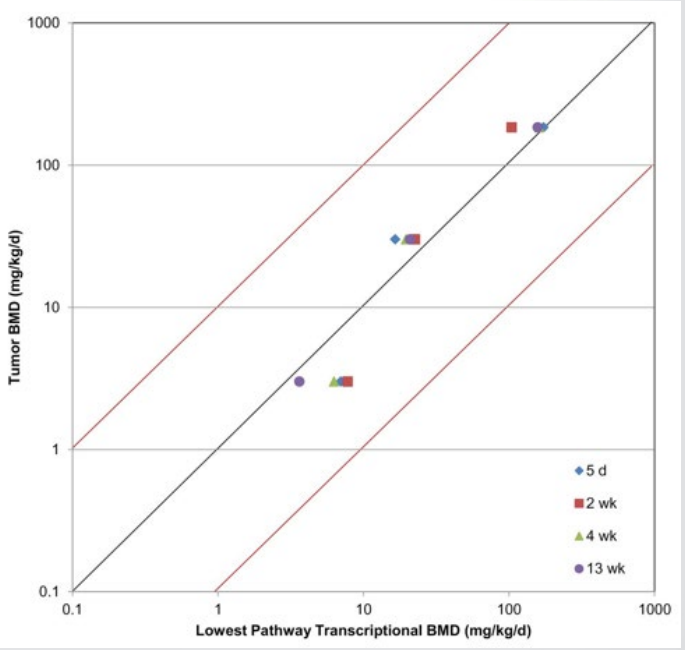
- Augment WOE (e.g., MOA/AOP)
- Opportunity to alert off-target effects
- Potential for reducing uncertainties

Concordance between Apical and Transcriptional PODs for Non-cancer and Cancer



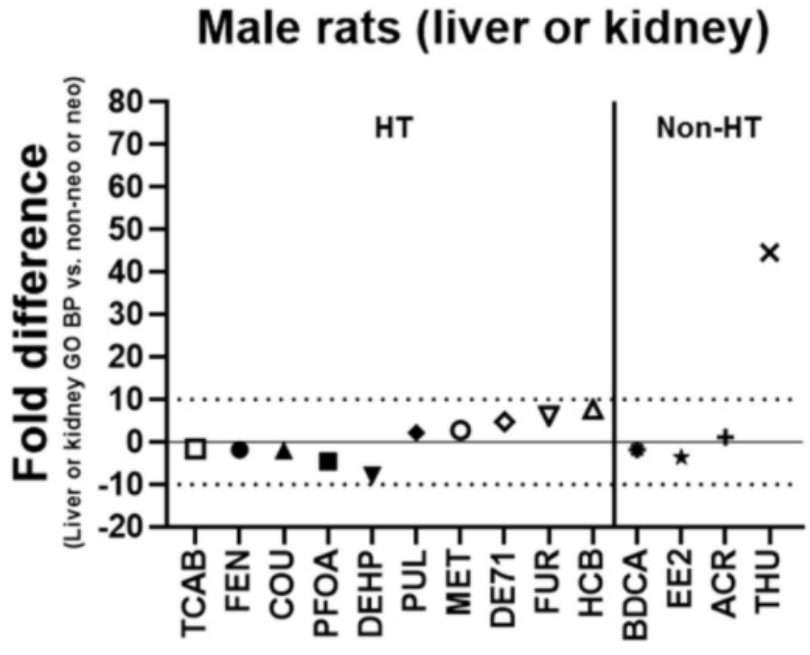
Thomas et al. (2011 and 2013). Toxicol Sci
https://pubmed.ncbi.nlm.nih.gov/21097997/
https://pubmed.ncbi.nlm.nih.gov/23596260/

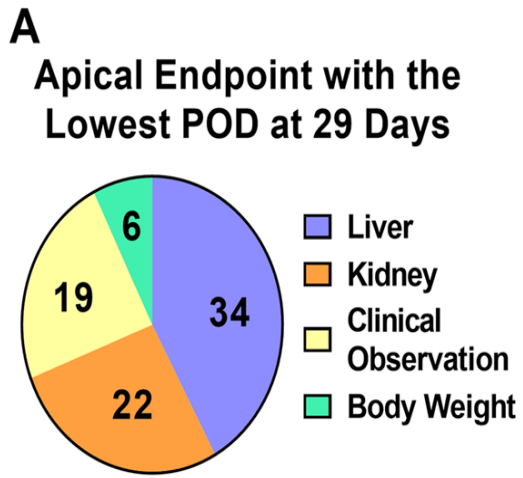
- 13-week exposures in rats or mice
- Transcriptome obtained from 'critical effect' tissues
- Lowest transcriptional pathway-based BMD from the 13-wks study vs. chronic non-cancer (left panel) or cancer (right panel) effect BMDs

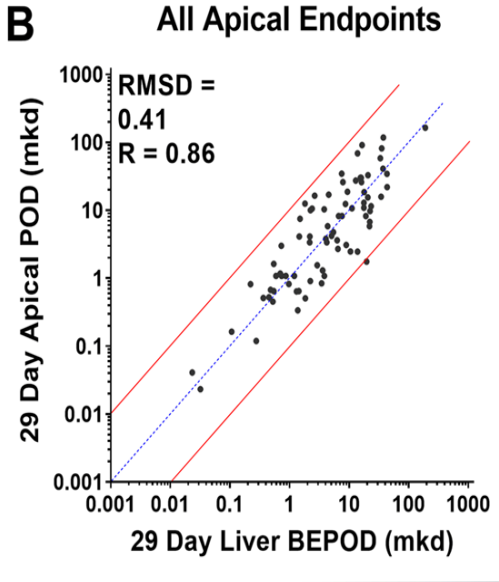


- Chemicals evaluated at 5d, 2, 4, or 13 wks in rats or mice
- Lowest transcriptional pathway-based BMD from the 5d, 2, 4, or 13-wks timepoints vs. chronic cancer effect BMDs

Concordance between Apical and Transcriptional PODs for Non-cancer and Cancer







- 18 chemicals evaluated in a 5-day in vivo oral exposure study in rats
- Lowest GO Biol Process BMD from the 5-day study vs. subchronic or chronic apical effect BMDs

Gwinn et al. (2020). Toxicol Sci 176(2):343-354
https://pubmed.ncbi.nlm.nih.gov/32492150/

- 79 chemicals from Open Toxicogenomics Project-Genomics Assisted Tox Evaluation (TG-GATES)
- Lowest liver biological effect POD (i.e., BMD from GO Biol Process) vs. 29-day apical effect BMDs

Johnson et al. (2020). Toxicol Sci 176(1):86-102
https://pubmed.ncbi.nlm.nih.gov/32384157/

Practical application of transcriptomic PODs: Bioactivity-exposure-ratio (BER)

- BERs are based on a margin-of-exposure (MOE) approach
- A MOE is calculated as the ratio of POD / Exposure for a given route (e.g., oral)
  - PODs for MOE calculations are typically based on traditional human or experimental animal toxicity dose-response data
  - BERs differ from MOE only in that PODs are derived from NAM data such as in vitro cell bioactivity (e.g., ToxCast; Tox21) or transcriptomics
  - A calculated BER is compared against a benchmark BER which is the product of the uncertainties associated with the chemical; for example:

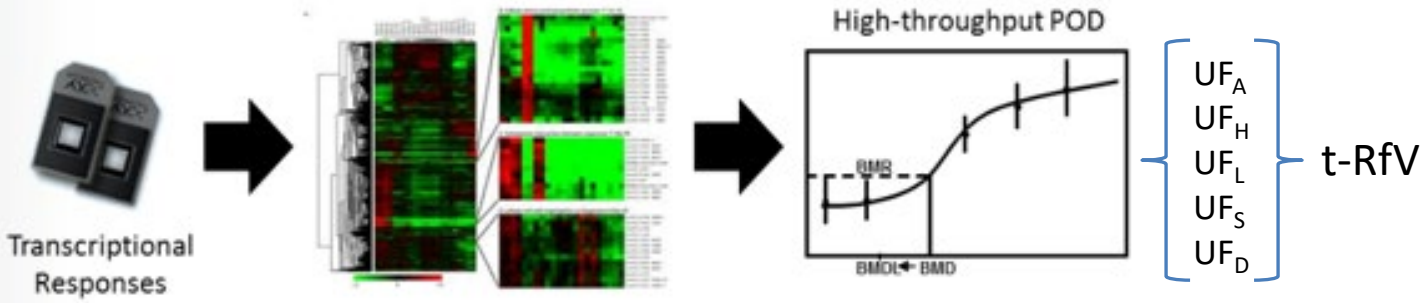
$$\text{Benchmark BER} = UF_A \times UF_H = 10 \times 10 = 100$$

Where
UF_A = uncertainty associated with animal-to-human extrapolation and,
UF_H = uncertainty associated with human interindividual variability

- The closer a calculated BER is to the benchmark BER, the greater the concern for risk of potential health outcomes in an exposed population

Practical application of transcriptomic PODs: Transcriptomic reference value (t-RfV)

- Non-cancer reference values in the U.S. EPA include an oral reference dose (RfD) and an inhalation reference concentration (RfC)
- An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.
- RfDs can be derived from a NOAEL, LOAEL, or benchmark dose (BMD), with uncertainty factors generally applied to reflect limitations of the data used.
- In the context of transcriptomics, a pathway (i.e., GO class)-based POD might facilitate derivation of a transcriptomic reference value (t-RfV)



Practical application of transcriptomic PODs: Mixtures assessment

- Chemicals co-occur in exposure media and/or internally
- Understanding and interpreting health risks associated with mixtures typically follows the priority of:
  - Whole mixture of concern
  - Sufficiently similar mixture
  - Component-based (integration of data across individual mixture chemicals)
- Due to significant lack of whole mixtures exposure and toxicity data, mixtures assessment typically falls under the component-based domain
- Component-based mixtures approaches such as the hazard index (HI) may be amenable to use of transcriptomic reference values
- Specifically, a screening-level HI does not require that mixture chemicals share a common target endpoint or health outcome; as such, individual hazard quotients (HQ) may be calculated across mixture chemicals using a measured or predicted exposure and a t-RfV

$$HI_{Screening} = \sum_{i=1}^{n} \frac{E_i}{t\text{-}RfV_i}$$

- The E_i and t-RfV_i are the human exposure metric and interim chronic noncancer t-RfV for the i^{th} mixture component, respectively, and n is the total number of mixture components
- If the $HI_{Screening}$ approaches or exceeds 1, there is indication of potential concern for human health outcomes associated with exposure to the mixture

Challenges

In moving forward…

- **Focus on Problem Formulation first**
  - Application of NAM data is dependent on “fit-for-purpose”
  - Big difference between “driving,” “filling,” and “nice-to-know”
- **Law of Parsimony**
  - “Enough precision to make a decision” (Tim Pastoor et al., 2014)
  - WOE link between adverse outcome and transcriptomic pathway events?? or,
  - Are biologically non-specific (but protective) transcriptomic PODs acceptable??
- **Understanding priors**
  - Existent hazard/dose-response/exposure/occurrence data??
  - Lack of (useful) quantitative data (this goes for hazard and exposure)

Acknowledgments

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- Lucina Lizarraga (EPA/ORD/CPHEA)

Questions?