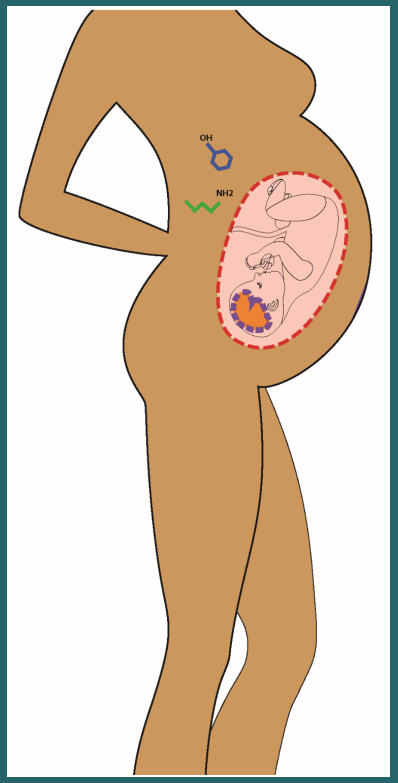
In vitro-in vivo extrapolation (IVIVE) for neurodevelopment: Toxicokinetics & *in vitro* point of departure evaluation of putative developmental neurotoxicants



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NCSOT PARC 01/19/2022

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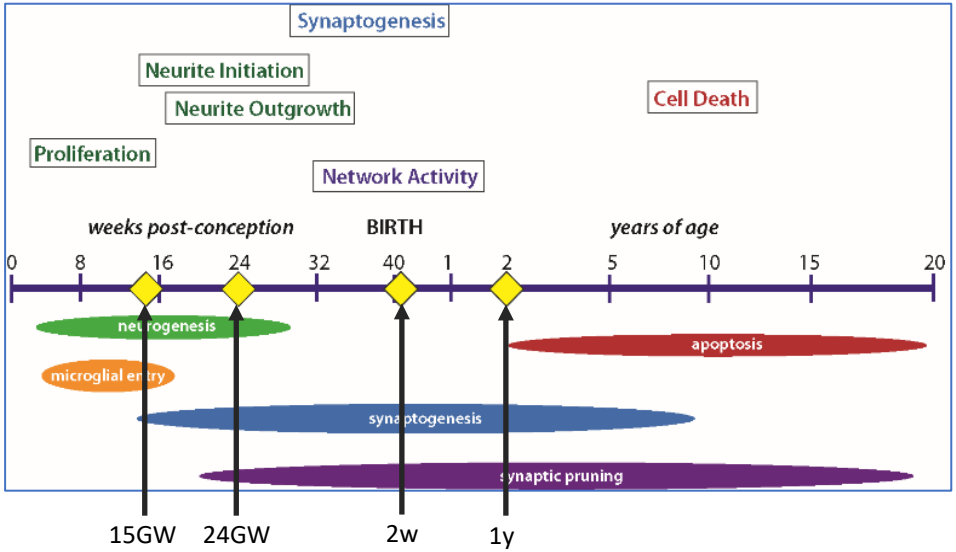
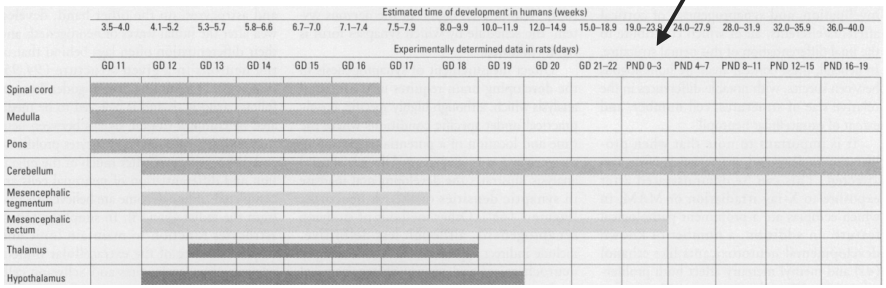
Timeline of neurodevelopment

Major windows of susceptibility span from 2nd trimester through 1st years of life



Cultures generated on postnatal day 0: ~19-23GW

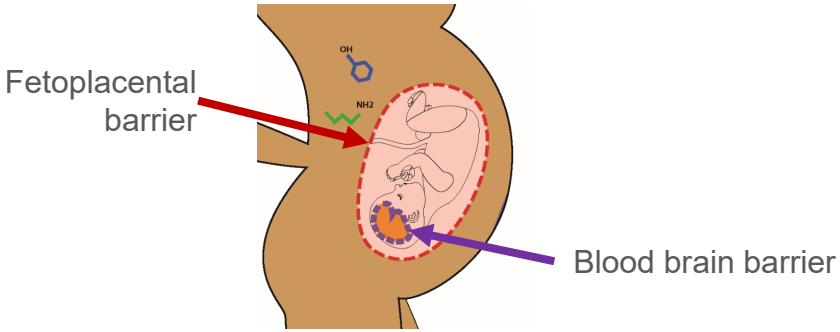


Rice & Barone, 2000

New Approach Methodologies increasingly employed to assess Developmental Neurotoxicity

- *In vitro* points of departure cannot be directly translated to *in vivo* dose metrics

- *In vitro* developmental neurotoxicity (DNT) new approach methodologies (NAMs) lack 2 critical barriers



→A specialized in vitro-in vivo extrapolation (IVIVE) approach combined with dosimetric modeling is required to estimate target site concentrations of relevance for DNT NAMs

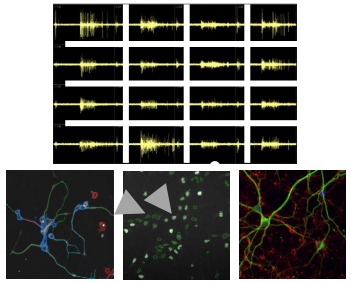
IVIVE approach

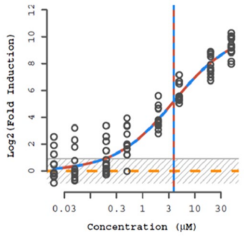
1. Identify compounds that elicited bioactivity in US EPA DNT NAMs
2. Toxicokinetic data gathered from prior efforts & collated
3. Physiologically based pharmacokinetic (PBPK) modeling performed using toxicokinetic data to predict C_{max}
4. Administered equivalent dose (AED) derived using reverse dosimetry based on DNT NAM bioactivity & C_{max} for lifestages of interest
5. Bioactivity exposure ratios (BERs) determined from comparison between AED & exposure

AED- administered equivalent dose
BER- bioactivity exposure ratio
C_{max}- maximal concentration
GW- gestation weeks
PBPK- physiologically-based pharmacokinetic

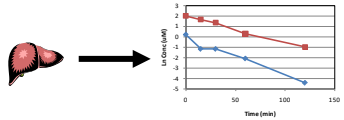
Data curation

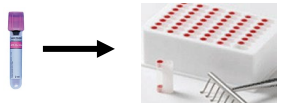
- *In vitro* Bioactivity
  - US EPA DNT NAMs
    - Microelectrode array (MEA)
      - Network activity & Cell death
    - High Content Imaging (HCI)
      - Proliferation, Apoptosis, Neurite outgrowth, Neurite maturation, Synaptogenesis
  - Identify compounds that elicited bioactivity



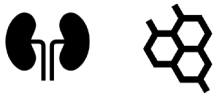


- Toxicokinetic data for 81 compounds that elicited bioactivity
  - Data generated in ToxCast PhI & II
    - Hepatic clearance
    - Plasma protein binding



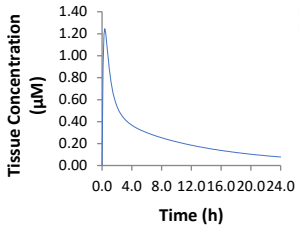


  - Additional toxicokinetic data collated
    - Nonmetabolic renal clearance
    - Physicochemical properties
    - Lifestage-specific physiologic information…



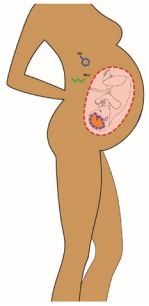
Physiologically Based Pharmacokinetic modeling

- Simcyp physiologically based pharmacokinetic (PBPK) model
  - Simulations to determine C_{max} in tissue compartments



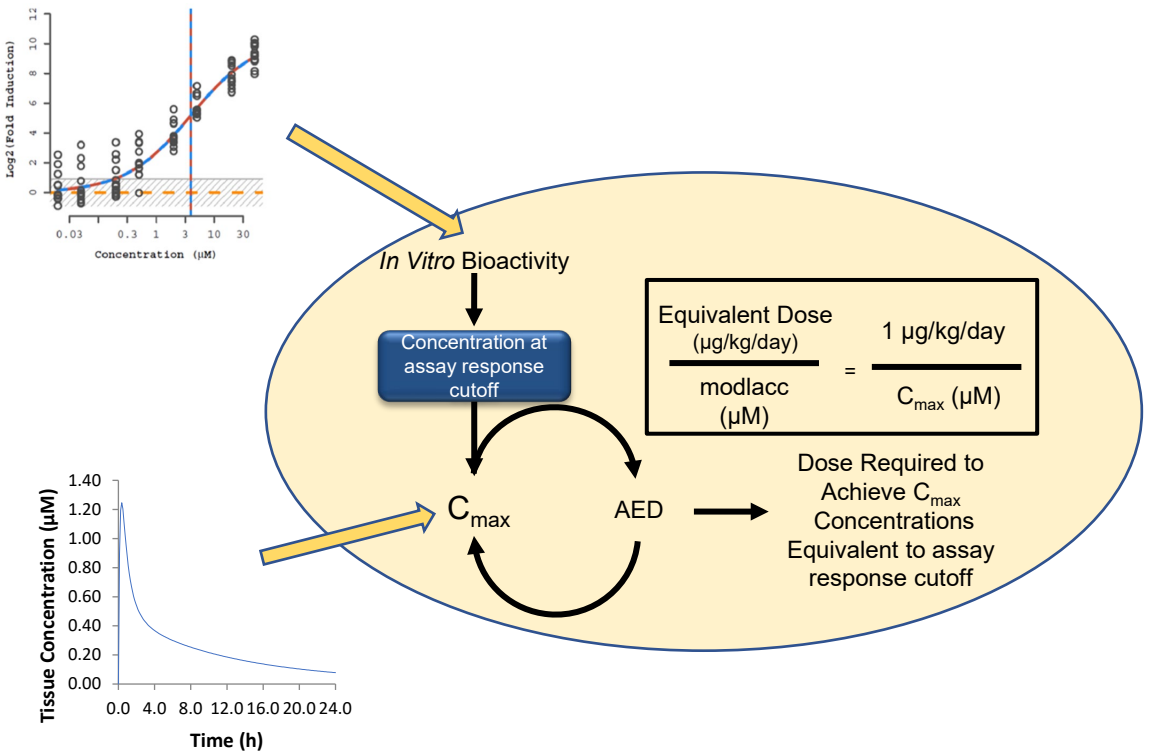
- Populations
  - Algorithms describe changes in anatomy, biochemistry, & physiology

    (Abduljalil *et al.* 2012, 2018, 2019; Gaohua *et al*. 2012)
  - Pregnancy
    - 15 & 24 gestation weeks (GW)
    - Fetoplacental compartment (fetus, placenta & amniotic fluid)
    - Maternal plasma & brain
  - Pediatric
    - 2w & 1y
    - Plasma & brain



Reverse dosimetry

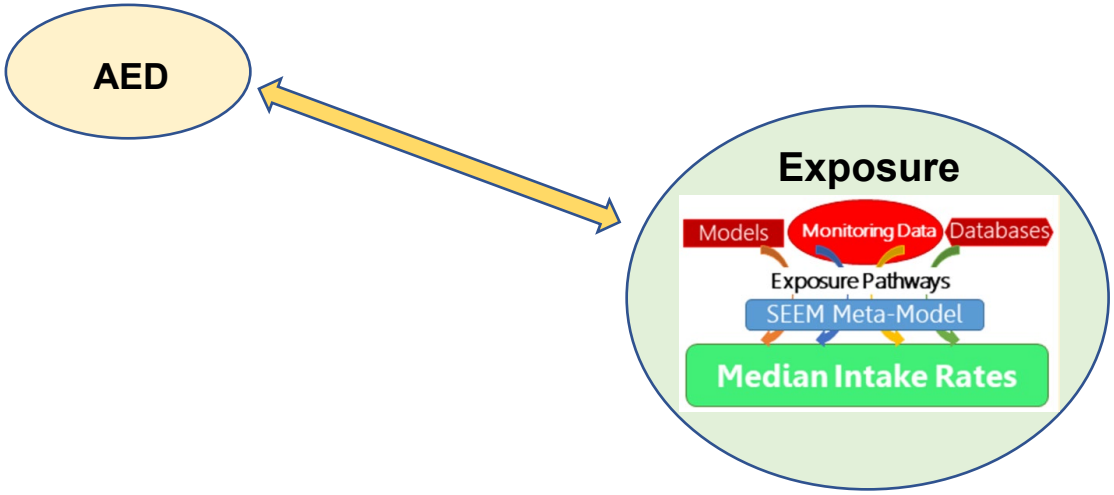
- Reverse dosimetry is used to derive administered equivalent doses (AEDs) for the lifestages of interest



Exposure

- Bioactivity Exposure Ratios (BER)
  - Provides comparison between anticipated external exposures & exposures needed to elicit bioactivity
- AEDs compared against exposure predictions
- Exposure predictions from Systematic Empirical Evaluation of Models (SEEM3; Ring *et al*. 2018)

$$BER = \frac{AED\left(\frac{mg}{kg}{d}\right)}{Exposure\ \left(\frac{mg}{kg}{d}\right)}$$



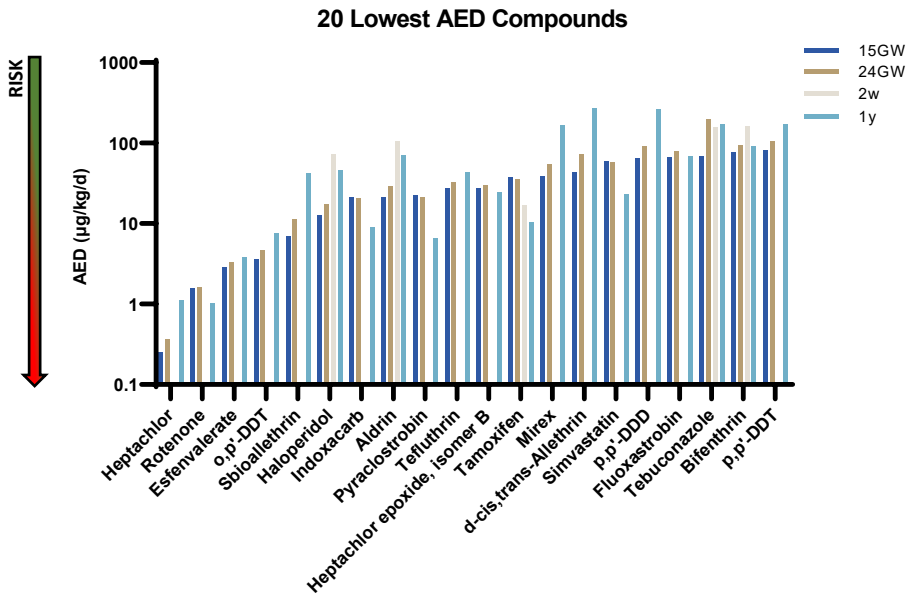
Results

Linking toxicokinetics to bioactivity: calculation of AEDs

$$AED = modlacc(\mu M) \times \left(\frac{1\mu g/kg/d}{\mu M\ Cmax}\right)$$

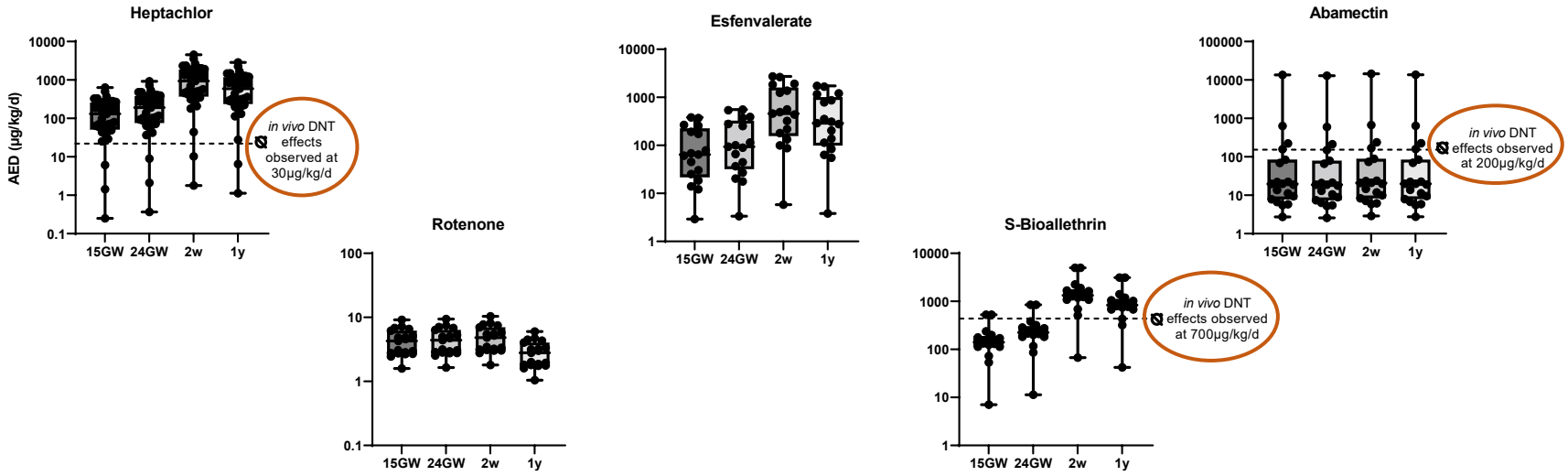
Key findings:

- AEDs ranged from 0.25 µg/kg/d for heptachlor to 8E6 µg/kg/d for triamcinolone
- Lowest AEDs generally found for the earliest lifestage



AEDs: relation to *in vivo* effects & BERs

Plots depict age-specific AEDs for all positive endpoints for 5 compounds with the lowest AEDs.



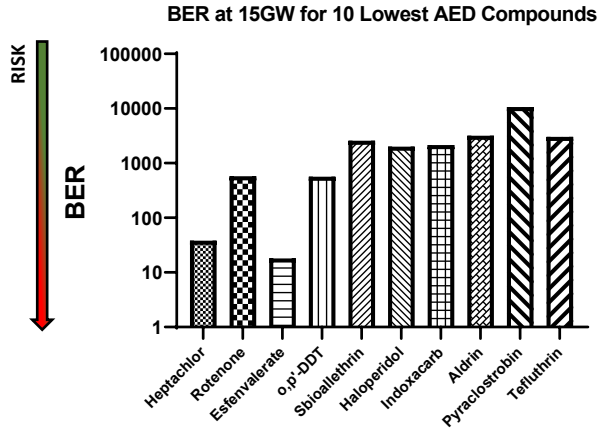
Key findings:

- *In vivo* effects found to be within range of AEDs that elicited bioactivity in DNT assays

Relating bioactive concentrations to exposures: estimations of BERs

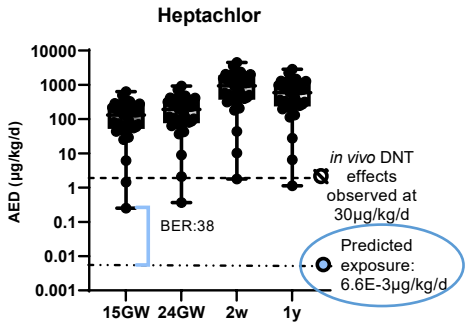
- Provides comparison between anticipated external exposures & exposures needed to elicit bioactivity
- Metric for margin of exposure

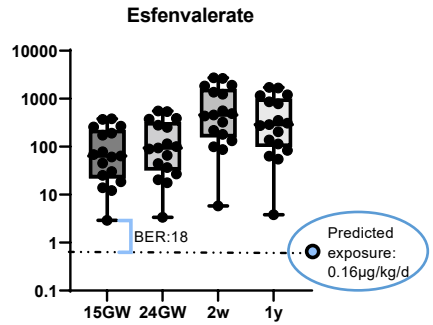
$$BER = \frac{AED(\frac{mg}{kg}{d})}{Exposure\ (\frac{mg}{kg}{d})}$$



Key findings:

- Two chemicals, heptachlor & esfenvalerate, show relatively low BERs of 38 & 18
  - Remaining chemicals have BERs of 500 or more



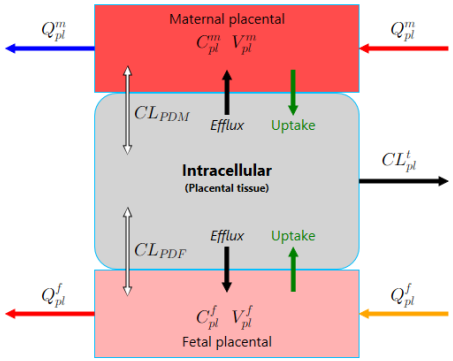


Expanding the model

1. Placental transfer model to determine fetal tissue-specific concentrations
2. Lactational model to assess the contribution of breastfeeding

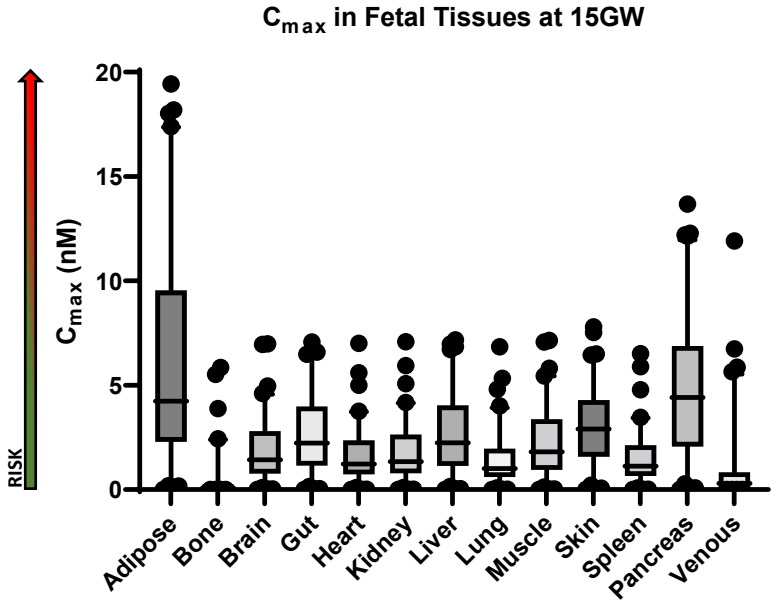
Placental transfer model

- Fetal tissue-specific concentrations



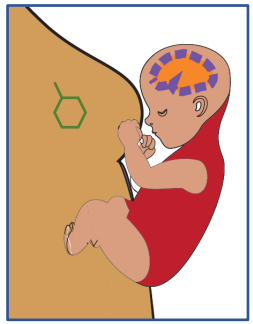
Key findings:

- Compounds preferentially partition into adipose
  - Followed by pancreas, skin, liver, gut
- Concentrations at 15GW > 24GW
  - Apart from bone & venous
  - Relative conc's in bone, **brain**, kidney, muscle, venous
- Fetal brain concentrations generally < fetoplacental
  - Apart from 25 compounds
  - 2 w/ AEDs <100
    - Emamectin Benzoate: 40
    - Tamoxifen: 38



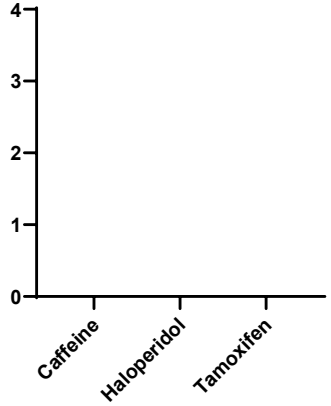
Lactational model

- Breastfeeding is an additional exposure pathway for infants & toddlers
- Lactational transport model in Simcyp
  - Infant daily dose (IDD) based on 200 mL/d intake
  - Relative infant daily dose (RIDD)- compared to maternal



Key Findings:

- IDDs spanned 8 orders of magnitude
  - 6E-7 µg/kg/d for triamcinolone to 11.4 µg/kg/d for reserpine
  - Corresponds to RIDD of 0.00004% to 856%
- Breastmilk levels are within 3X of *in vivo*
- Minimal contribution to exposure for most



Conclusions

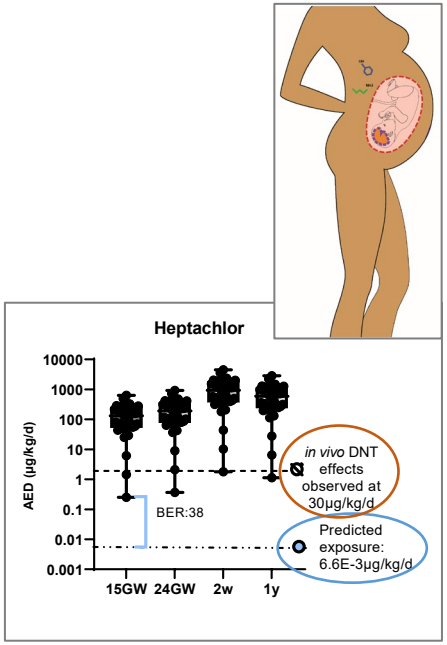
- Novel approach to relate concentration in *in vitro* DNT assays to *in vivo* levels during critical periods of brain development
- Allows for identifying *in vivo* exposures that could elicit bioactivity: AEDs
- *In vivo* DNT effects fall within range of AEDs & *in vivo* breastmilk levels fall within 3X, *supporting the predictivity of our novel approach*
- Approach could be used in risk assessment prioritization of chemicals of concern for DNT: BERs

Dosimetric models developed for this purpose need to consider:

- *Fetoplacental & blood-brain barrier transfer*
- *Dynamic nature of developing brain, barriers, & physiology*

Through future research we will additionally consider the impact of:

- *Metabolic ontogenies*
- *Passive & active permeability*
- *Additional chemicals*



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QUESTIONS?

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Chemical distribution

- Generally lipophilic
- Tissue accumulation
  - Brain C_{max} >Fetoplacental C_{max} >Plasma C_{max}
  - Highest concentrations in fetoplacental
- Placental transfer
  - Highest brain concentrations
    - High f_u
    - Low $logP_{o:w}$
- Breastmilk accumulation
  - > for weak monoprotic acids
  - < for neutral, highly lipophilic