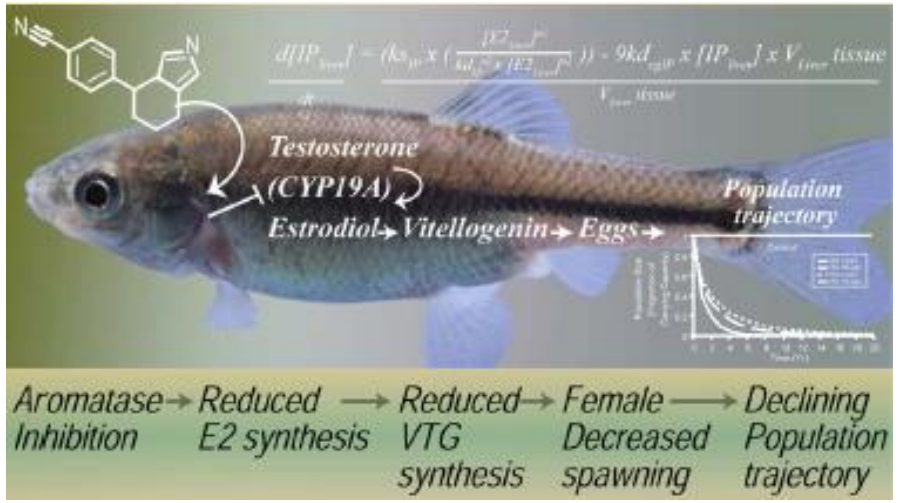
An Evolving View of Quantitative Adverse Outcome Pathways and Considerations for Application

Dan Villeneuve, US EPA*, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division, Duluth, MN, USA

**The contents of this presentation neither constitute, nor necessarily represent US EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

Quantitative AOPs



"Quantitative understanding of the relationships underlying transition from one KE to the next, as well as critical factors that can modulate those relationships, are sufficiently well-defined to allow quantitative prediction of the probability or severity of the AO occurring for a given activation of the MIE"

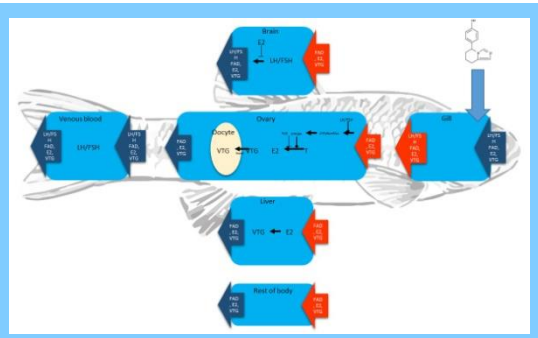
Conolly et al. 2017 – Quantitative adverse outcome pathways and their application to predictive toxicology. Environ. Sci. Technol. 51: 4661-4672.

Our First Quantitative AOP

coupled together multiple physiologically-based and/or statistical models

HPG axis model

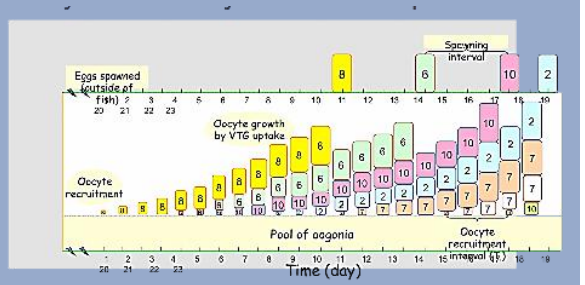
<u>Aromatase</u> Inhibition → <u>Granulosa</u> Reduced E2 synthesis → <u>Plasma</u> Reduced circulating E2 → <u>Hepatocyte</u> Reduced VTG production → <u>Plasma</u> Reduced circulating VTG



Cheng, et al. Computational modeling of plasma vitellogenin alterations in response to aromatase inhibition in fathead minnows. Toxicol. Sci. 2016, 154, 78–89.

Oocyte growth dynamics model

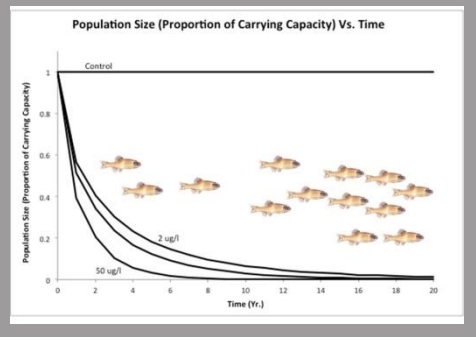
<u>Ovary</u> Impaired Oocyte Dev. → <u>Female</u> Decreased ovulation/spawning



Watanabe, et al. Predicting fecundity of fathead minnows (Pimephales promelas) exposed to endocrine disrupting chemicals using a MATLAB®-based model of oocyte growth dynamics. PLoS One 2016, 11, e0146594.

Population dynamics model

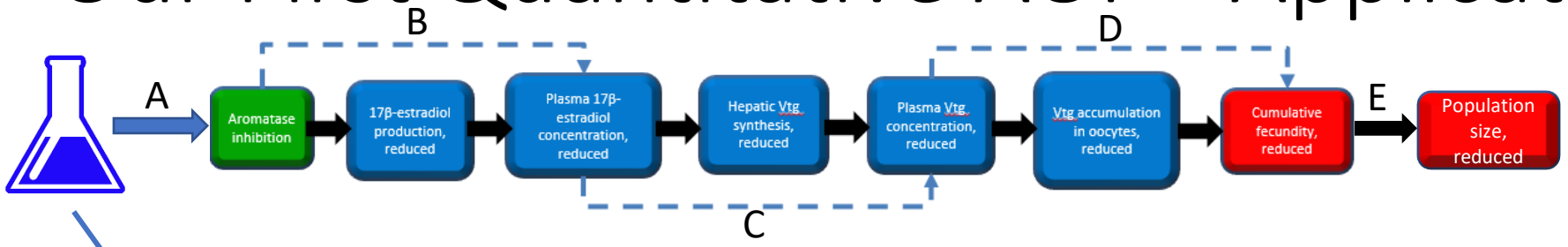
<u>Population</u> Declining Trajectory



Miller et al. Linkage of biochemical responses to population-level effects: A case study with vitellogenin in the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 2007, 26, 521–527.

Conolly et al. 2017 – Quantitative adverse outcome pathways and their application to predictive toxicology. Environ. Sci. Technol. 51: 4661-4672.

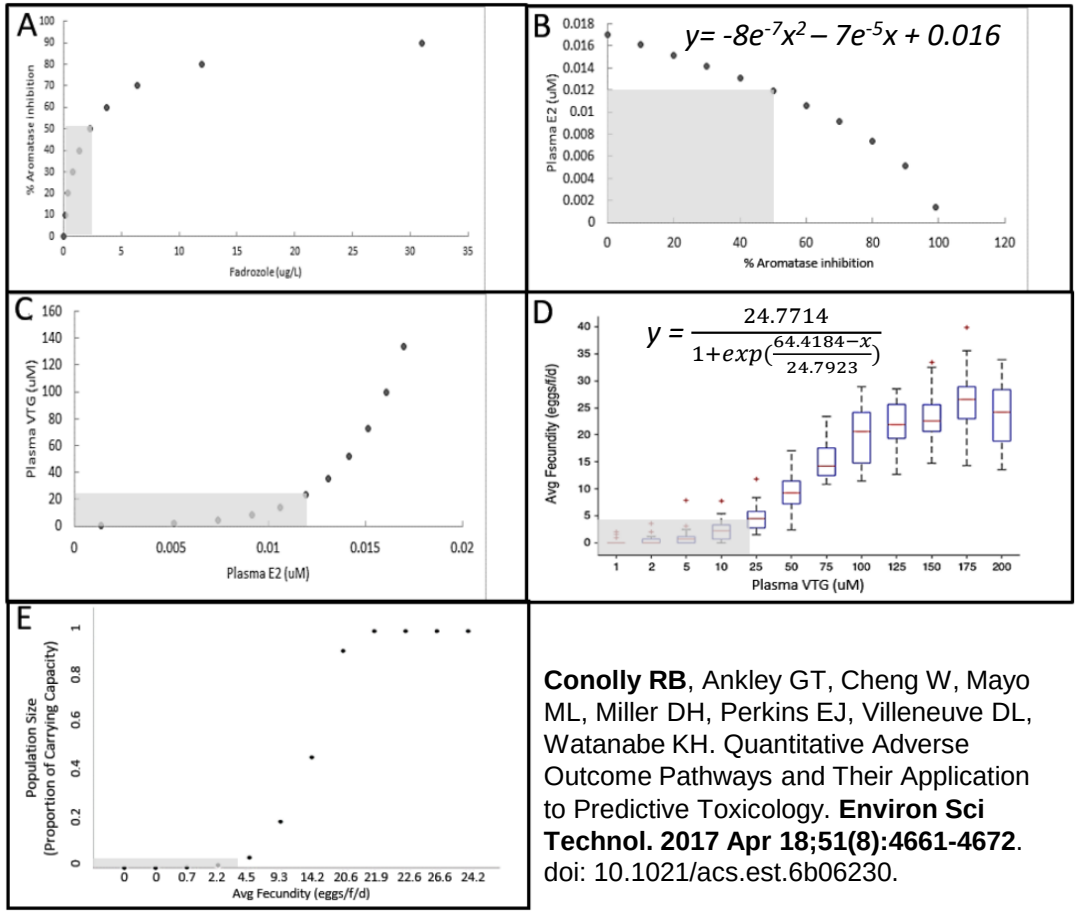
Our First Quantitative AOP - Application



Equipotent concentration of reference chemical

Model-derived response-response relationships for major KERs along the AOP.

Steady state, after compensation assumed.



Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology. **Environ Sci Technol. 2017 Apr 18;51(8):4661-4672**. doi: 10.1021/acs.est.6b06230.

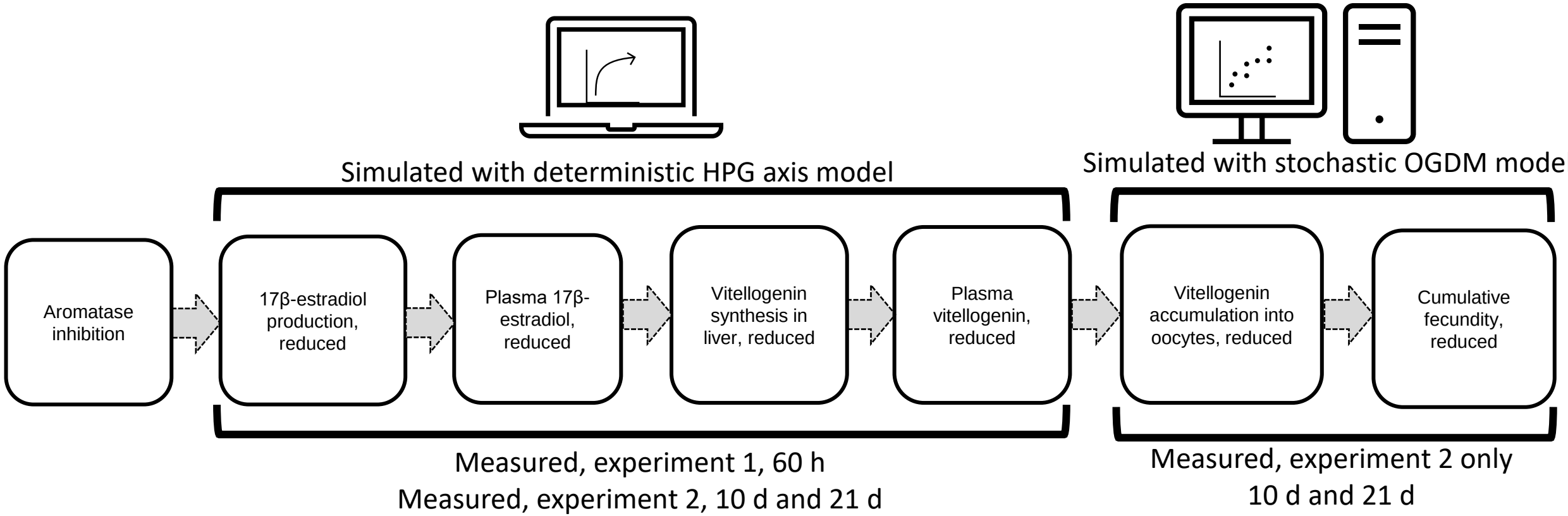
For a simplified, continuous exposure scenario, could simplify to a series of regression equations.

Response-response relationships

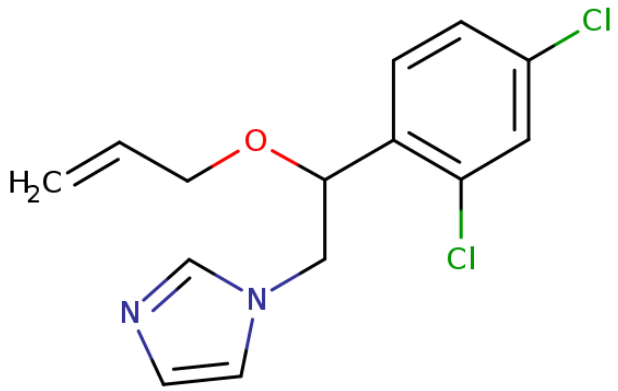
Testing our Quantitative AOP

Compare outcomes predicted via our qAOP construct to those measured empirically

Novel aromatase inhibitor identified via ToxCast (NVS and Tox21 assays)



Simulated dose-response

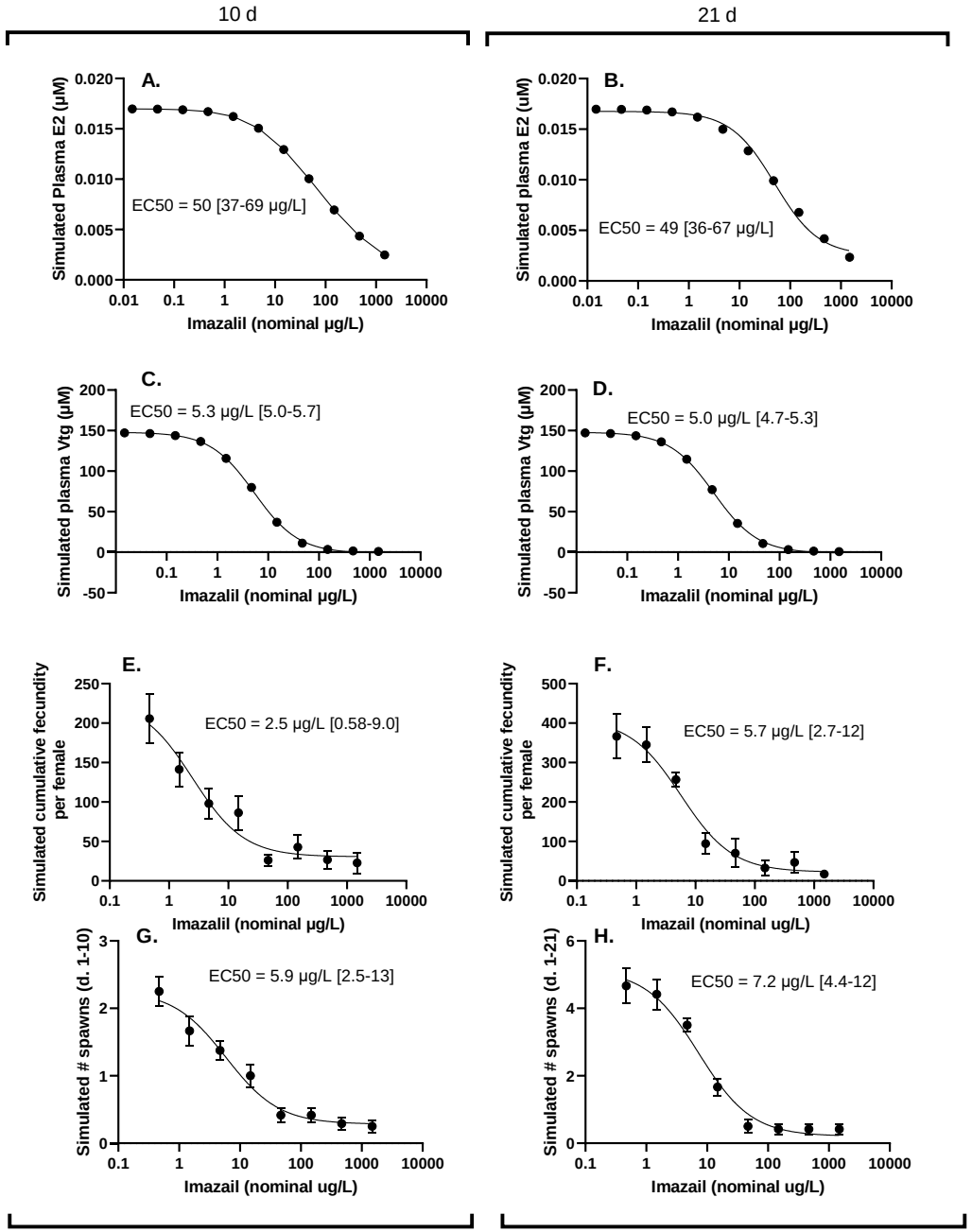


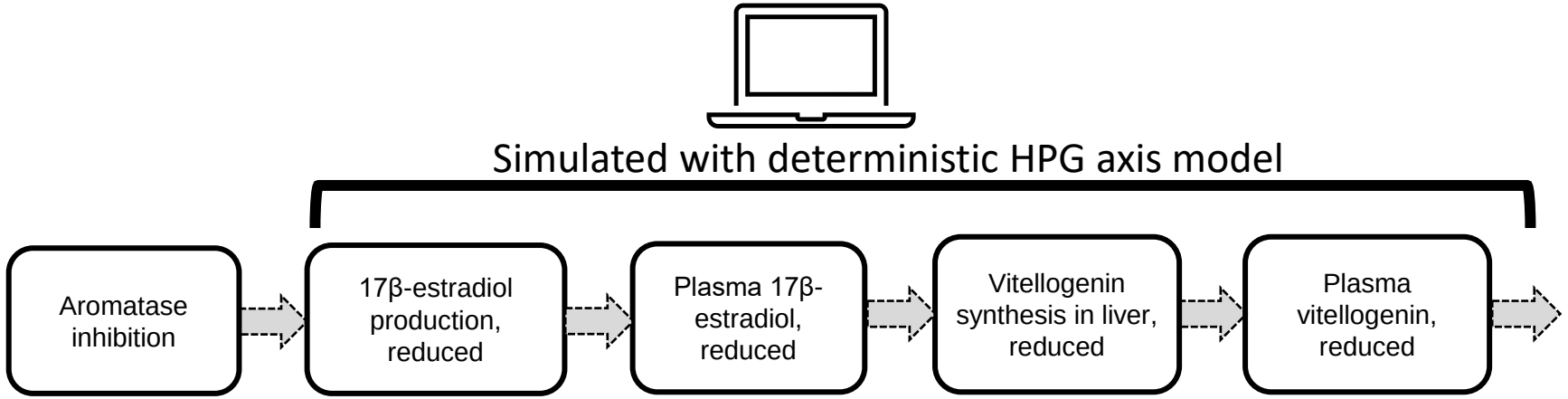
1 µg imazalil/L ≈ 0.012 µg fadrozole/L

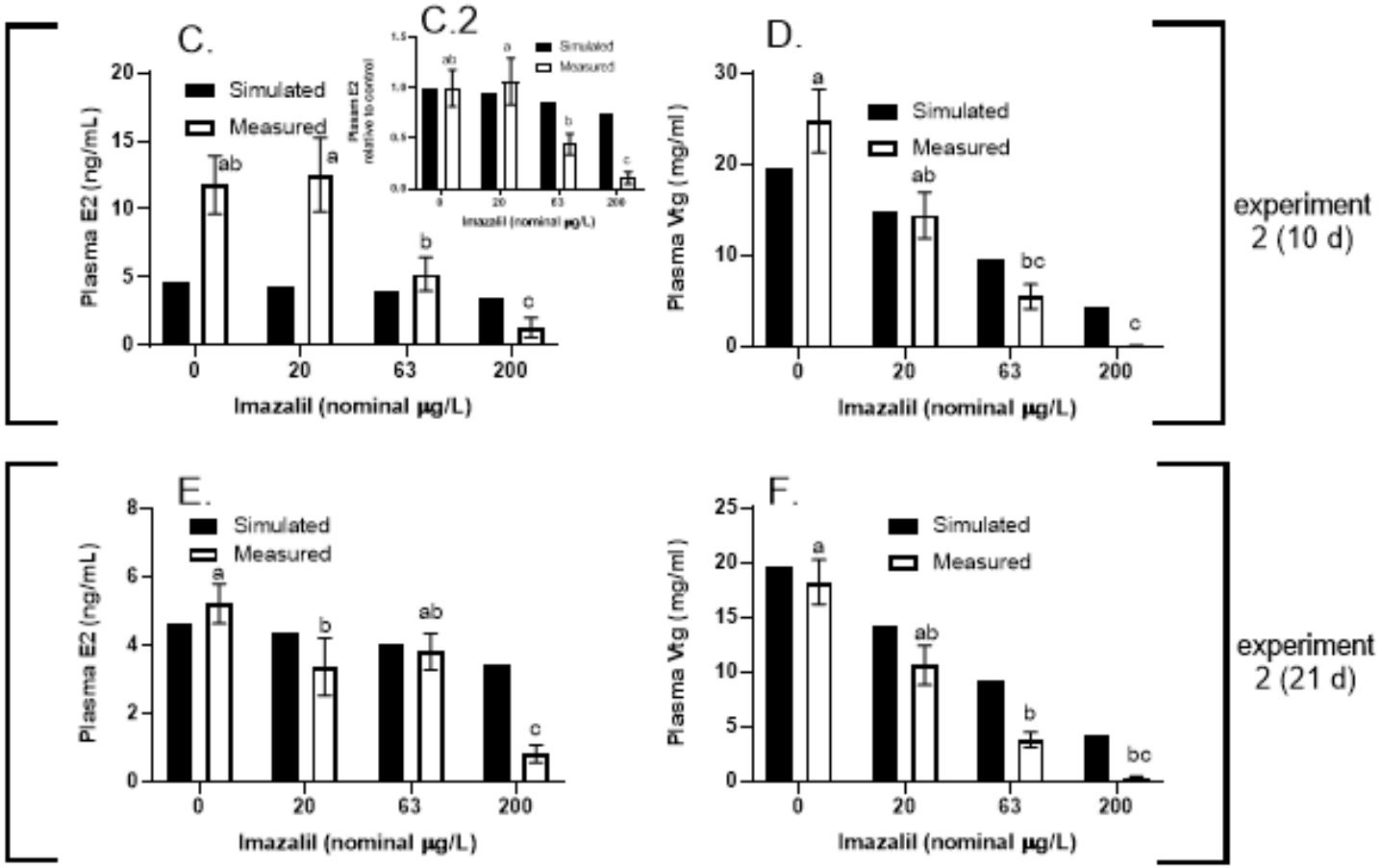
Assumptions:

1) Identical toxicokinetics
2) Identical toxicodynamics
3) Single mode of action

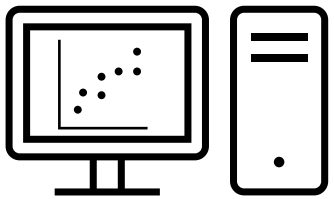
All are likely incorrect assumptions



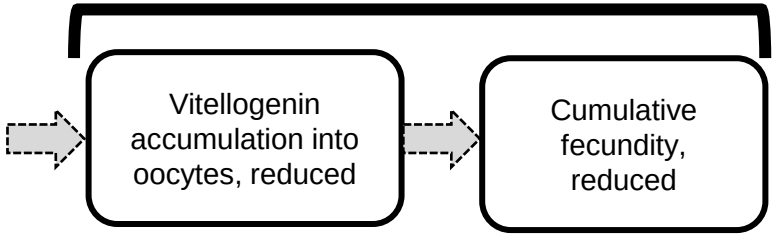


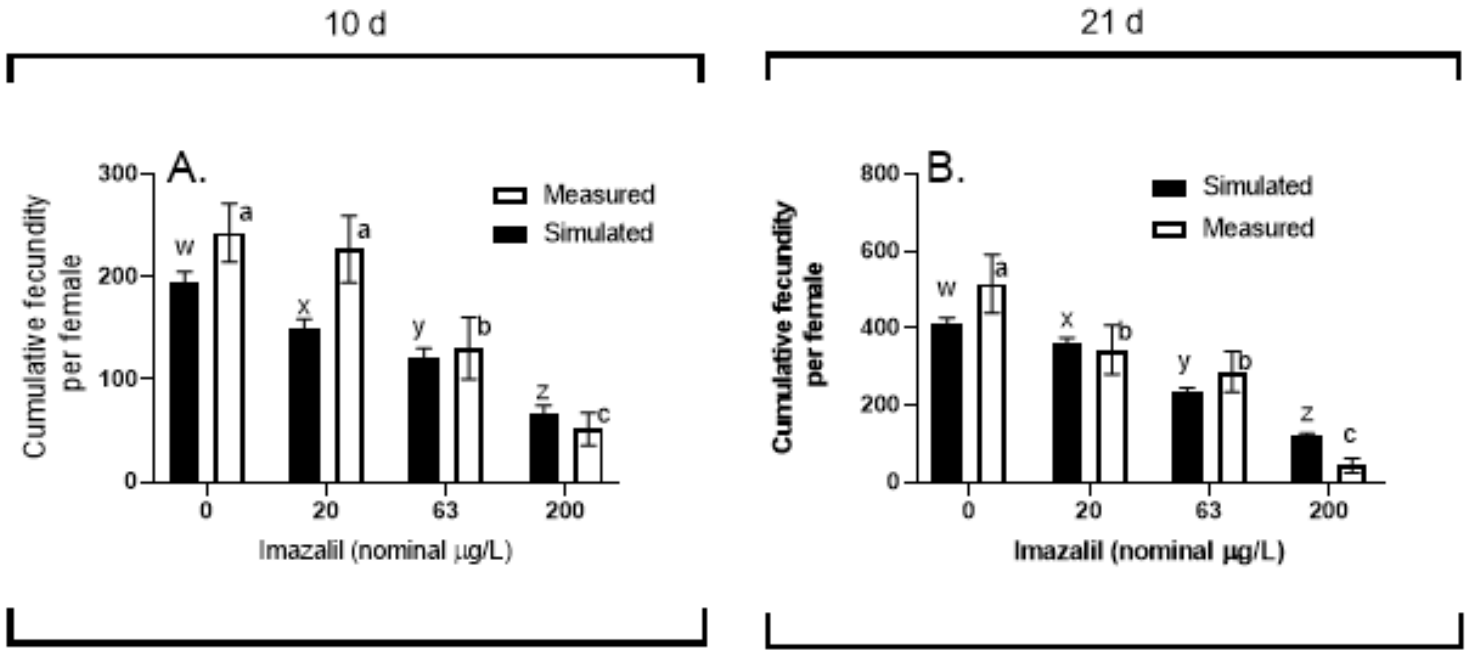


- Simulations generally underestimated concentrations of E2 and Vtg.
- Underestimated the magnitude of effect, particularly at higher concentrations.
- Possibly due to imazalil's effects on additional steroidogenic enzymes



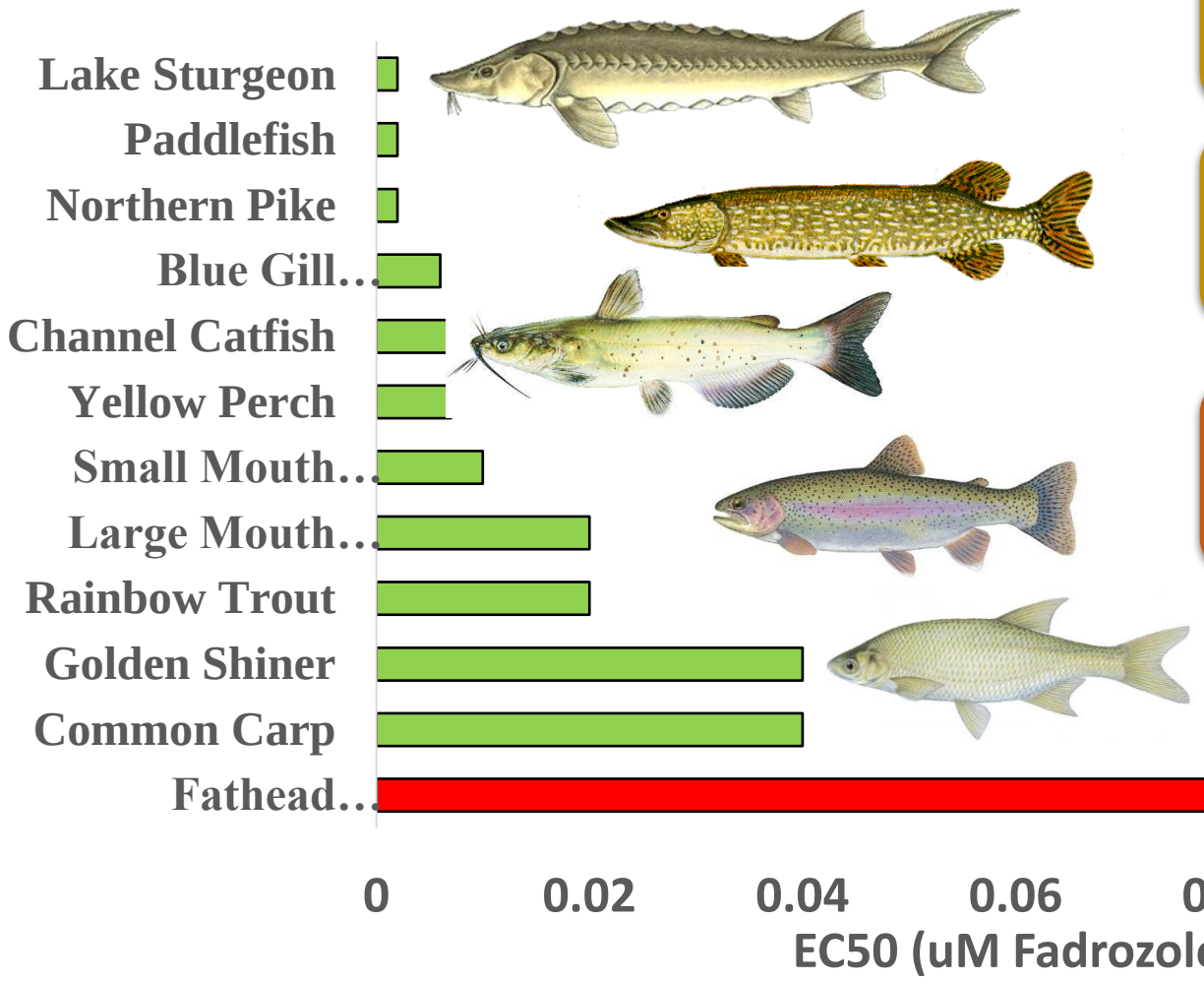
Simulated with stochastic OGDM model





- Simulations did a reasonable job of predicting effects on cumulative fecundity
- 10 d LOEC was about 10-fold greater than simulated EC50
- 21 d LOEC was just 4-fold greater than simulated EC50.

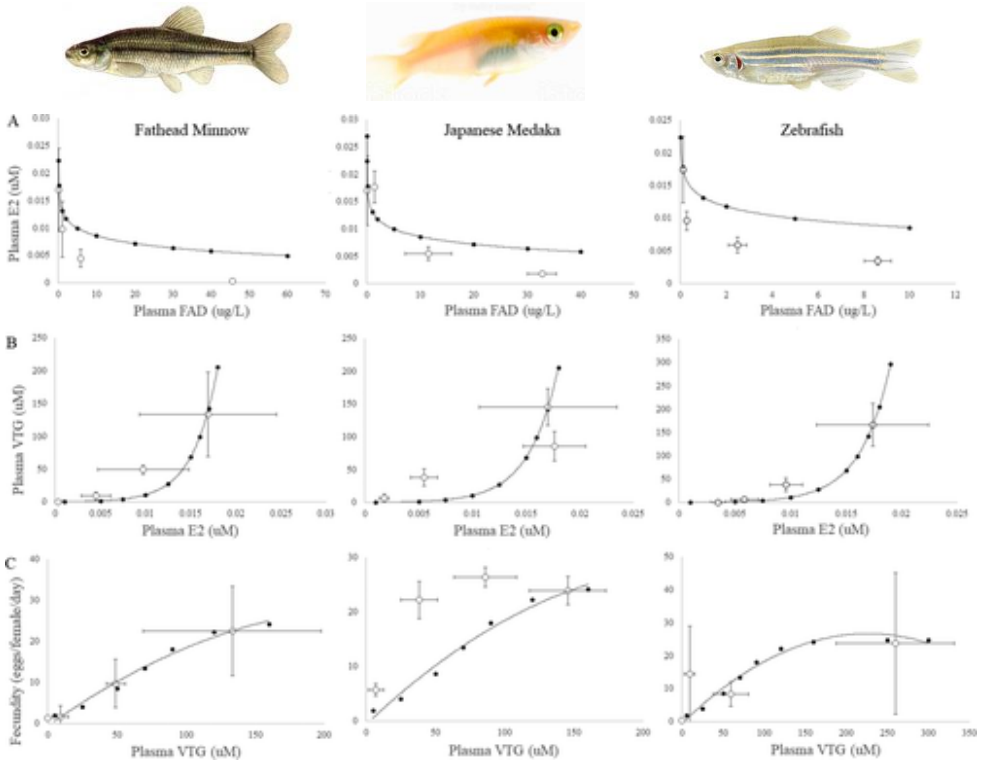
Adjustment for intrinsic susceptibility to aromatase inhibition – allows for cross-species application of the R-R regression equations.



Plasma Reduced circulating E2

Plasma Reduced circulating VTG

Female Decreased cumulative fecundity



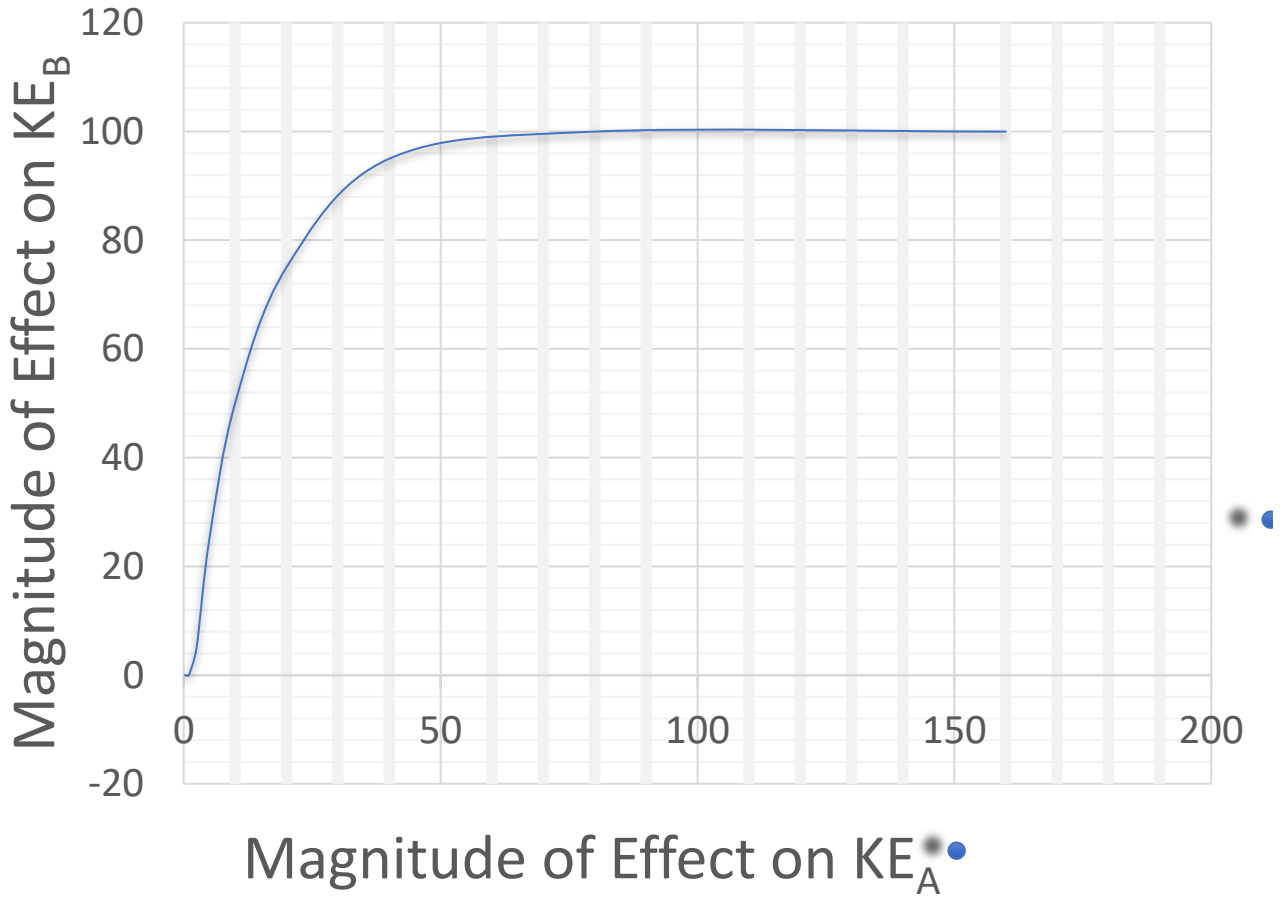
Doering et al.. Environ Sci Technol. 2019 53(17):10470-10478. doi: 10.1021/acs.est.9b02606.

Doering et al. Toxicol Sci. 2019 170(2):394-403. doi: 10.1093/toxsci/kfz115.

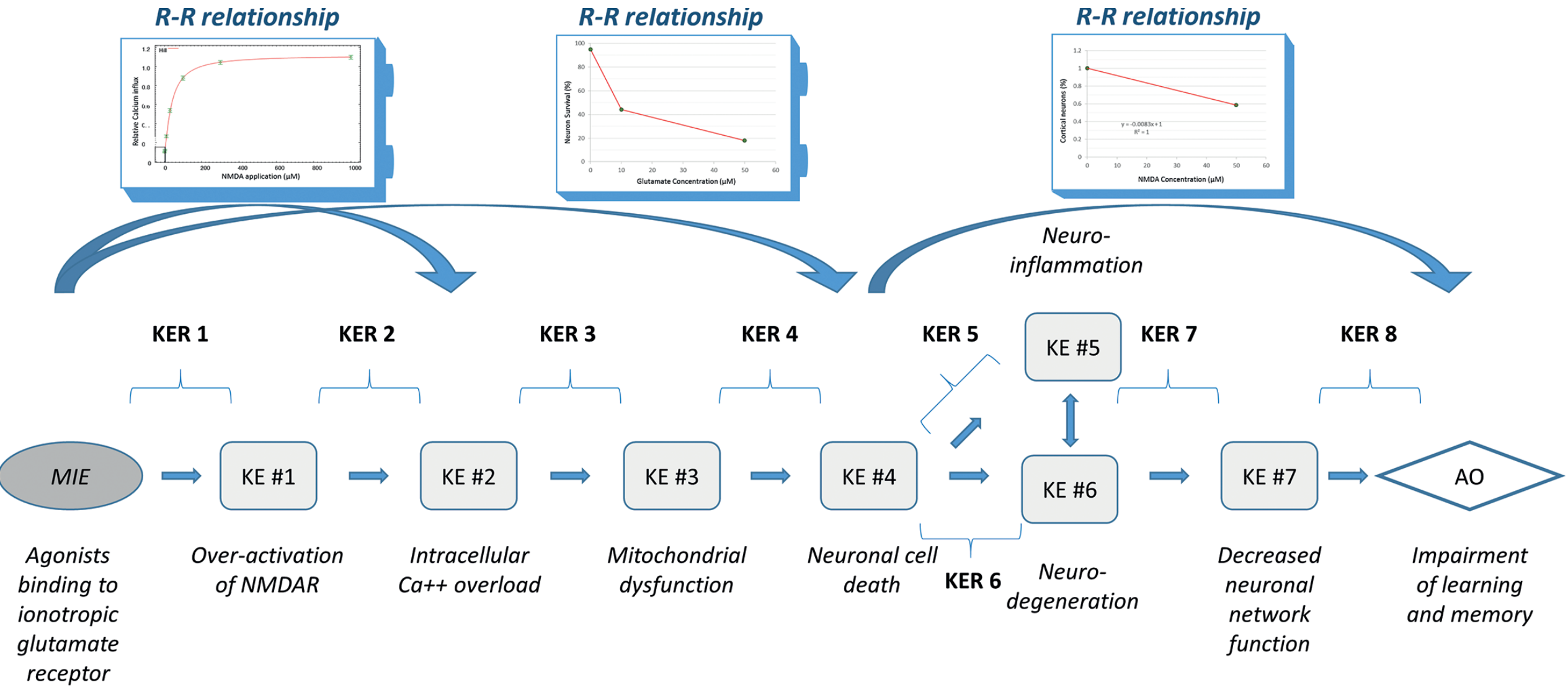
Limitations to our previous approach

- Very resource intensive to develop the models
  - 10-15 years of research
  - Novel experimentation

- Not practical to replicate for many AOPs



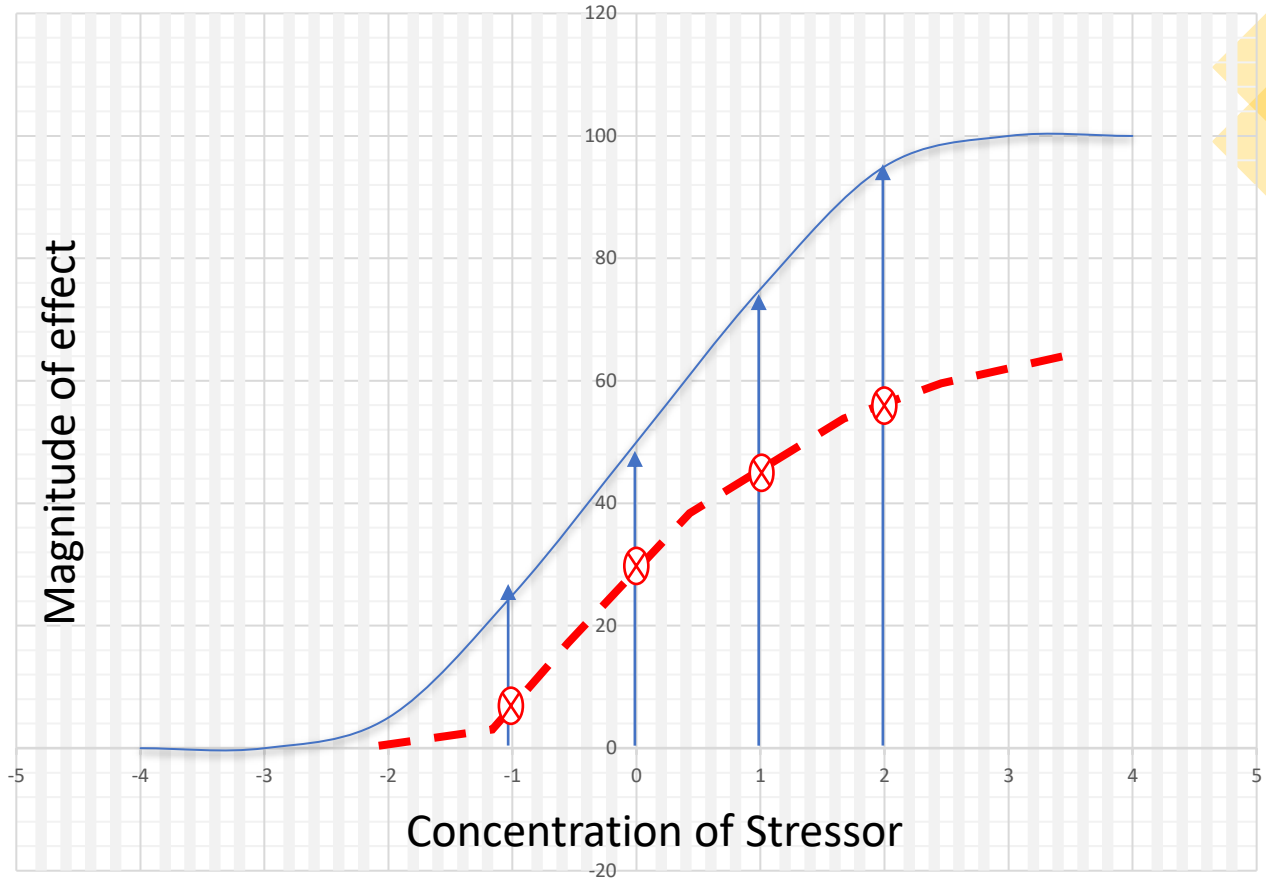
Modular, KER-driven approach to qAOP development



Foran, C., Rycroft, T., Keisler, J., Perkins, E., Linkov, I. and Garcia-Reyero, N. (2019) “A modular approach for assembly of quantitative adverse outcome pathways”, *ALTEX - Alternatives to animal experimentation*, 36(3), pp. 353-362. doi: 10.14573/altex.1810181.

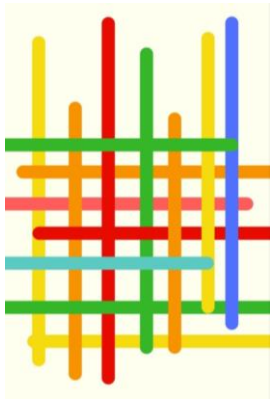
Alternative

- Can we take a more empirically-based approach
- Leverages the kinds of data we tend to have available (dose-response)
- R-R-R can be derived from concentration response information for two different KEs, as long the stressor is the same.



$$\frac{\text{Effect magnitude KE1}}{\text{[prototypical stressor]}} \times \frac{\text{[prototypical stressor]}}{\text{Effect magnitude KE2}} = \frac{\text{Effect magnitude KE1}}{\text{Effect magnitude KE2}}$$

(R-R-R)

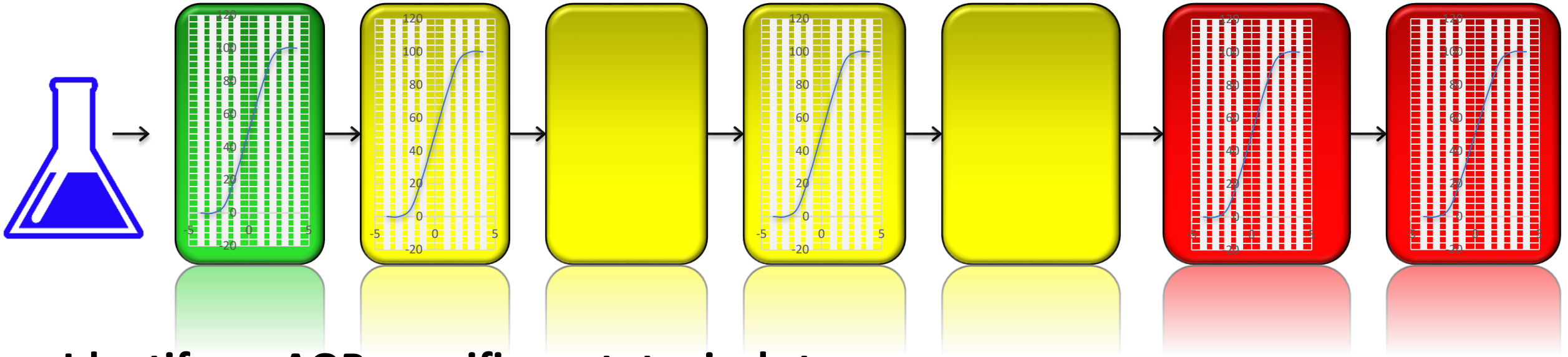


Prototypical Stressor - Defined

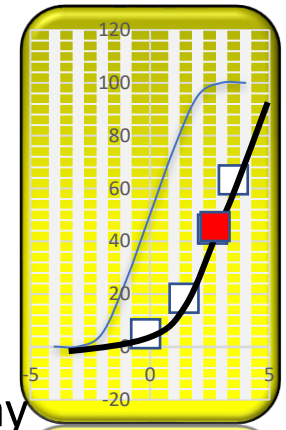
Prototypical stressor: A stressor that is known to trigger the molecular initiating event (MIE) (or the earliest key event in the pathway) and for which there is an extensive database with respect to its impacts on the downstream key events (KEs) such that experimental evidence related to that stressor's effects provided considerable support for key event relationships (KERs) along the pathway and the AOP as a whole.

- Prototypical stressors often serve as a focal point for literature searches and other assembly of empirical support
- Prototypical stressors are not necessarily chemicals (e.g., radiation)

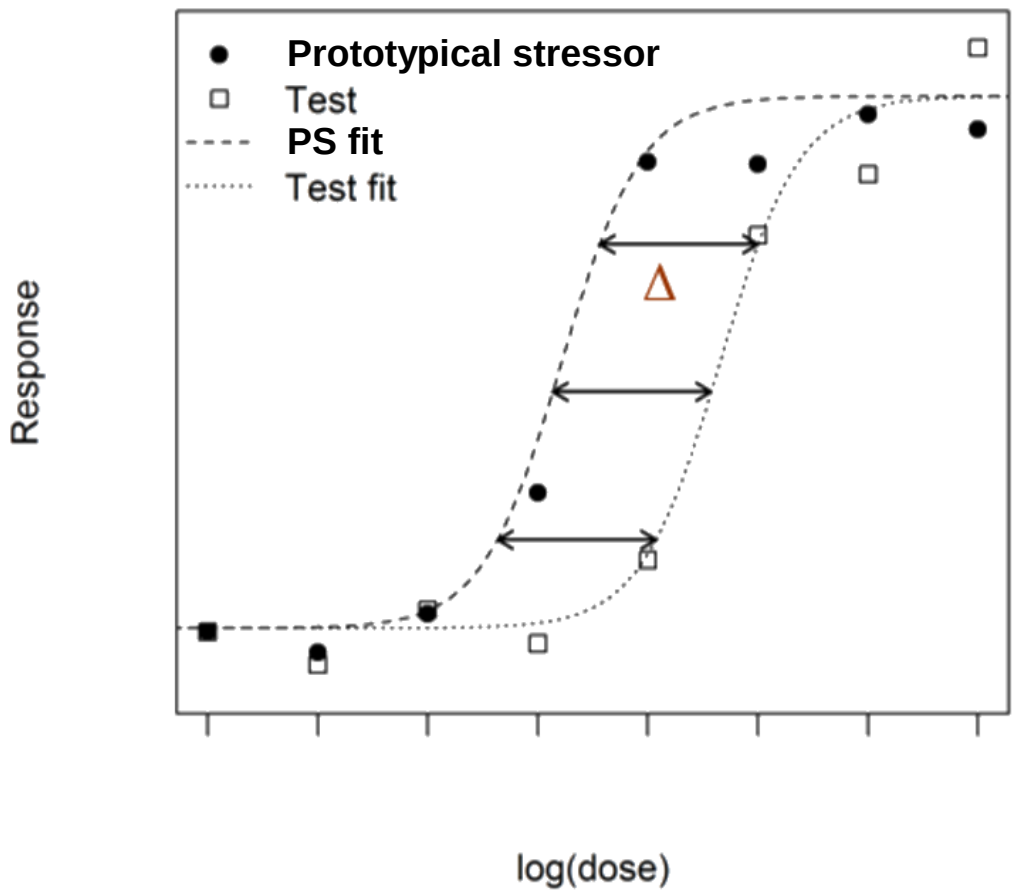
Prototypical Stressor Approach



- **Identify an AOP-specific prototypical stressor**
  - Document concentration-response for prototypical stressor across as many KEs as possible
- **For any new stressor**
  - Define relative potency at any one (or more) KEs along the pathway
  - Calculate “equivalent” concentration of prototypical stressor
  - Extrapolate to dose response curves for prototypical stressor at KEs farther down the pathway



Toxic Equivalency Approach



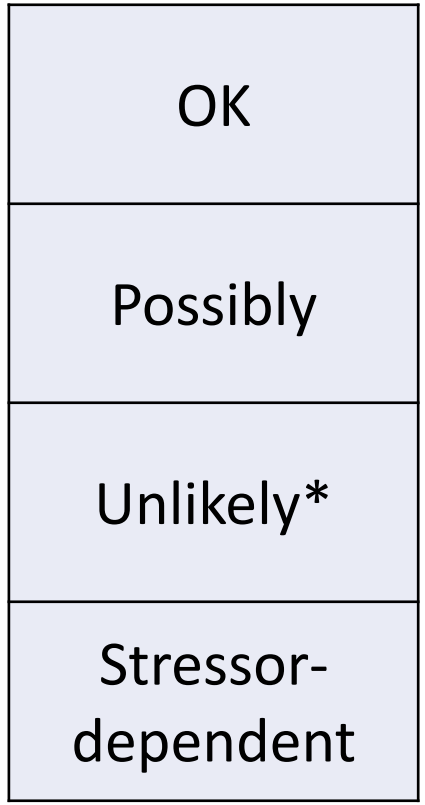
$$TEQ = \sum n\,(Ci \times TEFi)$$

- Widely used for risk assessment of mixtures of dioxin-like compounds.
- 2,3,7,8-TCDD as index chemical
- Potency of other congeners expressed relative to dioxin.

Assumptions

Assumptions implicit in the TEF approach include:

- The individual compounds all act through the same biological or toxic pathway;
- The effects of individual chemicals in a mixture are essentially additive at submaximal levels of exposure;
- The dose-response curves for different congeners should be parallel
- Target organ(s) in terms of fate/distribution for all congeners is the same over the relevant range of doses



*Uncertainty associated with violating can be estimated

Safe, Stephen H., Lea Pallaroni, Kyungsil Yoon, Kevin Gaido, Susan Ross, and Donald McDonnell. "Problems for risk assessment of endocrine-active estrogenic compounds." *Environmental Health Perspectives* 110, no. suppl 6 (2002): 925-929.

Prospects

- Deviations from assumptions of TEF approach will yield inaccuracies
  - Quite likely
  - Uncertainty can be estimated to at least some extent
- The generalizability of response-response relationships for different species, stressors, etc. is also relatively uncertain.

- Same limitations apply to the computational model-based approaches we've employed previously.
- Assembly of data to support a "prototypical stressor" approach is likely more achievable in the near terms than robust and generalizable R-R-Rs.

Summary

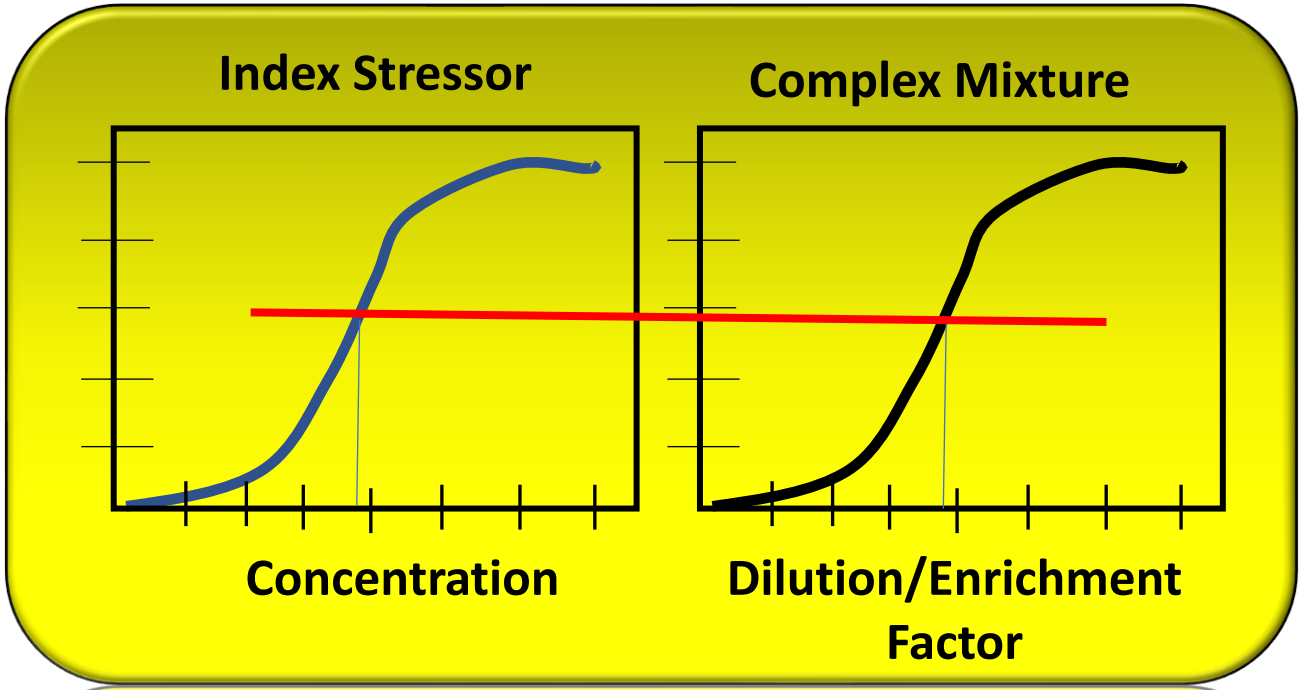
- qAOP allows one to estimate the probability or severity of an AO based on the magnitude/duration of perturbation of one or more KEs
- qAOP must be coupled with chemical-specific information (e.g., potency; ADME) for use in predictive risk assessment
- Once envisioned as the “most advanced” stage of AOP development, qAOP now viewed through lens of “fit-for-purpose”
- Variety of qAOP development approaches and strategies have been employed, based on the available data and intended application. (fit-for-purpose)
- Quantitative understanding of the KERs provides an effective, modular, foundation for qAOP development.

Acknowledgements

These are not all my ideas.
They reflect contributions and conversations I've had with innumerous colleagues.
Want to acknowledge all that contributed to the research and concepts presented.

Prototypical Stressors and Mixtures

Chemical Mixture (individual exposome)



Biological response equivalence at same KE

- Calculate Equivalency factor
- Extrapolate along AOP assuming same behavior as index stressor