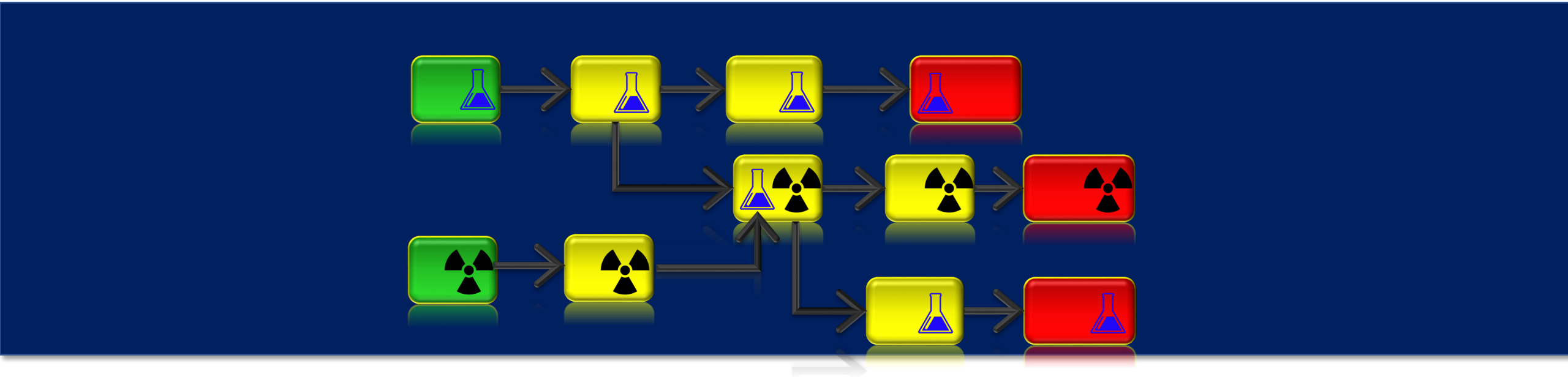
Incorporating index stressors with the AOP framework to aid quantitative application



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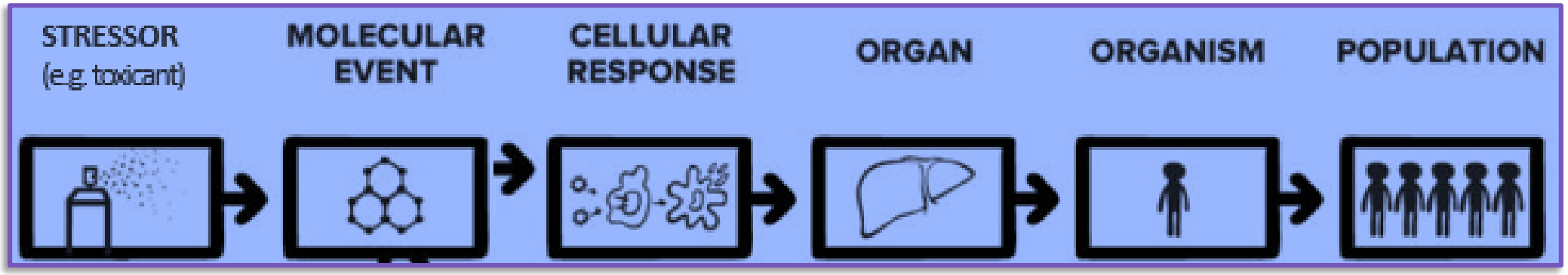
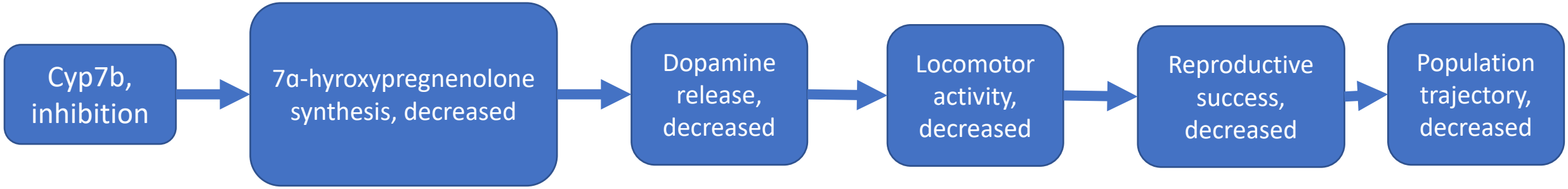
Adverse Outcome Pathways



An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.
(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

- **Organize and assemble the specialized scientific knowledge required to interpret results from new approach methodologies (NAMs).**
- Present it in a simple to follow graphical and narrative format
  - Supported by scientific literature and evidence
  - Searchable, globally accessible, and transparent

HAZARD ≠ RISK



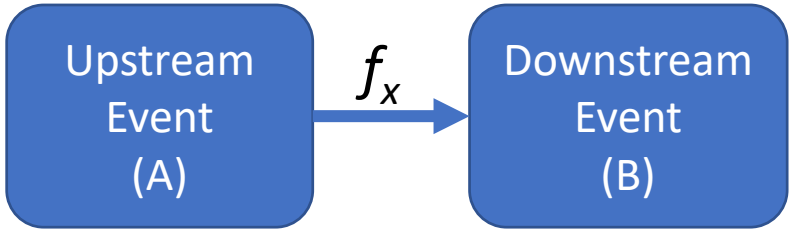
Not enough to know this could happen and why

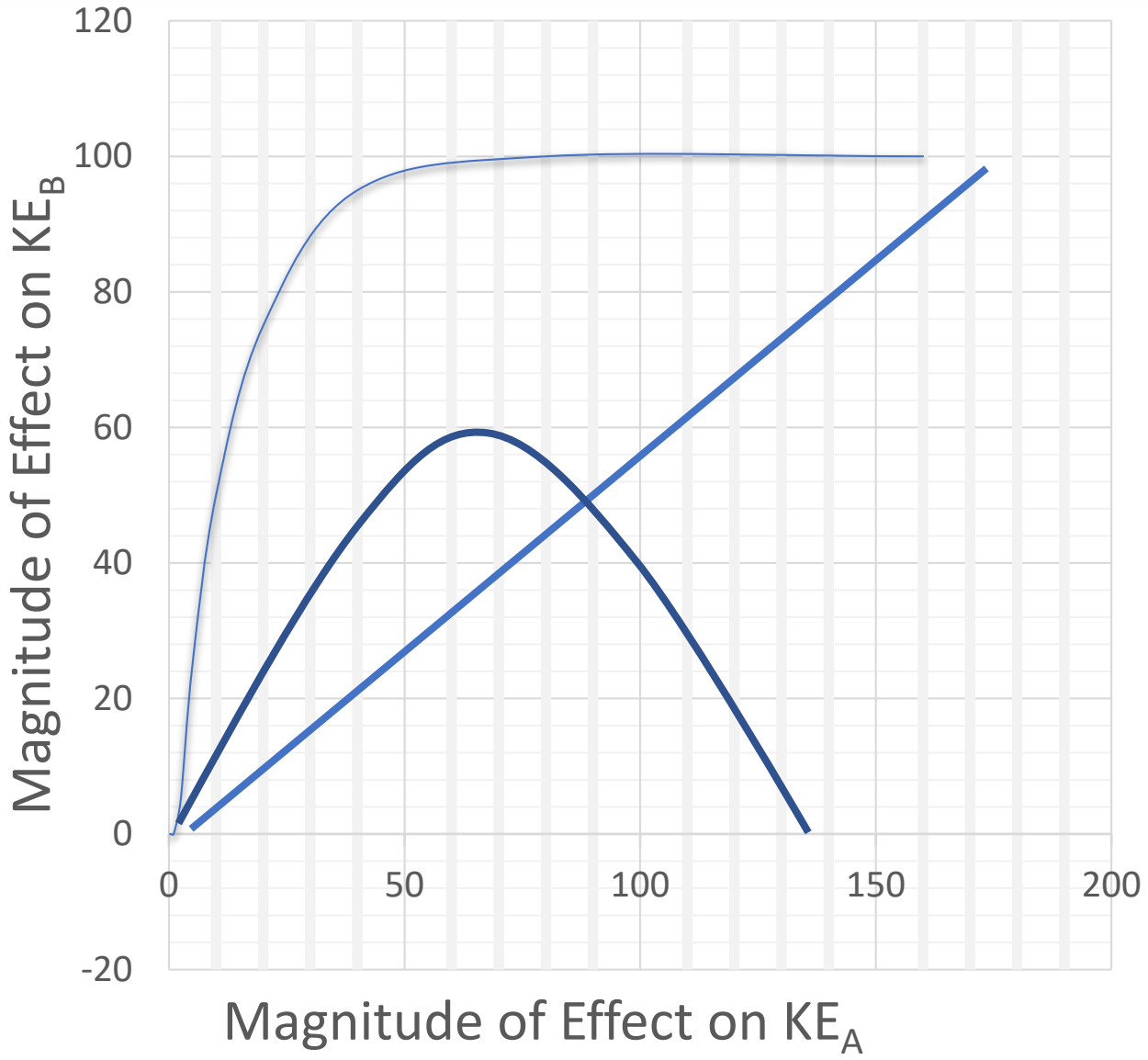
Need to know whether it is likely to happen in a given scenario.

Risk = probability

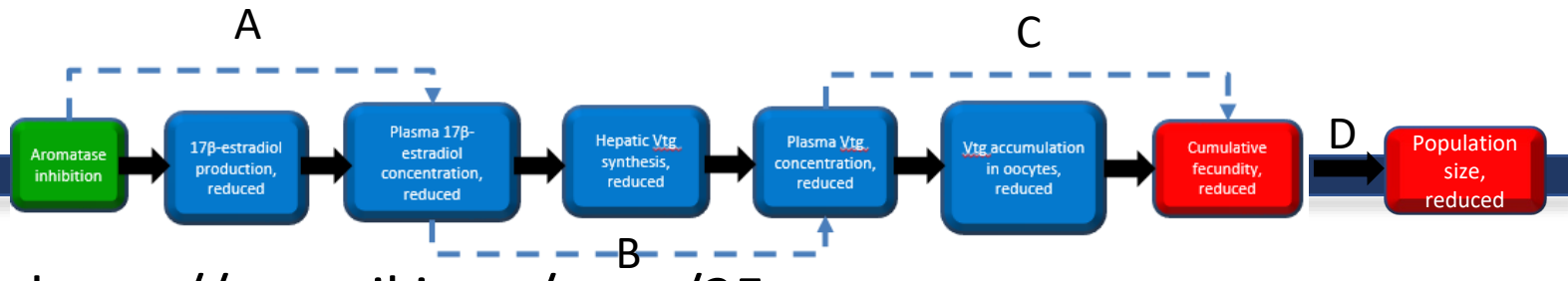
Response-Response Relationship

Change in a biological measurement (KE_B) as a function of change in another biological parameter (KE_A) on which it is dependent.





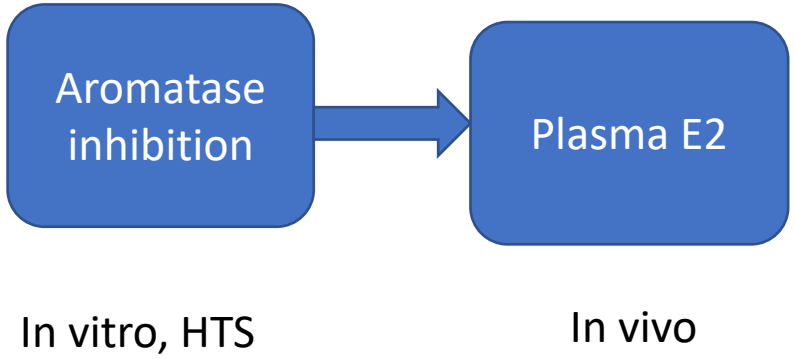
Response-Response Relationships

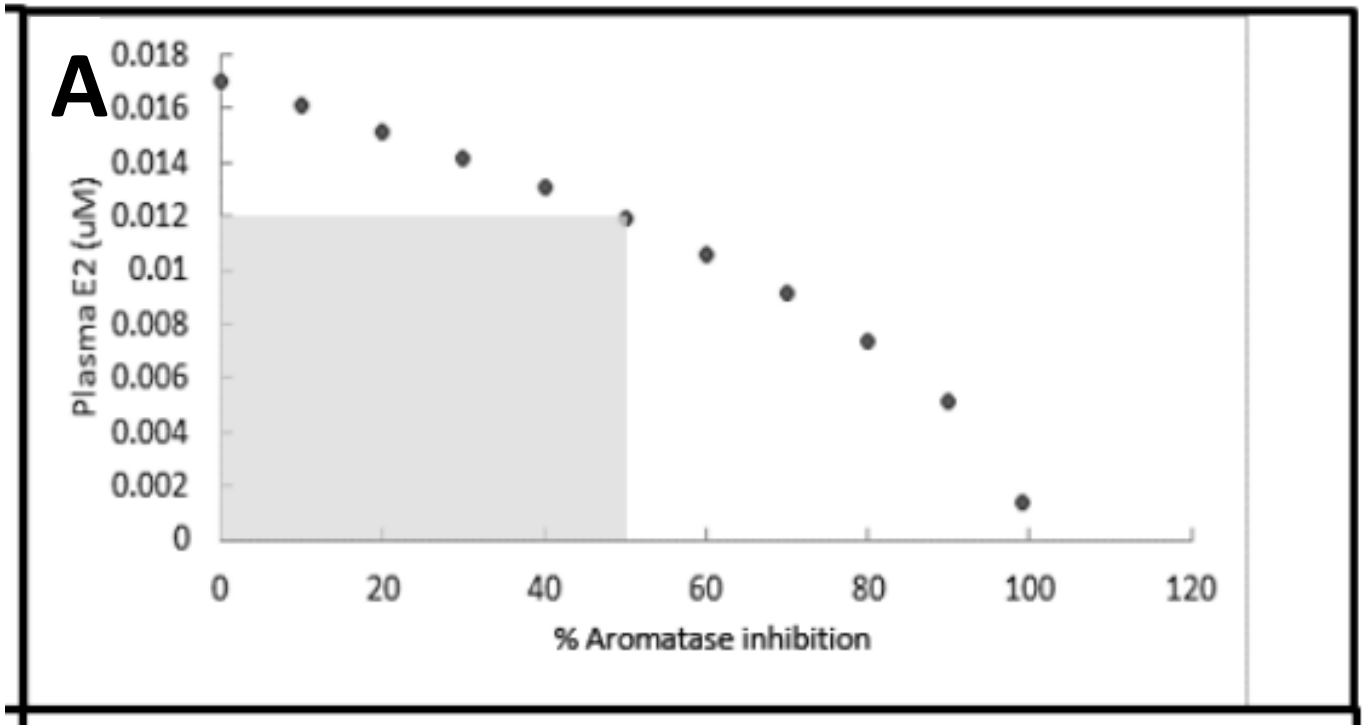


https://aopwiki.org/aops/25

Response-Response Relationship

Example

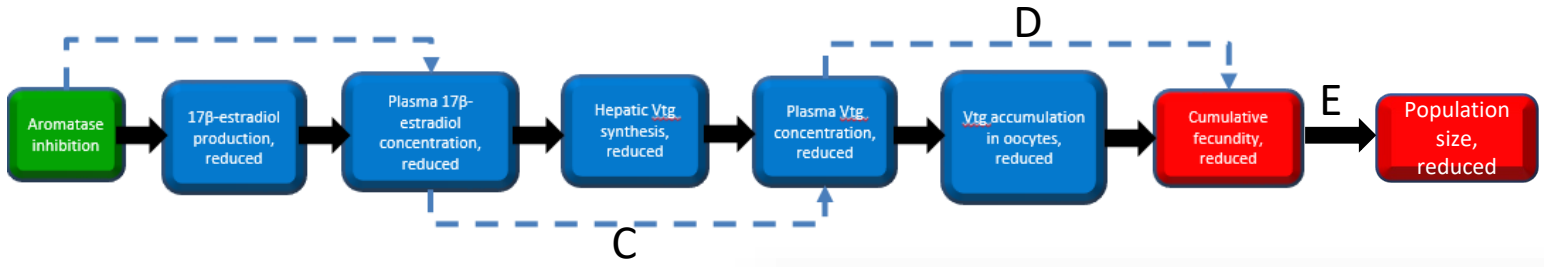




$$y = -8e^{-7}x^2 - 7e^{-5}x + 0.016$$

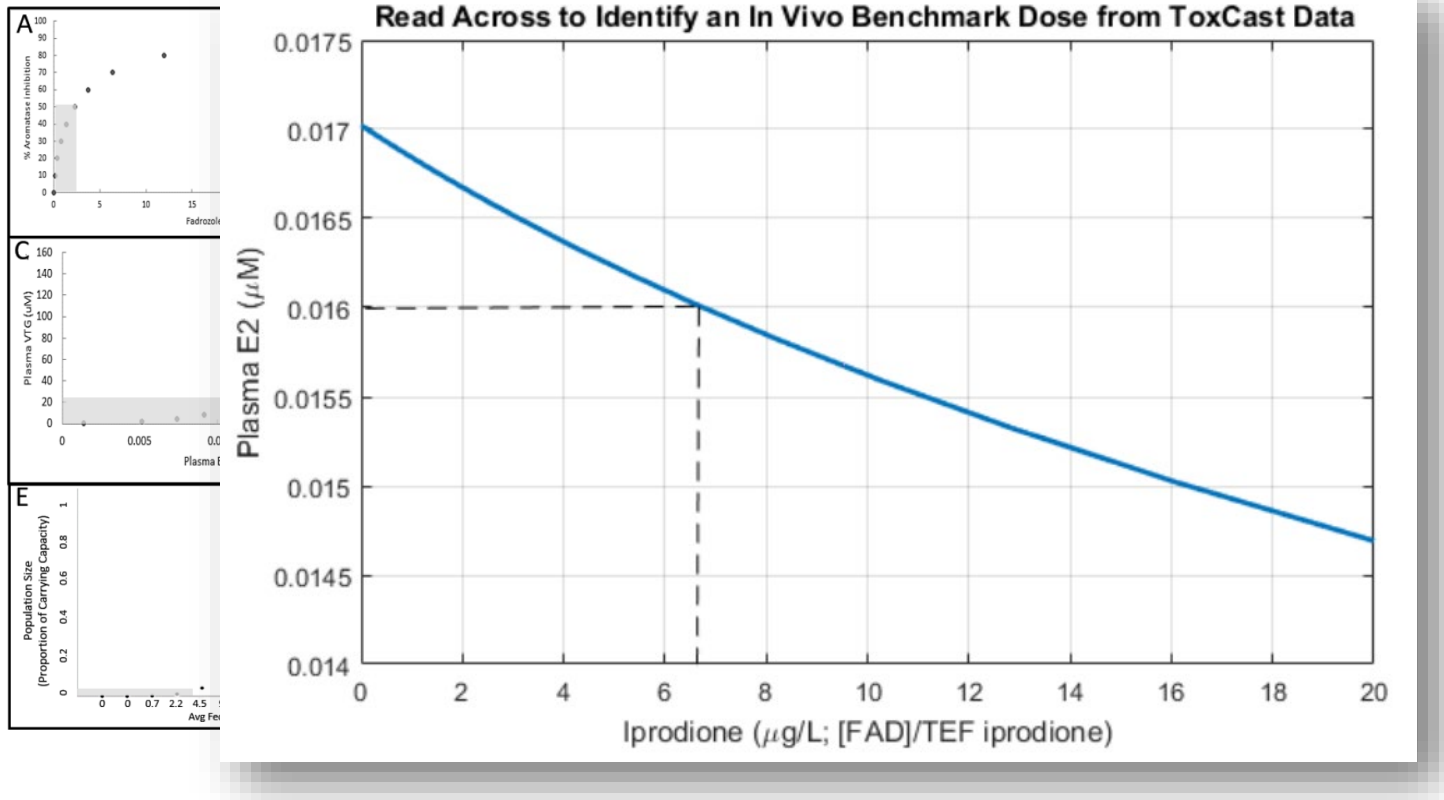
Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology. Environ Sci Technol. 2017 Apr 18;51(8):4661-4672. doi: 10.1021/acs.est.6b06230.

Quantitative AOP



A series of generalizable response-response relationships that span the AOP.

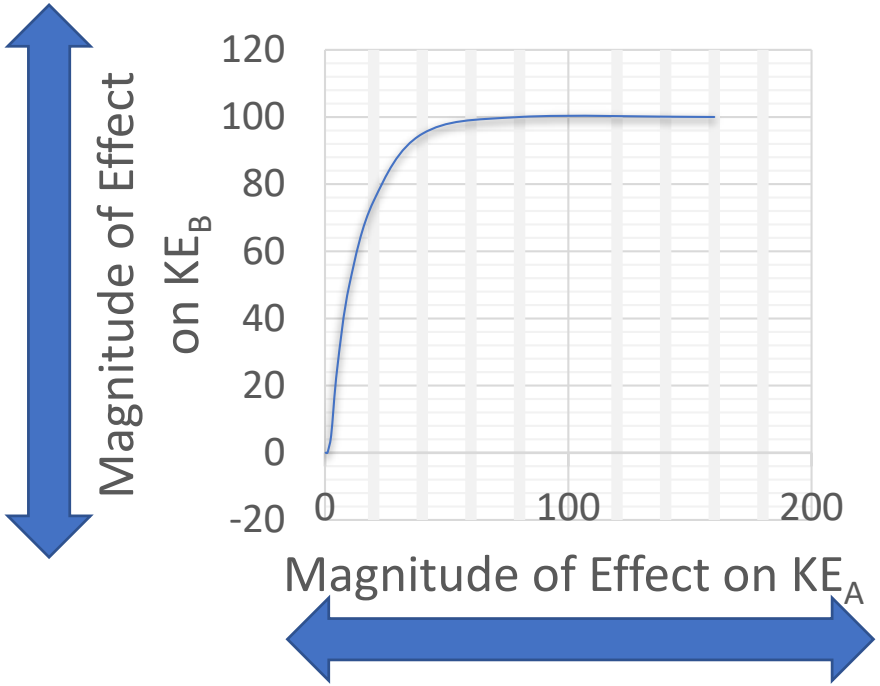
Allow for estimation of downstream effect from measured magnitude of effect on an upstream KE.



Conolly et al. Environ Sci Technol. 2017 Apr 18;51(8):4661-4672. doi: 10.1021/acs.est.6b06230.

Challenge: Experiments are rarely designed to capture R-R

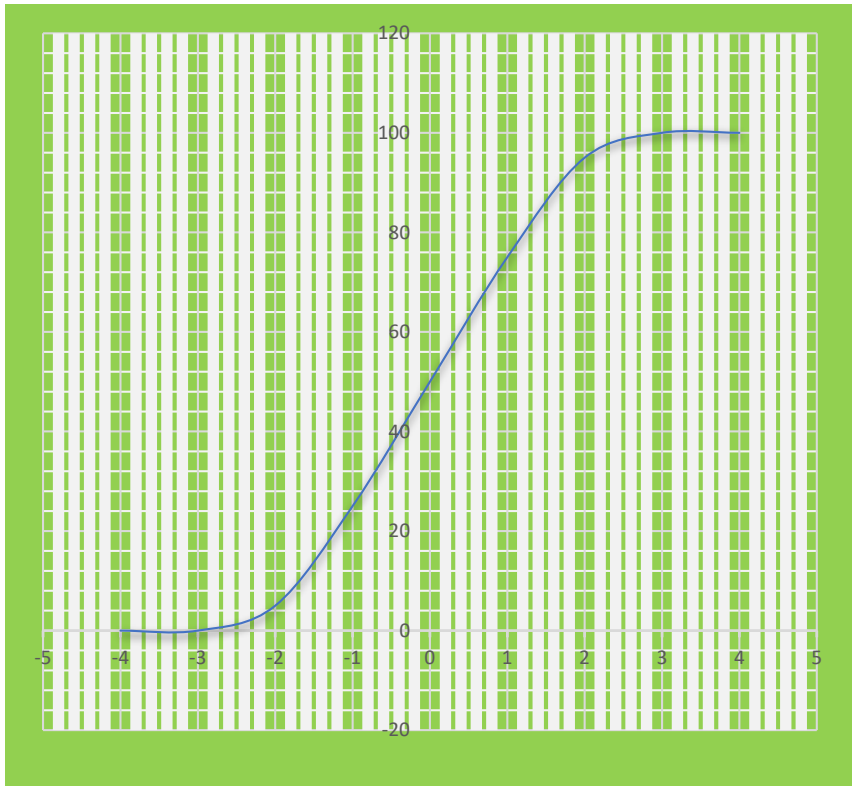
- Toxicology has traditionally focused on endpoint responses as a function of the concentration or magnitude/duration of stressor
- Rarely cover a broad range of perturbation in both upstream and downstream KEs
- Time-courses are needed to account for anticipated lag in time-to-effect as progress along the AOP.
- Measure multiple KEs, ideally in the same experiment.



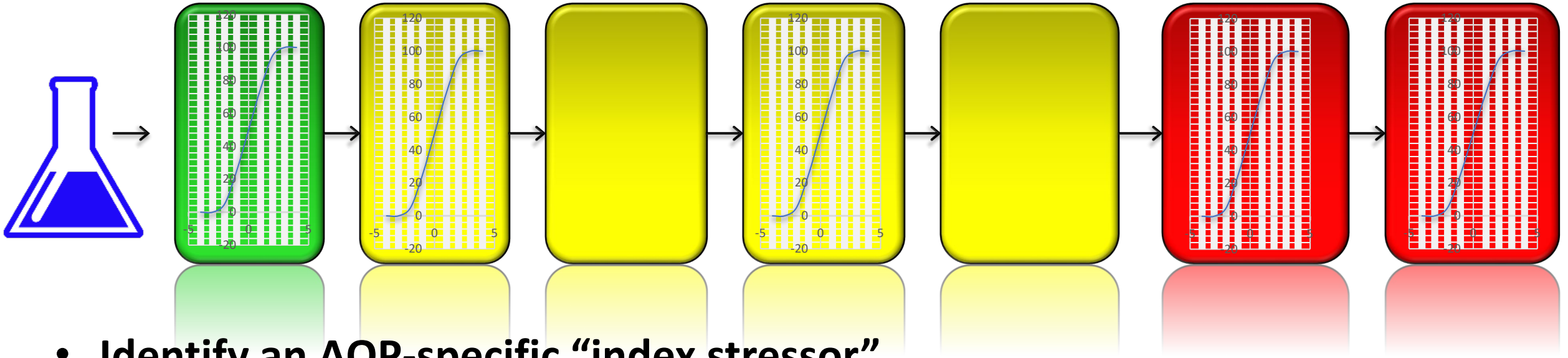
An Alternative/Complementary Approach

How can we leverage the types of data that are more readily available to support quantitative extrapolation along an AOP?

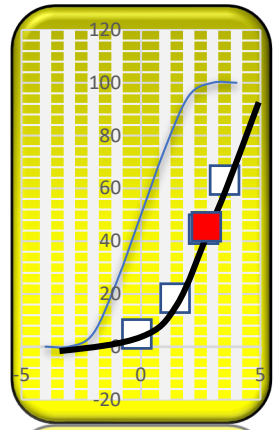
Recognizing, AOPs are not stressor-specific



An Alternative/Complementary Approach



- **Identify an AOP-specific "index stressor"**
  - Document concentration-response for "index stressor" across as many KEs as possible



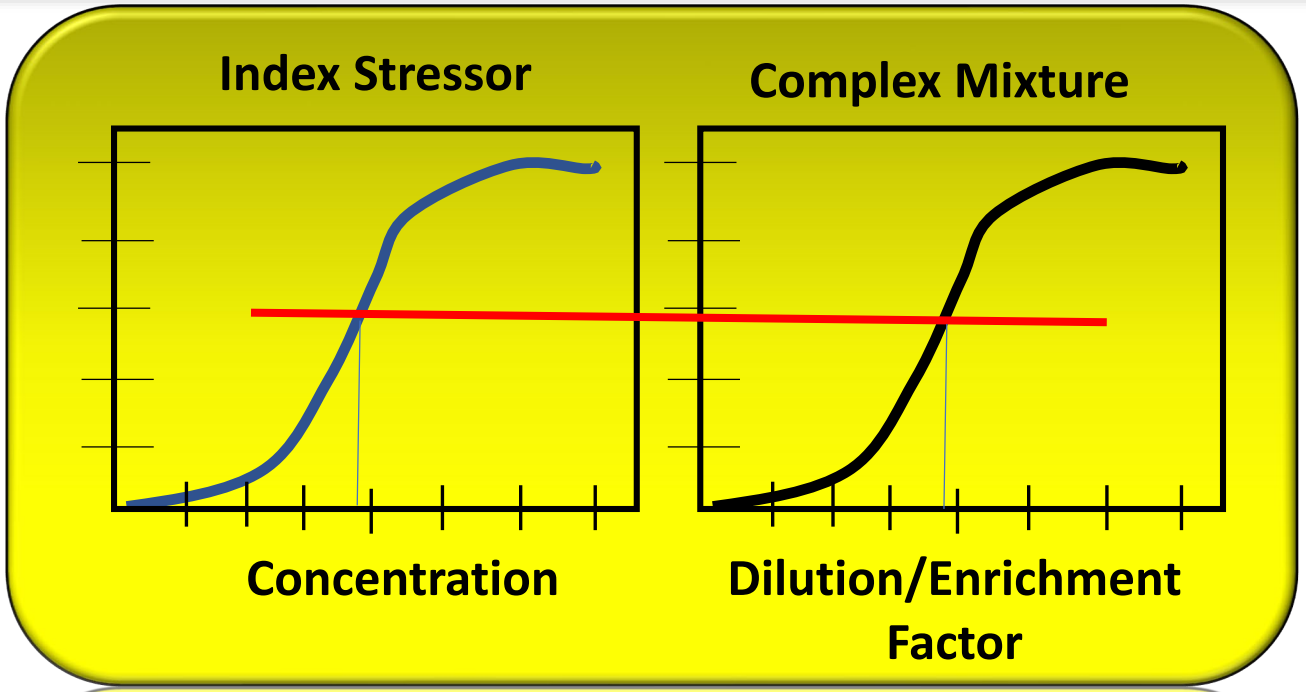
- For any new test chemical
  - Define relative potency at any one (or more) KEs along the pathway
  - May be based on full dose-response characterization or a single point estimate
  - Calculate "equivalent" concentration of index stressor
  - Extrapolate to dose response curves for index stressor at KEs farther down the pathway

An Alternative/Complementary Approach

Known components

Unknown components

Chemical Mixture



Biological response equivalence at same KE

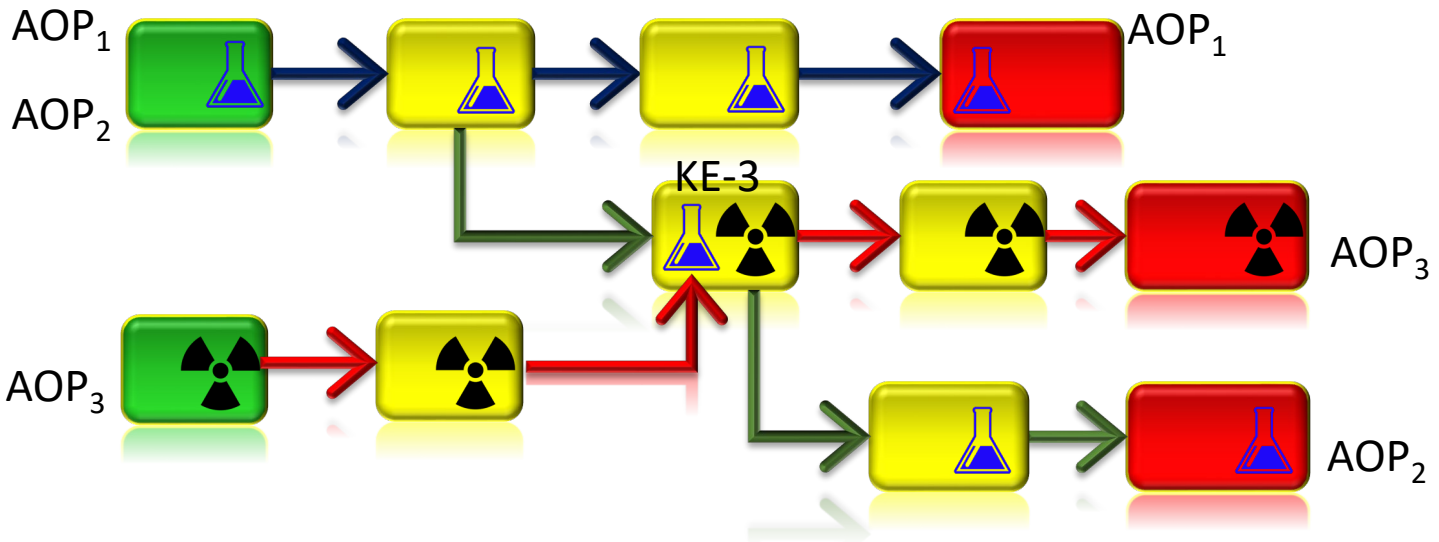
- Calculate Equivalency factor
- Extrapolate along AOP assuming same behavior as index stressor

An Alternative/Complementary Approach

Index stressors for different AOPs can be interconverted at any KE for which the concentration-response has been defined for both.

AOPs 1 and 2 developed using a chemical index stressor

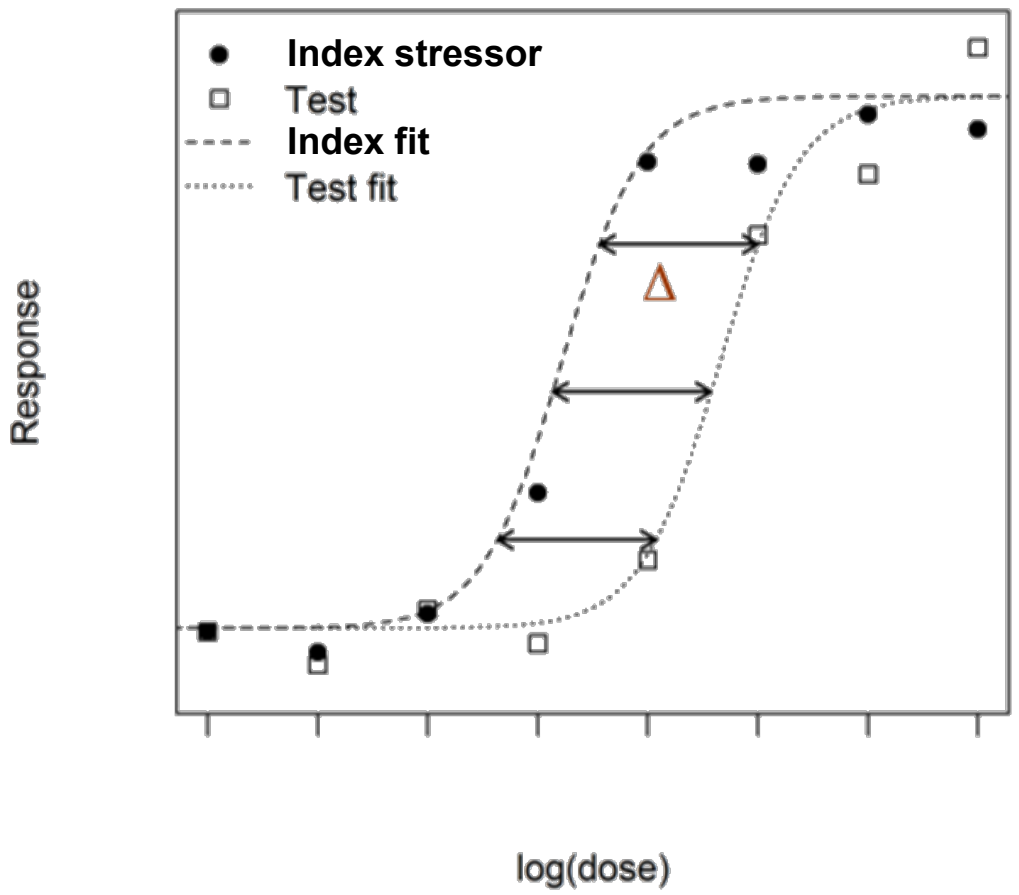
AOP 3 developed using a radiation index stressor



AOPs 2 and 3 can be compared against either the chemical-based threshold or the radiation-based threshold

New opportunities for multiple stressor evaluations

Precedent for the approach: Relative potency factors



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

- 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/RPF approach include:

- The individual compounds all act through the same biologic 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

OK
Possibly
Unlikely*
Stressor-dependent

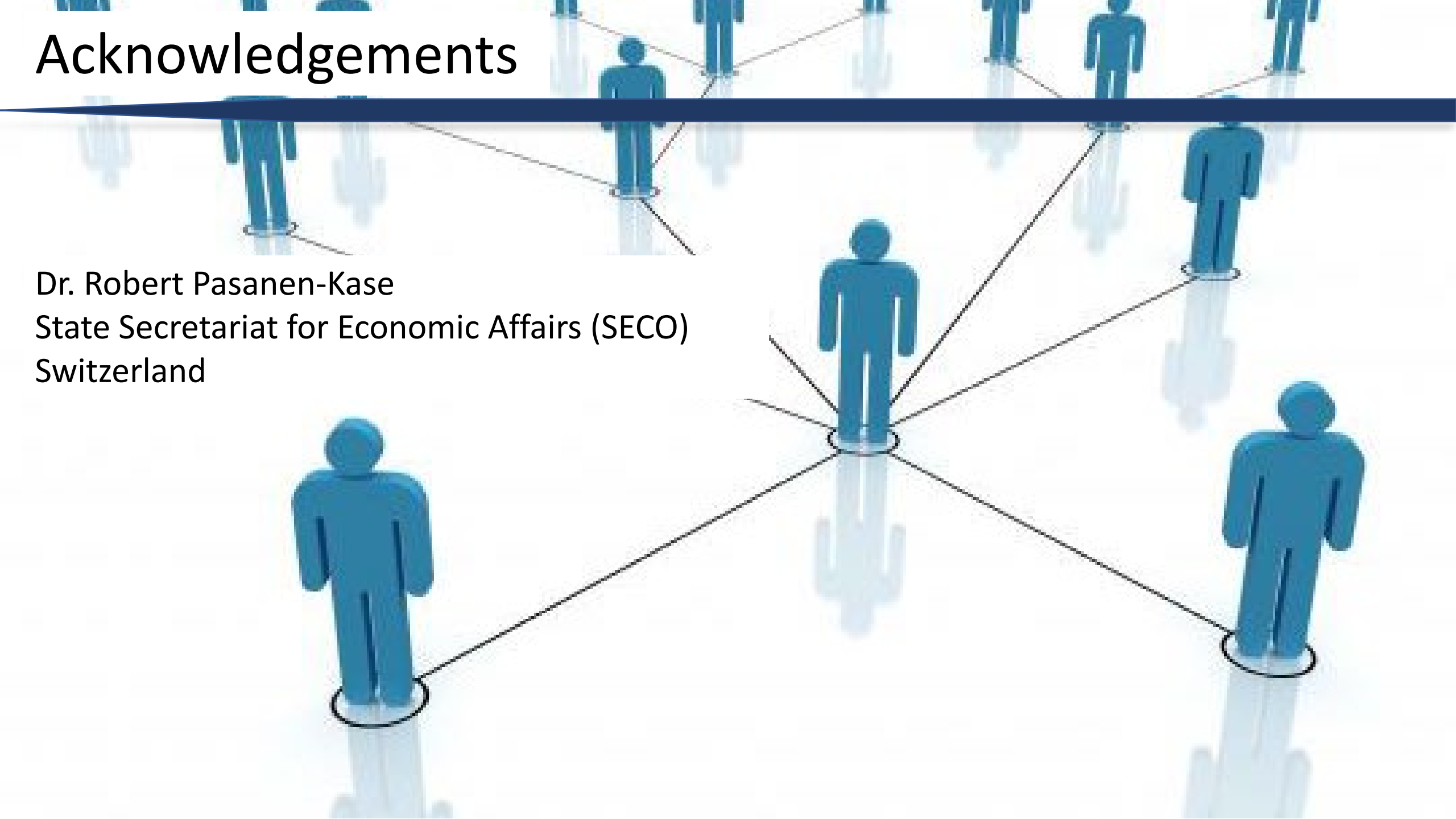
*Uncertainty associated with violating can be estimated

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.

Conclusions

- Error and uncertainty can be expected with deviation from the assumptions of the TEF approach – but probably no worse than current qAOP approaches.
- Incorporation of identification and dose-response characterization for index stressors into the AOP framework has potential to significantly enhance quantitative application.
- Provides the potential to compare relevant effects of radiation against established thresholds for chemicals or vice versa and facilitates multi-stressor evaluations.
- Case studies to pilot the concept are needed to inform possible incorporation into the AOP framework

Acknowledgements



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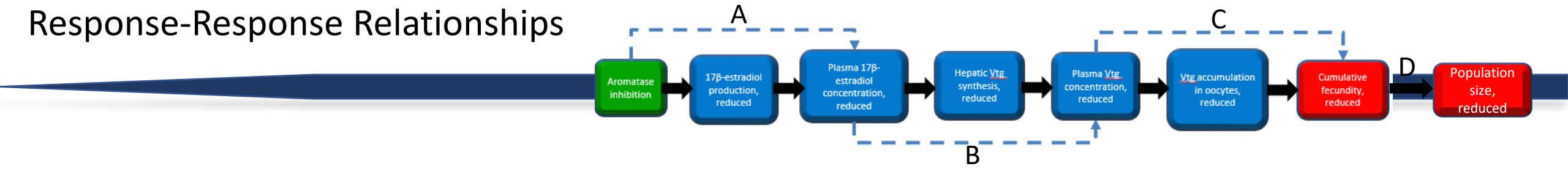
The kinds of data we get from “new approach methodologies” (NAMs)

The kinds of effects we care about.

https://aopwiki.org/aops/218

Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity

Response-Response Relationships



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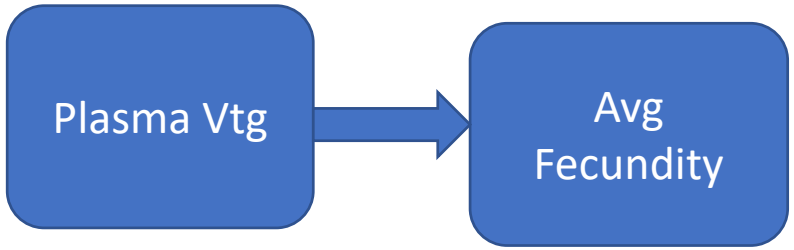
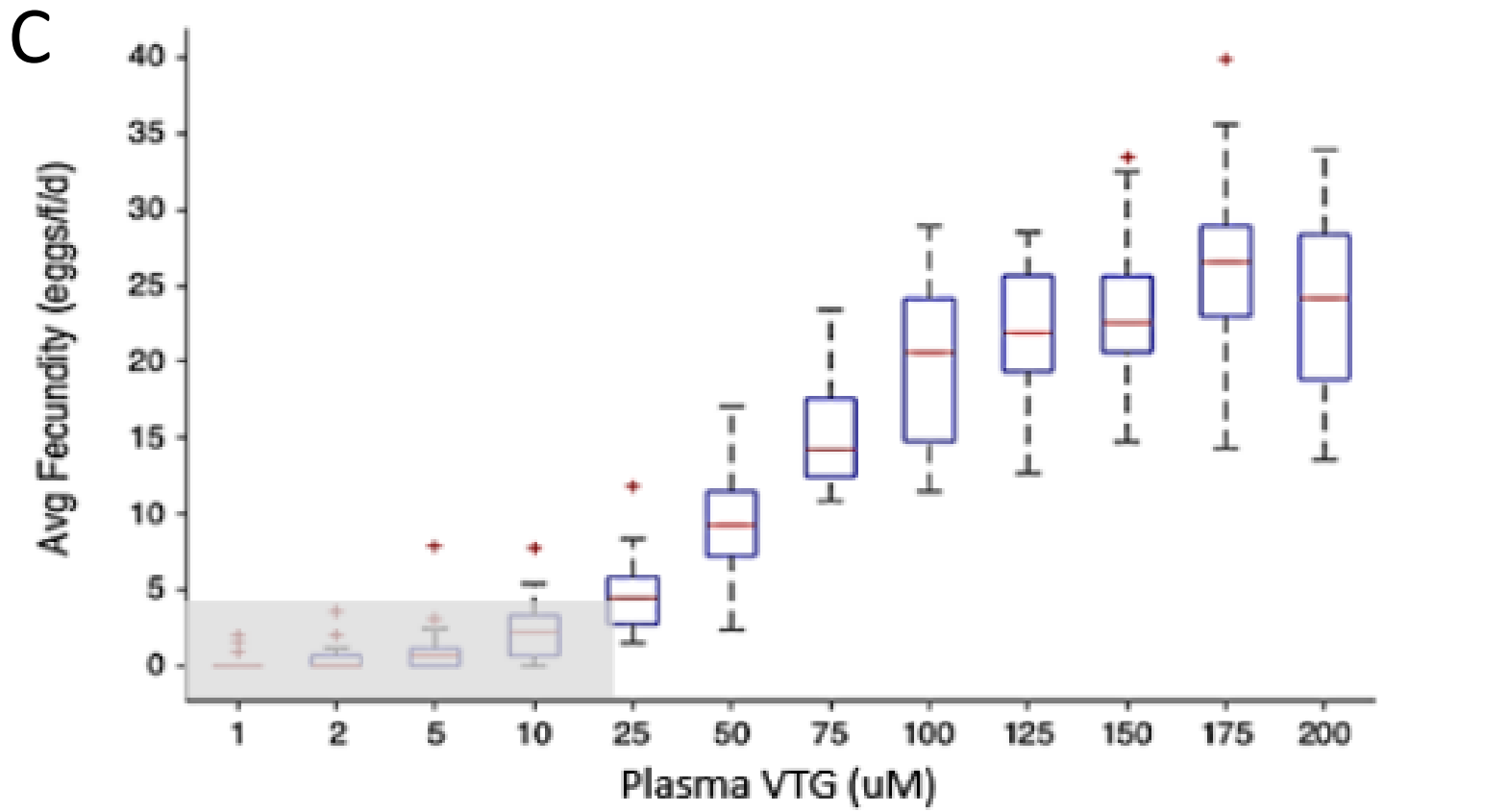


In vivo

In vivo

$$y = \frac{24.7714}{1+exp(\frac{64.4184-x}{24.7923})}$$



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Prospects

- Deviations from assumptions of TEF approach will yield inaccuracies
  - Quite likely
- The generalizability of response-response relationships for different species, stressors, etc. is also relatively uncertain.
  - QAOP-based predictions may not be any better
- Assembly of data to support an “index stressor” approach is likely more achievable in the near terms than robust and generalizable R-R-Rs.