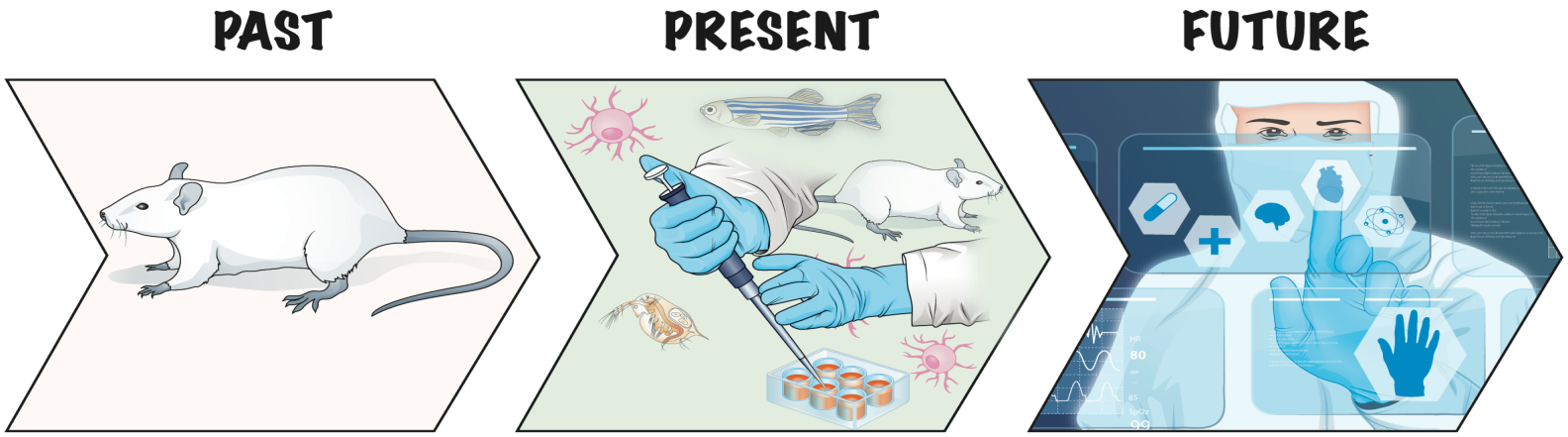


Approaches and tools for AOP assembly and an example of a Bayesian network approach to predicting steatosis

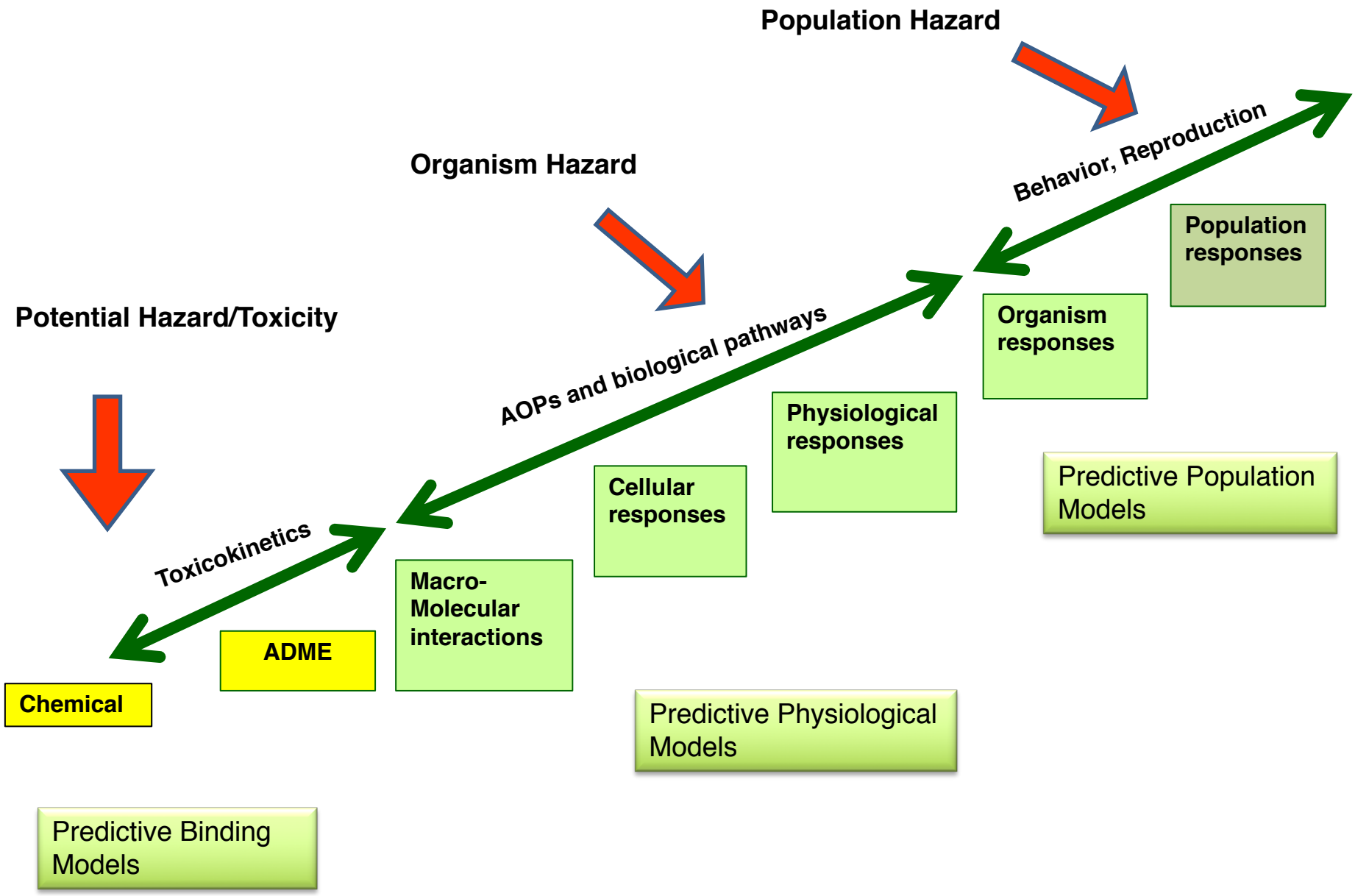
Natàlia G Reyero Vinas
US Army ERDC

SOT, Baltimore (MD)
March 13th 2019

Outline

1. AOP Development
2. Modular Approach
3. AOP Networks

Development of tools for rapid hazard assessment (AOPs)



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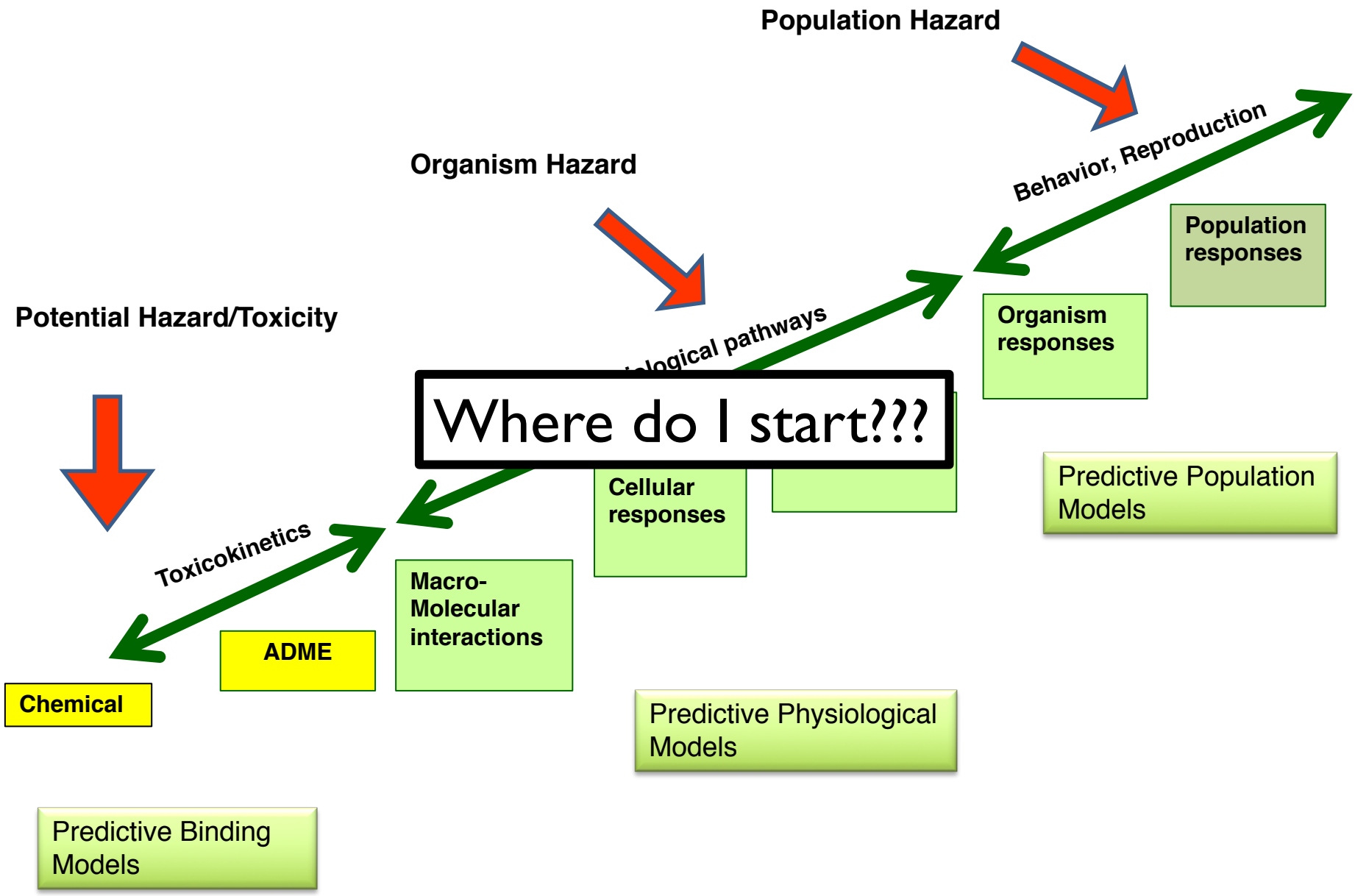
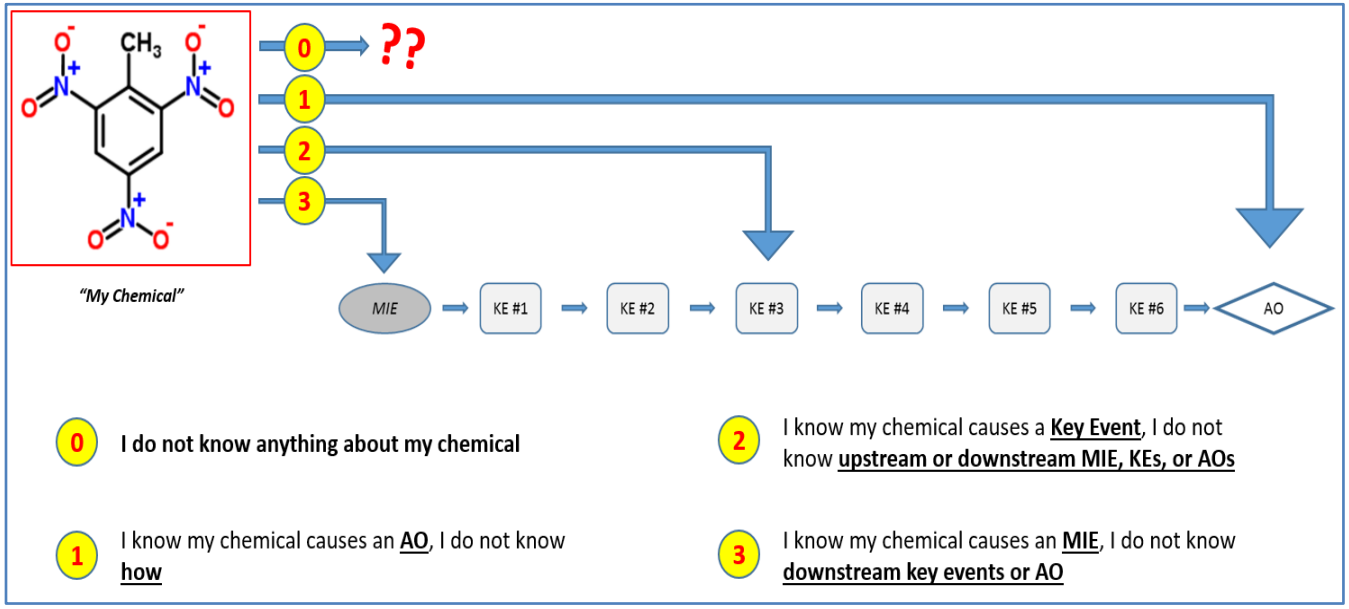


Defining the Initial Knowledge State: AOPERA (teaser!)

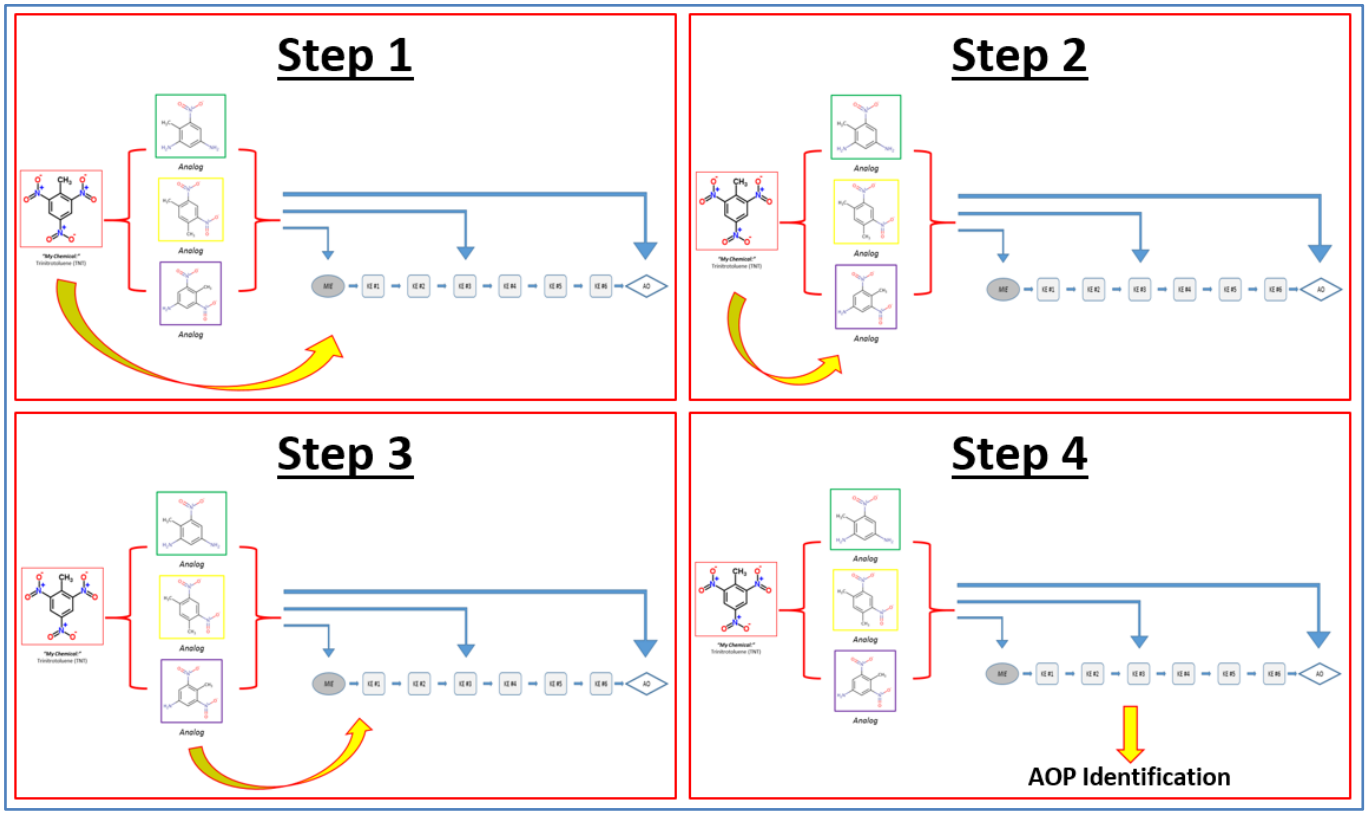
- Level 0 – the practitioner does not know anything about the toxicity of their chemical.
- Level 1 – the practitioner knows that their chemical causes an AO but does not know the upstream MIE or KEs.
- Level 2 – the practitioner knows that their chemical causes a KE but does not know upstream or downstream KEs or the MIE and AO.
- Level 3 – the practitioner knows their chemical causes an MIE but does not know the downstream KEs or AO.



Rycroft et al, in prep.

Initiating the Process

- Step 1 – link the uncharacterized chemical directly to MIEs, KEs, or AOs.
- Step 2 – identify analogs for the uncharacterized chemical.
- Step 3 – link the characterized chemical (initial chemical if characterized, analog if initial chemical is uncharacterized) to MIEs, KEs, or AOs.
- Step 4 – identify AOPs that contain the MIEs, KEs, or AOs that were found in Steps 1 and 3.



Summary of resources that support the proposed four-step process and their applicability to each step

Name	Step 1	Step 2	Step 3	Step 4
VEGA QSAR	X	X	X	
OECD QSAR Toolbox	X		X	
Toxicity Estimation Software Tool (TEST)	X	X	X	
OncoLogic	X		X	
EPA's *New Chemical Categories*	X		X	
Mcule	X	X	X	
Chemistry Dashboard ("CompTox")		X		
Analog Identification Methodology (AIM)		X		
ChemSpider		X		
eMolecules		X		
PubChem		X		

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ChemSpider		X		
eMolecules		X		
PubChem		X		

STAY TUNED!

AOPs

- AOPs are a way of organizing information and transferring knowledge
- Help to harness and utilize existing knowledge
- AOPs can help more effectively use HTS data in regulatory decision-making
- AOPNs might be more predictive of real-life scenarios

Understanding mechanistic relationships is necessary but not sufficient

Qualitative AOPs as Conceptual Models are a Good Start

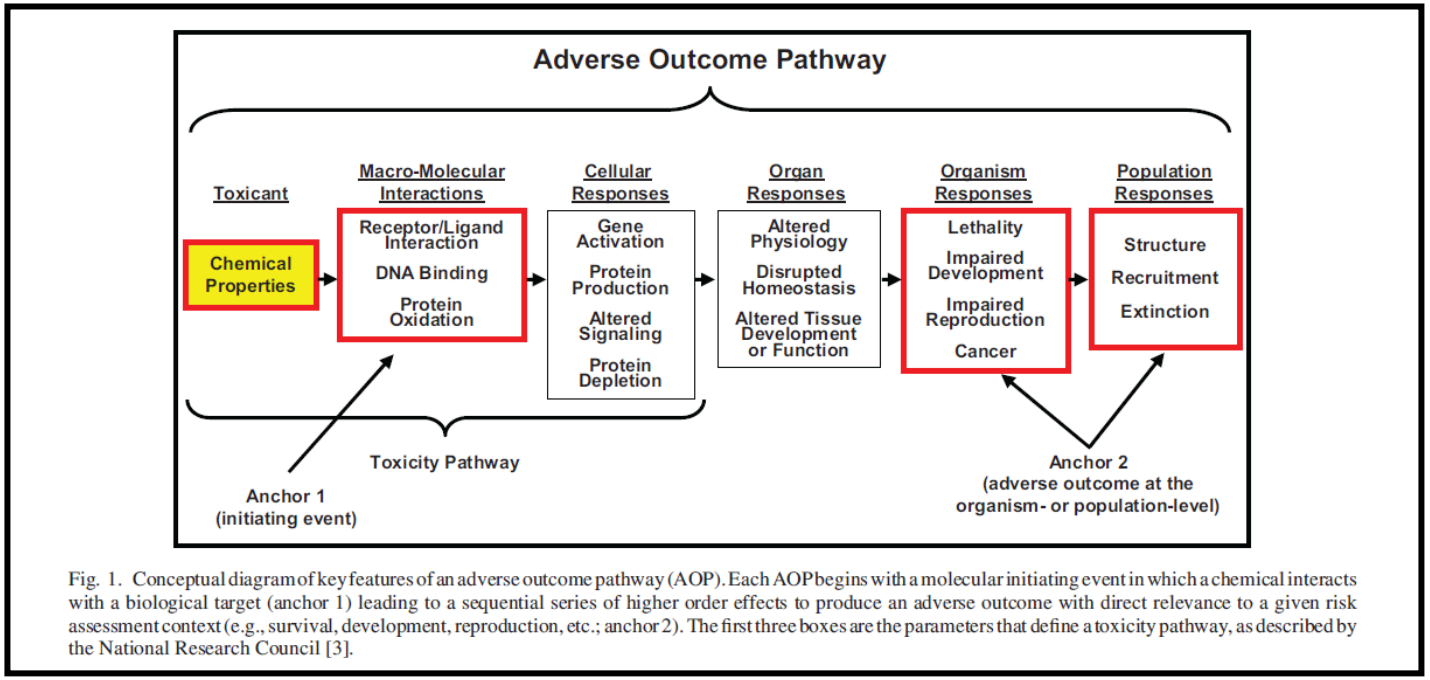


Fig. 1. Conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects to produce an adverse outcome with direct relevance to a given risk assessment context (e.g., survival, development, reproduction, etc.; anchor 2). The first three boxes are the parameters that define a toxicity pathway, as described by the National Research Council [3].

But we need more than logical relationships and proxies

V. Forbes

Challenges for QAOPs

- Linkages need to be both mechanistic and quantitative
- Need to know if/which details can be ignored and/or incorporated in (complex) models
- Need to include feedbacks
- Inter-species extrapolation is still a major challenge

V. Forbes

Understanding mechanistic relationships is necessary but not sufficient

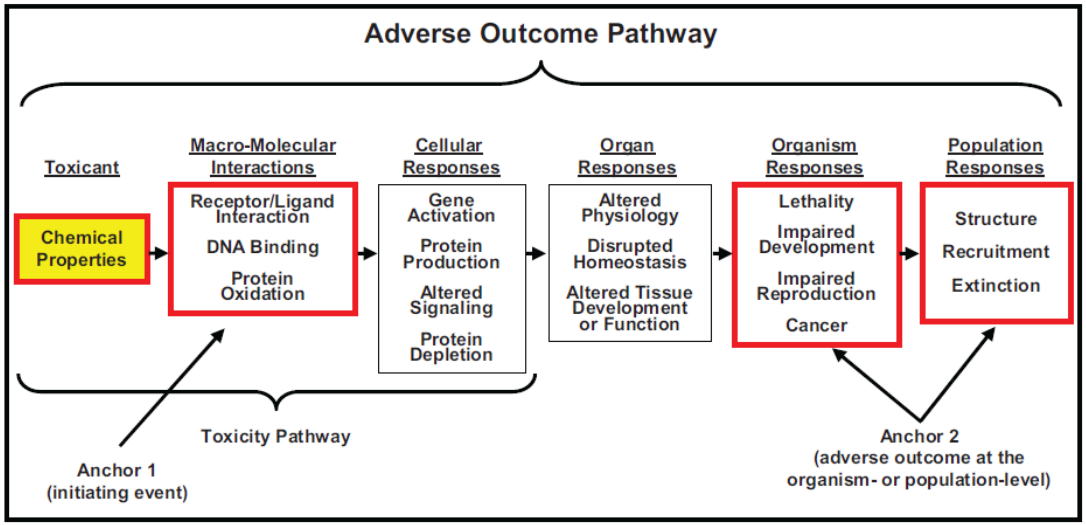


Fig. 1. Conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects to produce an adverse outcome with direct relevance to a given risk assessment context (e.g., survival, development, reproduction, etc.; anchor 2). The first three boxes are the parameters that define a toxicity pathway, as described by the National Research Council [3].

Regardless, SOME information is better than NO information

Just need to be aware of UNCERTAINTY

- Quantitative, predictive AOPs are necessary for ***screening emerging contaminants*** and potential substitutes to inform their prioritization for testing.

- A ***modular approach*** for assembly of quantitative AOPs, based on existing knowledge, would allow for rapid development of biological pathway models to screen contaminants for potential hazards and prioritize them for subsequent testing and modeling.

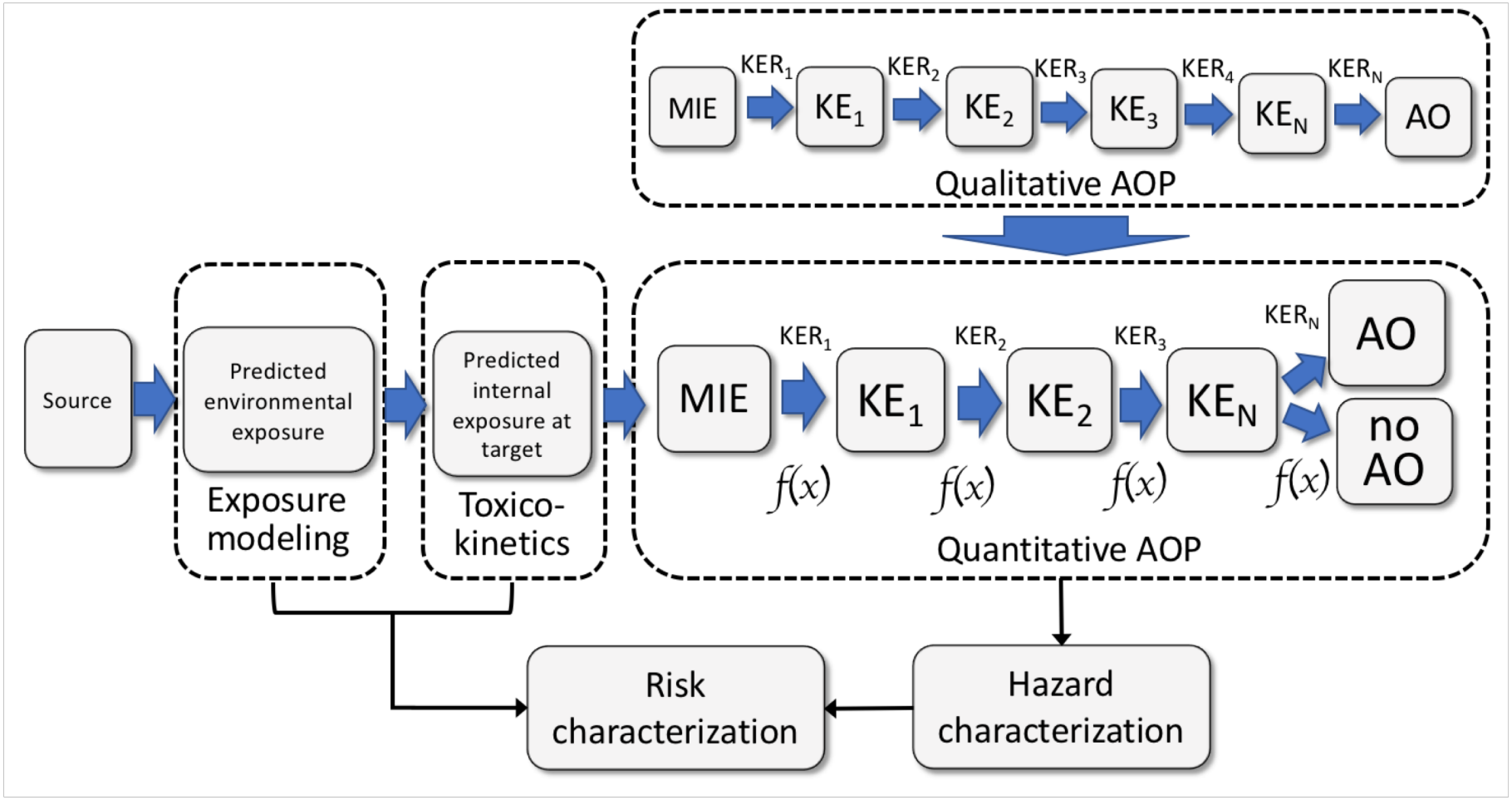
- For each pair of KEs, a quantitative KE relationship (KER) can be derived as a *response-response function* or a *conditional probability matrix* describing the anticipated change in a KE based on the response of the prior KE.

- This transfer of response across KERs can be used to assemble a quantitative AOP.

- Here we demonstrate the use of proposed approach in two cases: inhibition of cytochrome P450 aromatase leading to reduced fecundity in fathead minnows and ionic glutamate receptor mediated excitotoxicity leading to memory impairment in humans

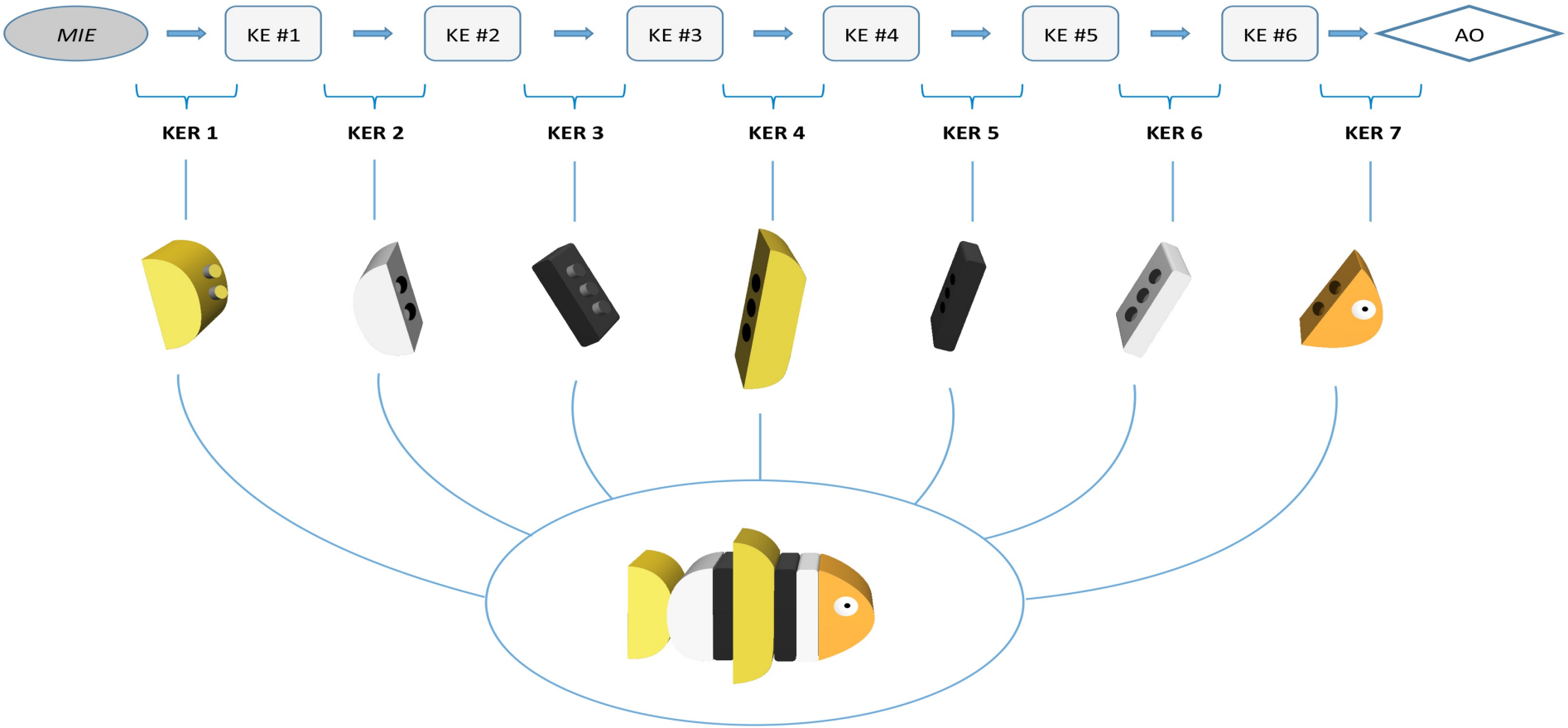
- This approach to simplistic, modular AOP models has wide applicability for rapid development of biological pathway models.

Translation of an AOP into a quantitative and computational AOP model



A qAOP captures response-response relationships between Key Events

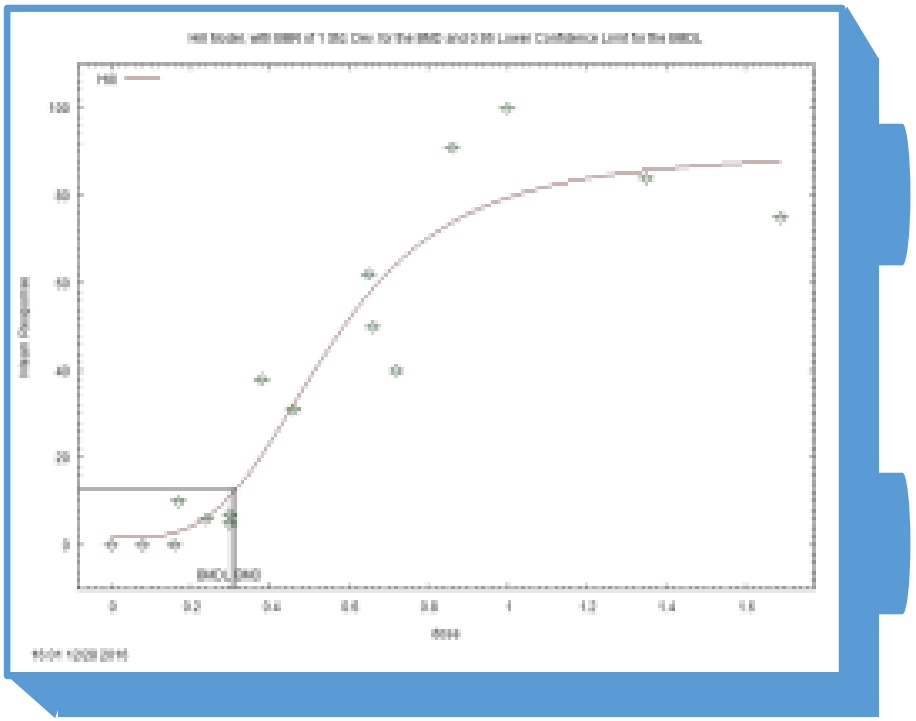
A modular approach



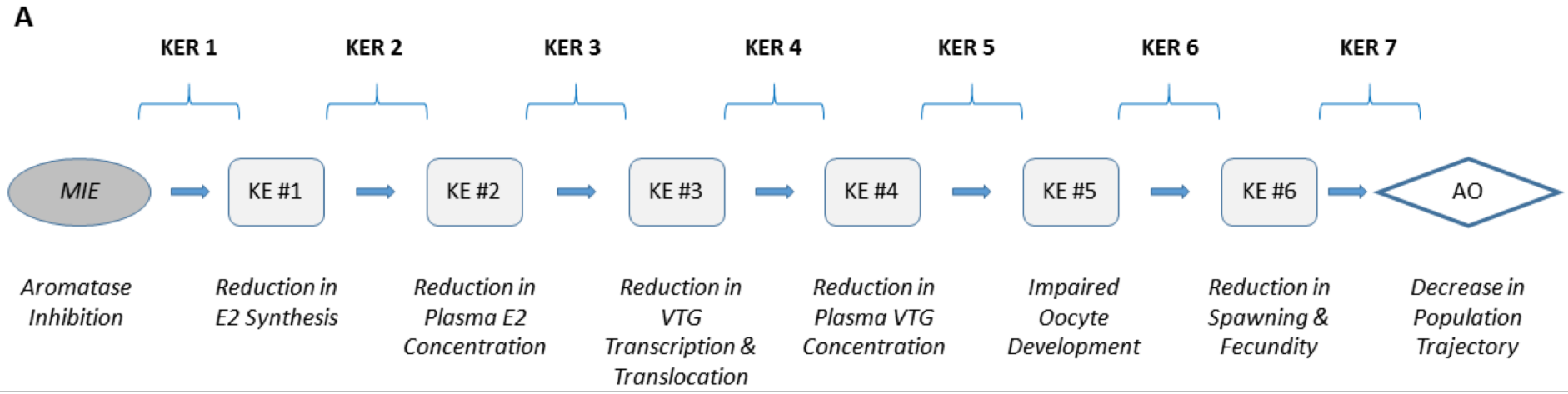
The ideal approach would allow construction of different AOPs from an assortment of modules describing response relationships between KEs. This modular approach, although coarser and more uncertain, would facilitate rapid prototyping and updating of both modules and complete AOP models, making it better-suited for screening and prioritization than the more detailed and resource-intensive mechanistic models referenced above.

A quantitative relationship between key events can be used as a piece of a model to chain together the activity of a biological pathway into a simple qAOP model.

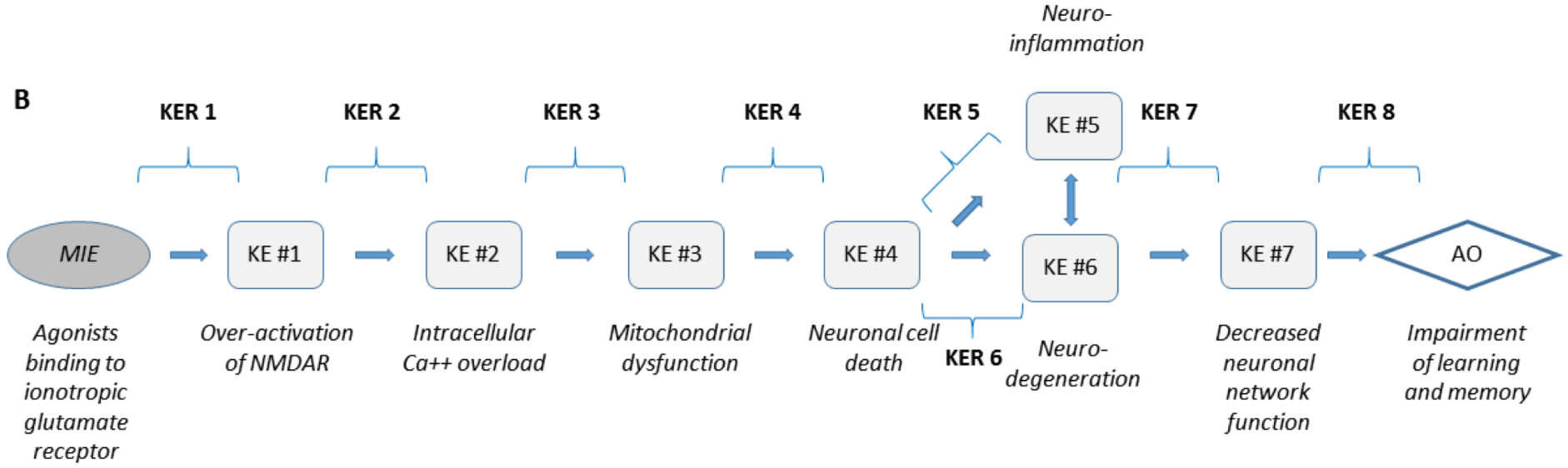
R-R relationship



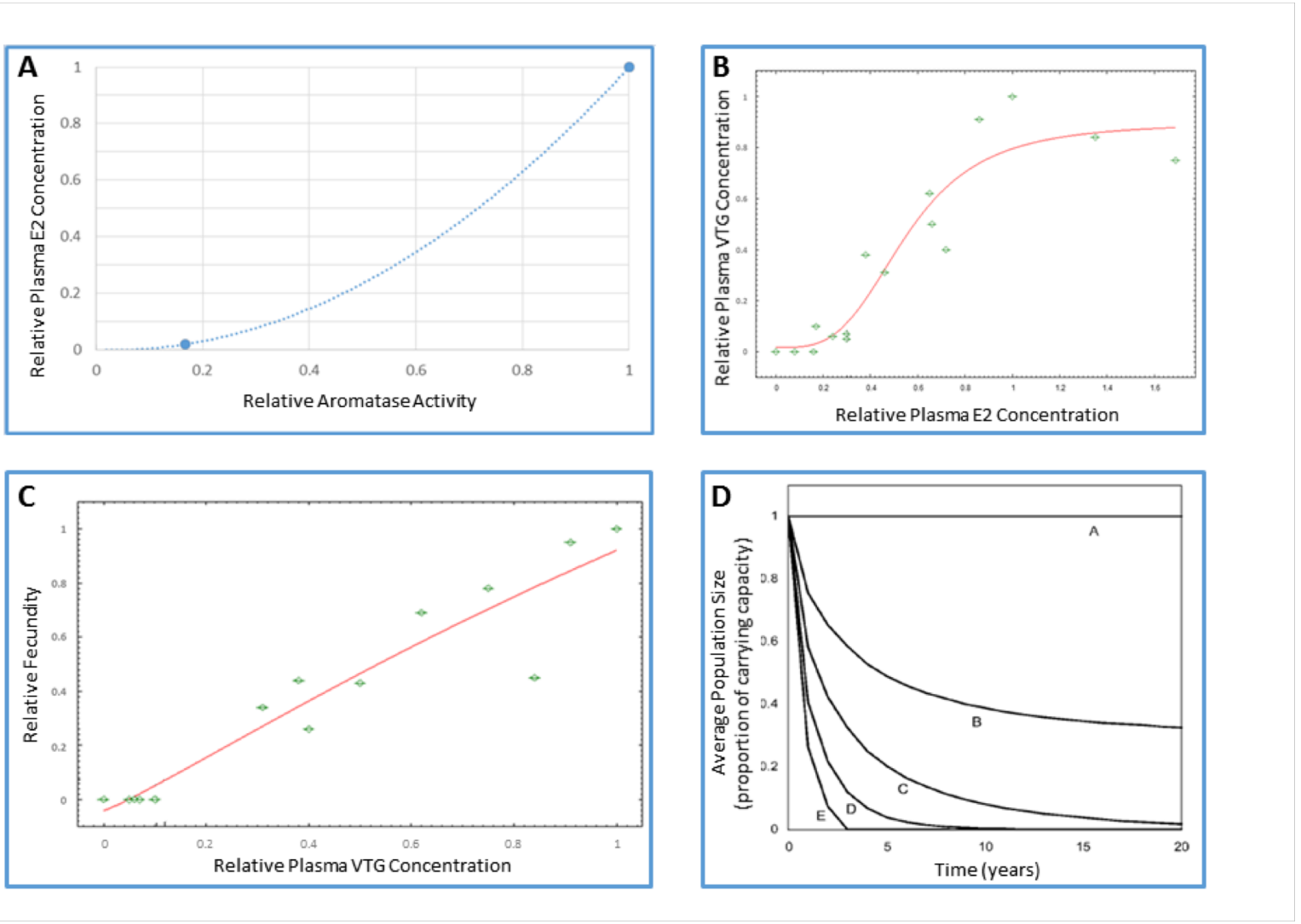
Aromatase inhibition leading to reproductive dysfunction in fish



Excitation of NMDA receptors leading to impairment of memory



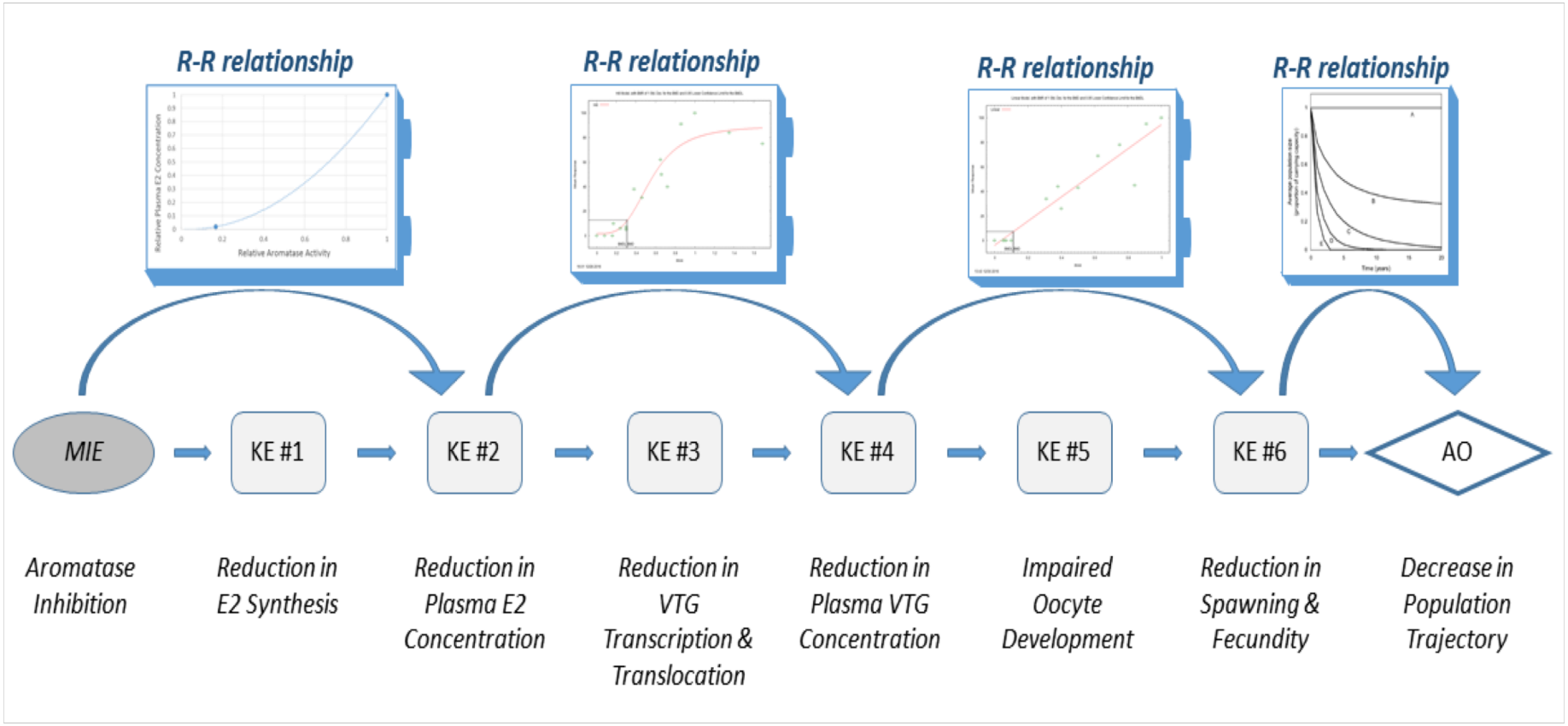
Response-response (R-R) relationships used to establish qKERs describing the influence that each KE has on its dependent KE



Aromatase inhibition leading to reproductive dysfunction in fish

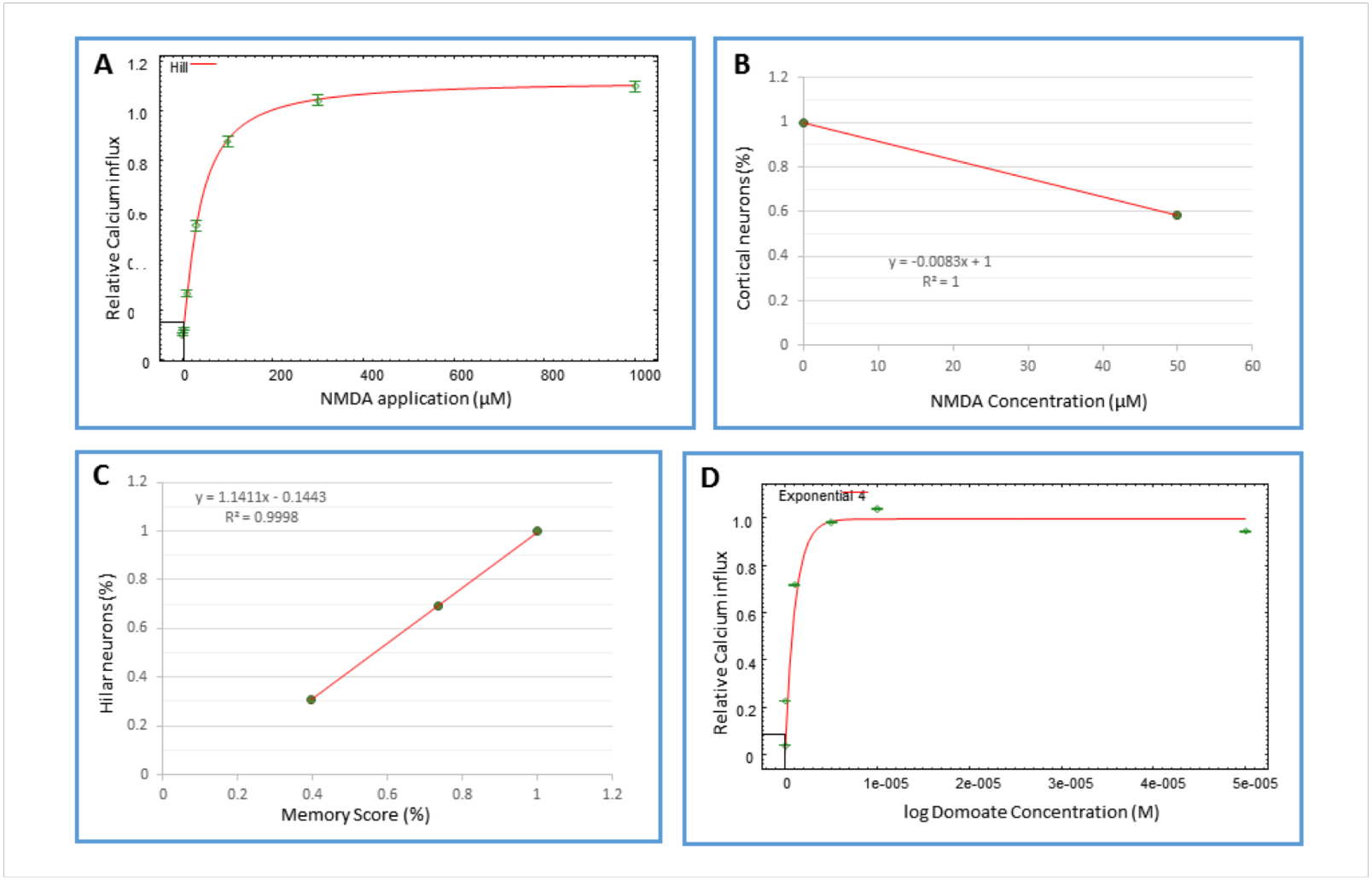
Foran et al 2018

Modular R-R relationships were used to transfer "activation" along the AOP for aromatase inhibition leading to reproductive dysfunction in fish, resulting in a prediction of the change in fish population.



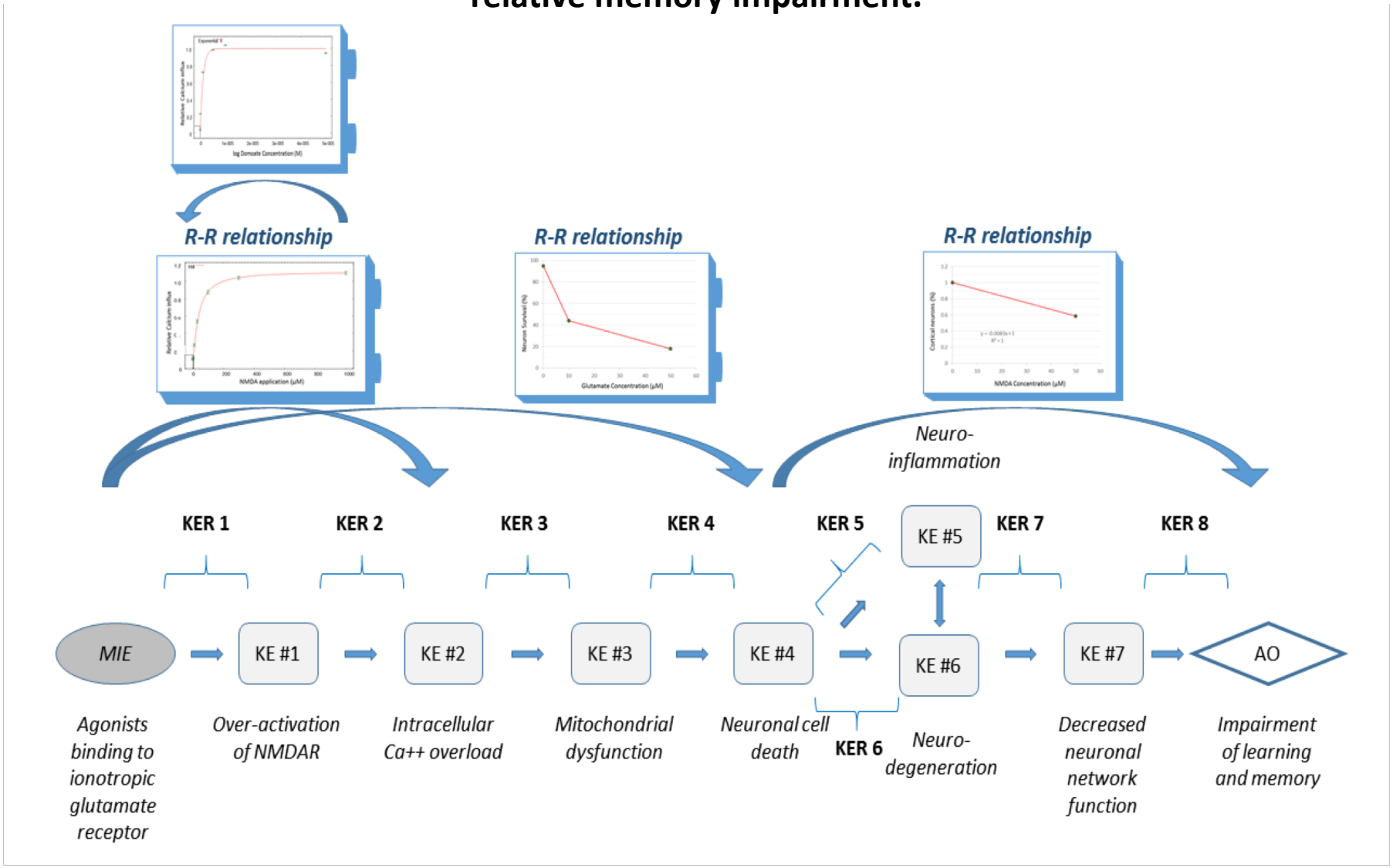
Foran et al 2018

Response-response relationships estimating qKERs that reflect the influence that a KE has on its dependent KE for the pathway from glutamate agonism to impaired memory



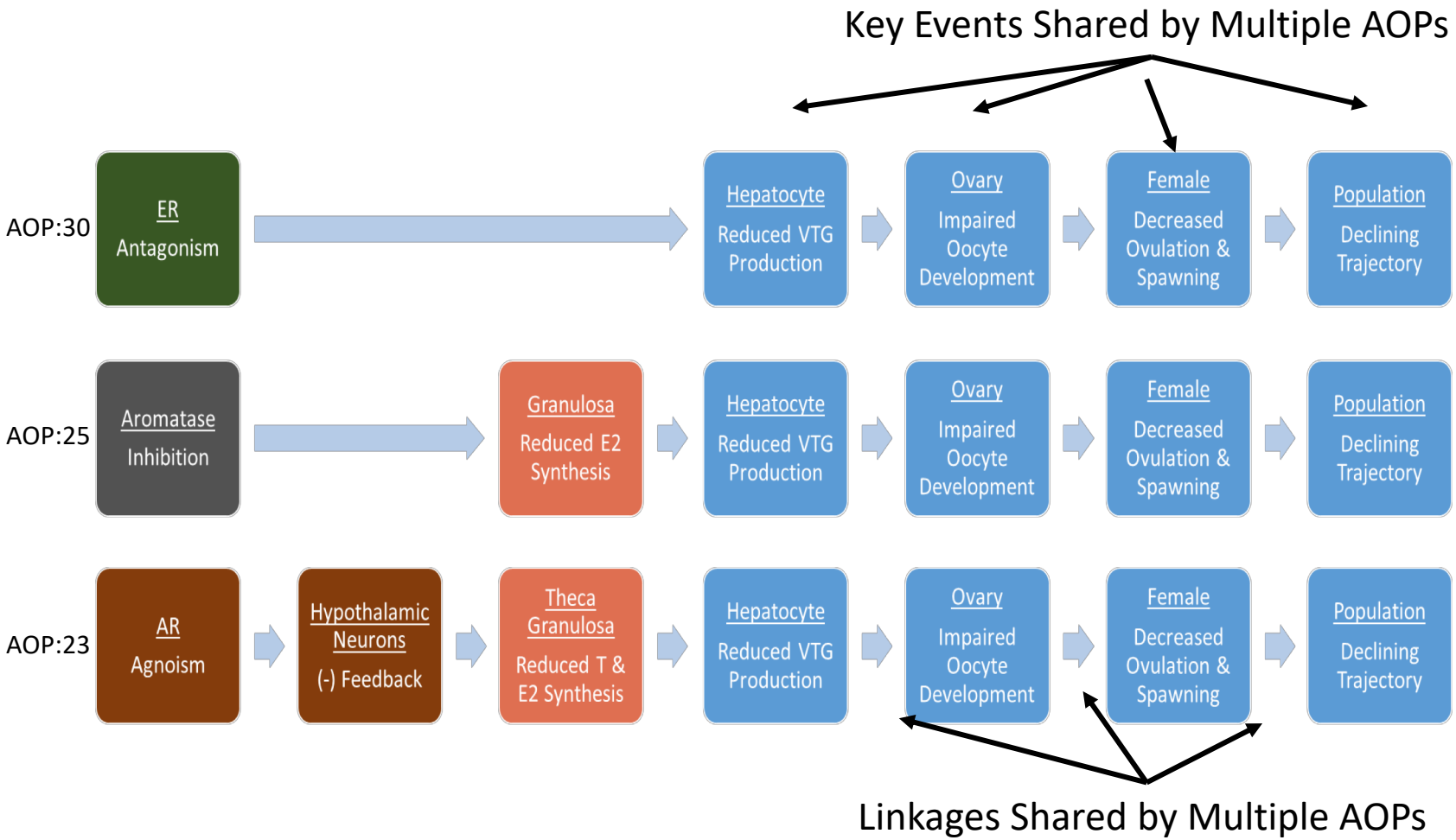
Foran et al 2018

Modular R-R relationships were used to transfer "activation" along the AOP for glutamate agonism through activation of NMDAR resulting a loss of neurons and a prediction of relative memory impairment.



Foran et al 2018

AOPs Form Networks Through Shared Key Events

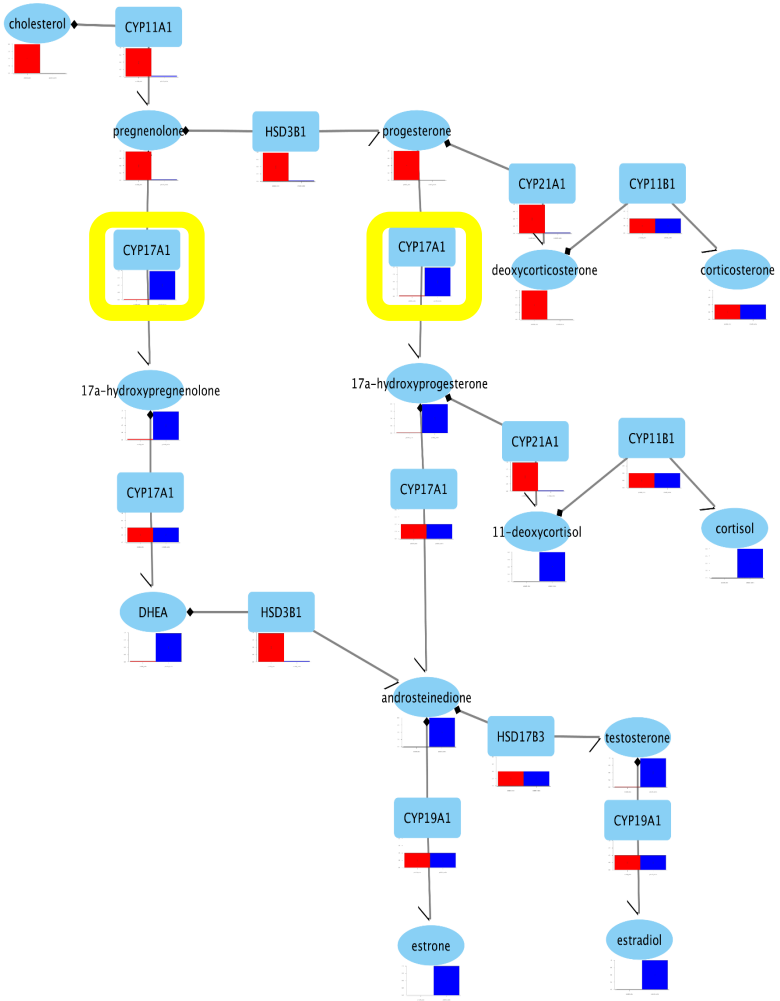


Courtesy of Dan Villeneuve

Modeling AOP networks for hazard screening

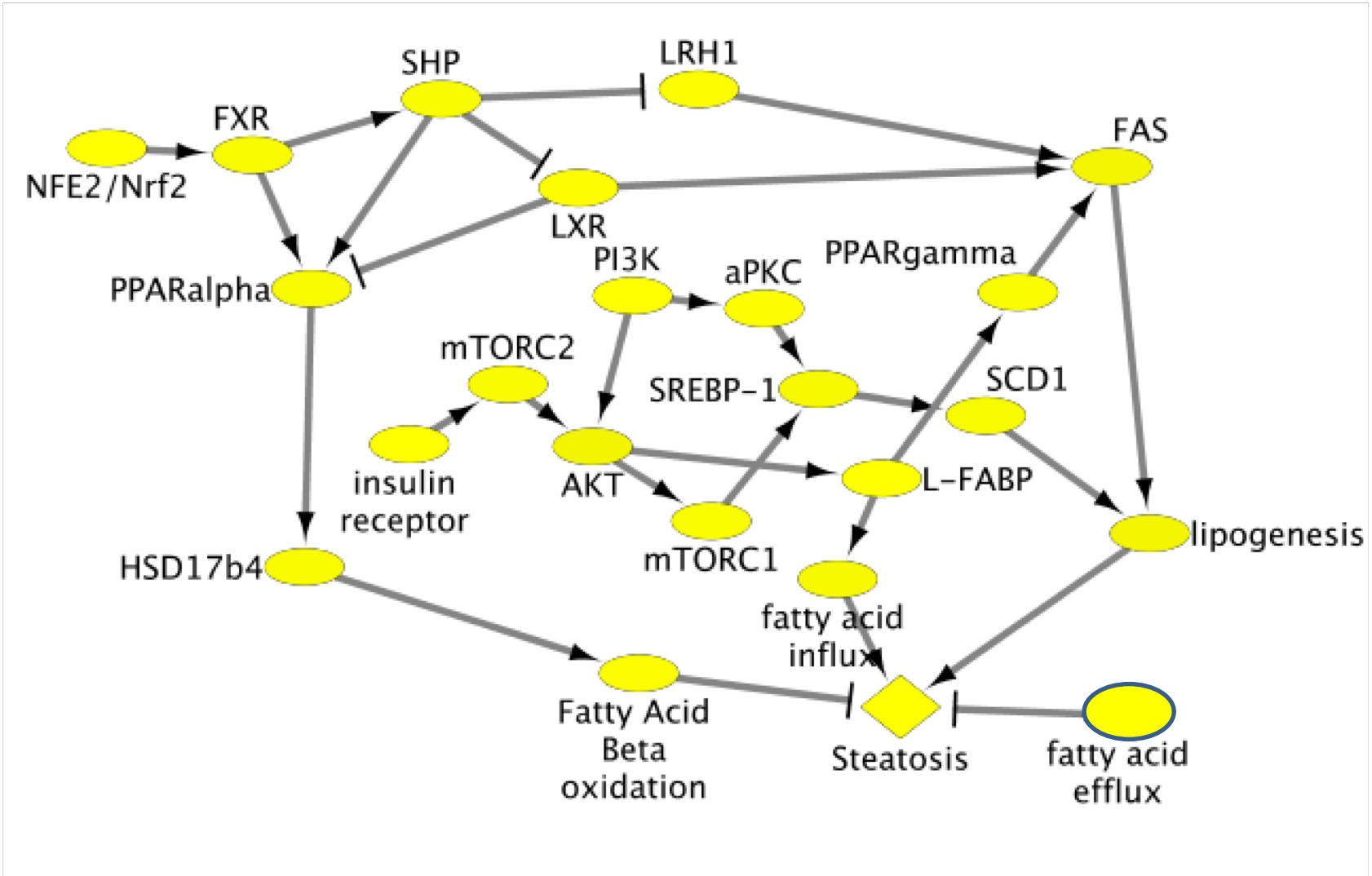
adverse outcome pathway bayesian networks

predict probability of adverse outcomes



L. Burgoon

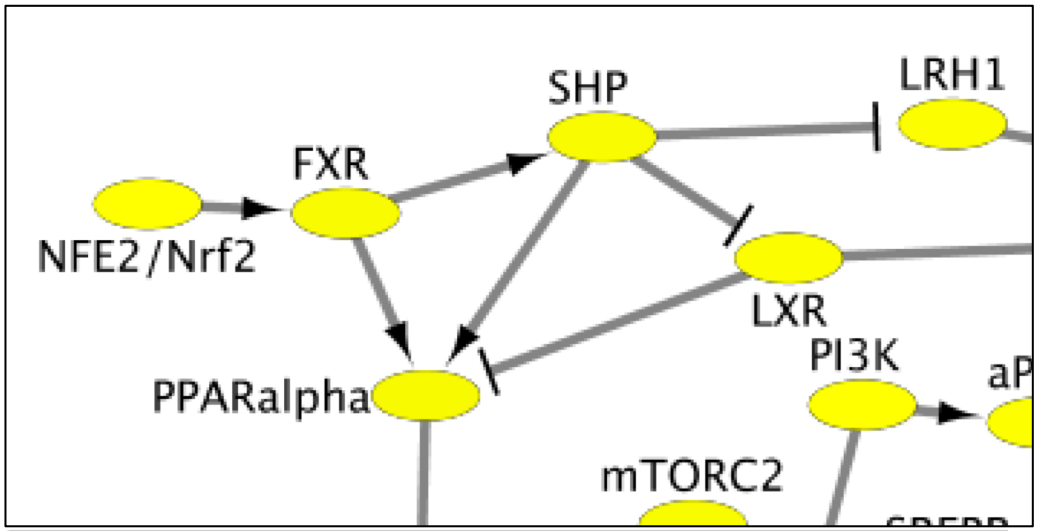
Adverse Outcome Pathway network for Liver Steatosis



Derived from Angrish et al., 2016; Burgoon et al., 2016

Bayesian network modeling

Causal network



Probability table

Nrf2

Nrf2	
Active	Inactive
0.50	0.50

FXR

Nrf2	FXR	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

SHP

FXR	SHP	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

LRH1

SHP	LRH-1	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

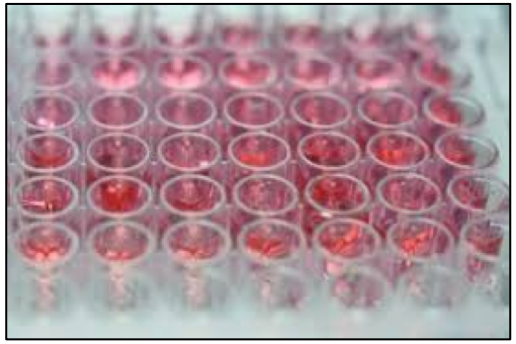
LXR

SHP	LXR	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

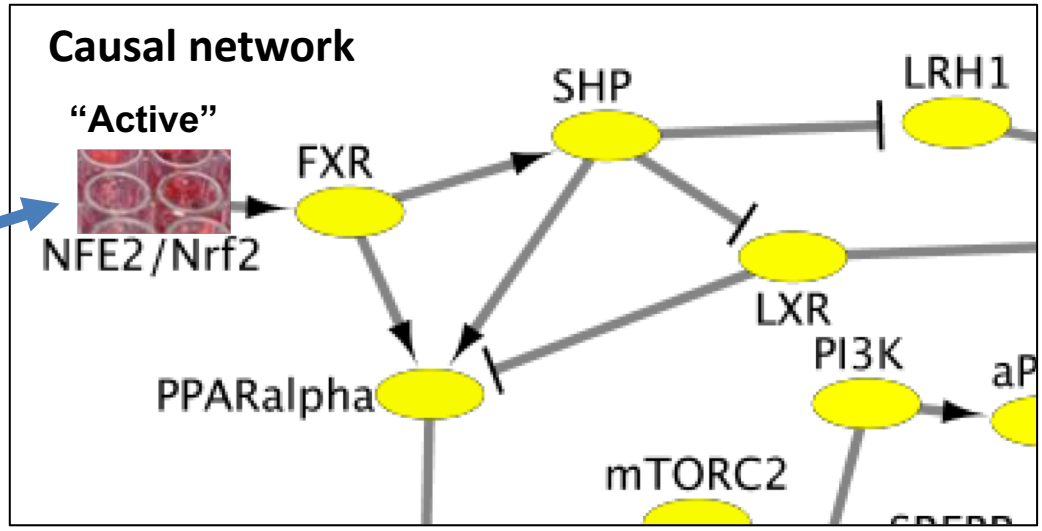
PPARalpha

FXR	SHP	LXR	PPAR-a	
			Active	Inactive
Active	Active	Active	0.50	0.50
Active	Active	Inactive	0.95	0.05
Active	Inactive	Active	0.05	0.95
Active	Inactive	Inactive	0.95	0.05
Inactive	Active	Active	0.50	0.50
Inactive	Active	Inactive	0.95	0.05
Inactive	Inactive	Active	0.05	0.95
Inactive	Inactive	Inactive	0.50	0.50

Bayesian network modeling



In vitro assay results can be used to determine activity of an event



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SHP

FXR	SHP	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

LRH1

SHP	LRH-1	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

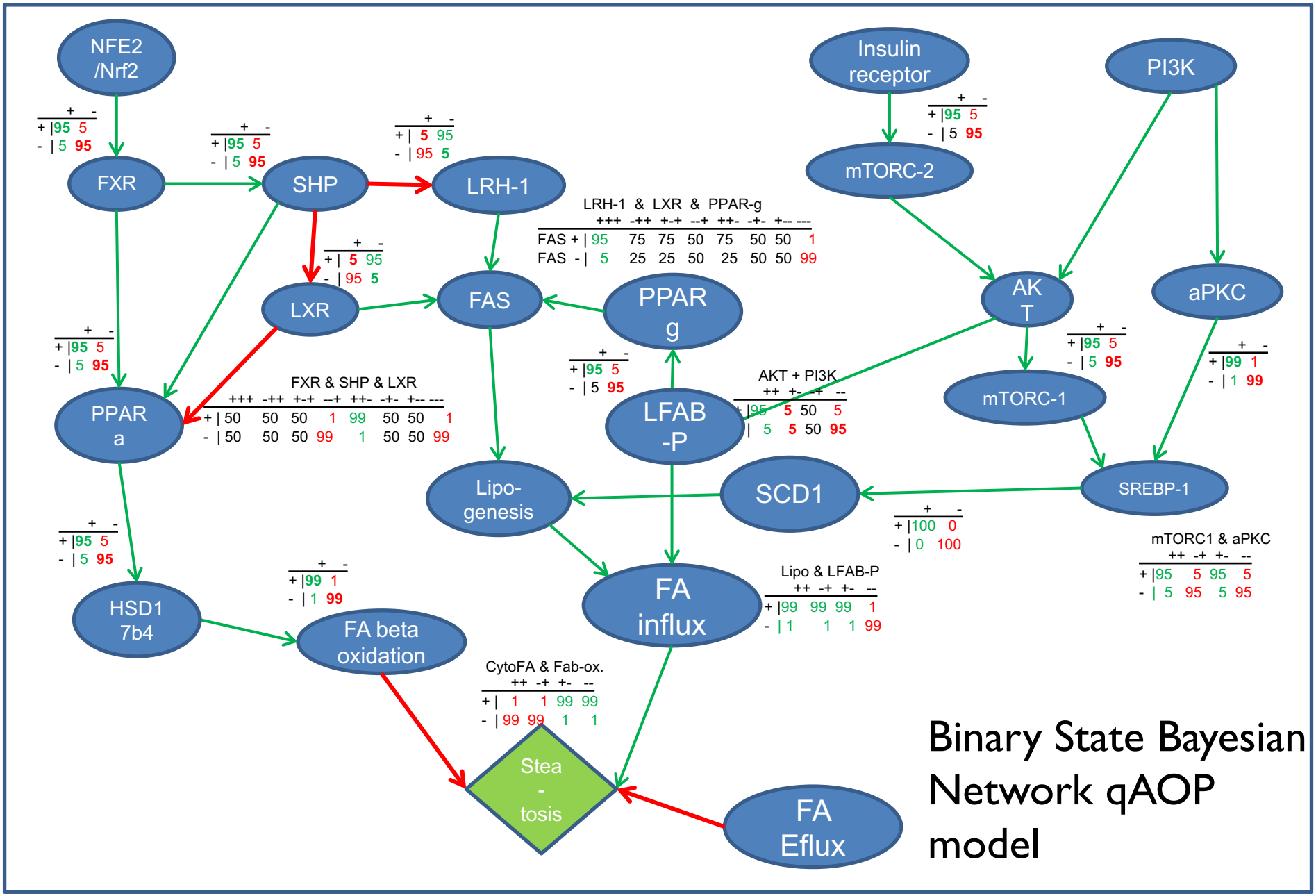
LXR

SHP	LXR	
	Active	Inactive
Active	0.95	0.05
Inactive	0.05	0.95

PPARalpha

FXR	SHP	LXR	PPAR-a	
			Active	Inactive
Active	Active	Active	0.50	0.50
Active	Active	Inactive	0.95	0.05
Active	Inactive	Active	0.05	0.95
Active	Inactive	Inactive	0.95	0.05
Inactive	Active	Active	0.50	0.50
Inactive	Active	Inactive	0.95	0.05
Inactive	Inactive	Active	0.05	0.95
Inactive	Inactive	Inactive	0.50	0.50

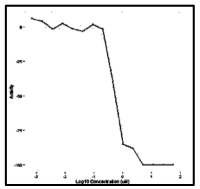
Predicting effect of assay measurements of events in an AOP network



Single chemical hazard screening: Benzo(k)fluoranthene

Incorporating in vitro assay data into a qAOP Bayes Net

In vitro assay



qHTS Assay for Inhibitors of HSD17B4

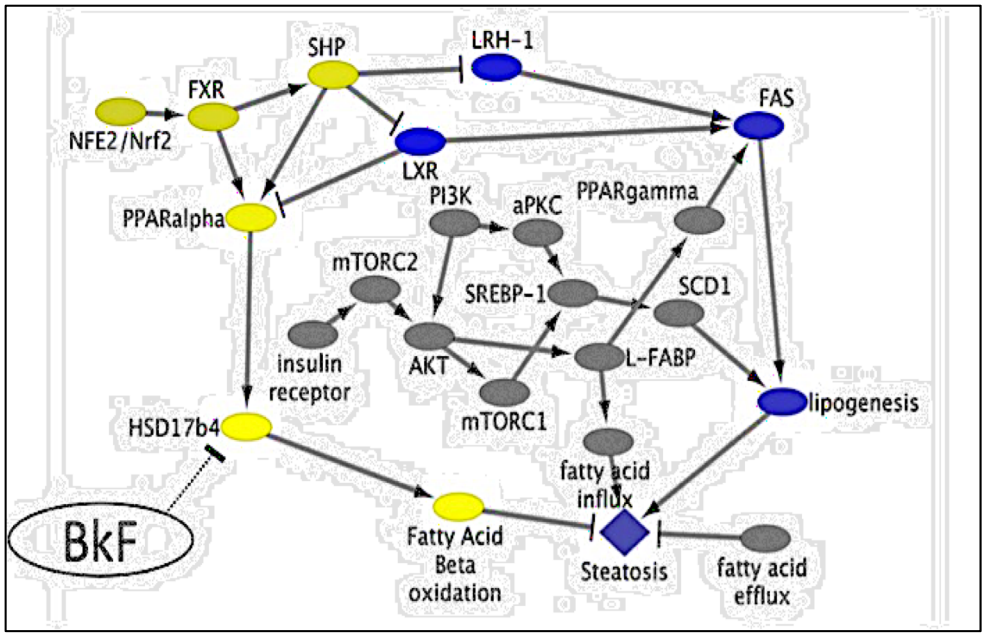
National Library of Medicine's Pub-Chem Database

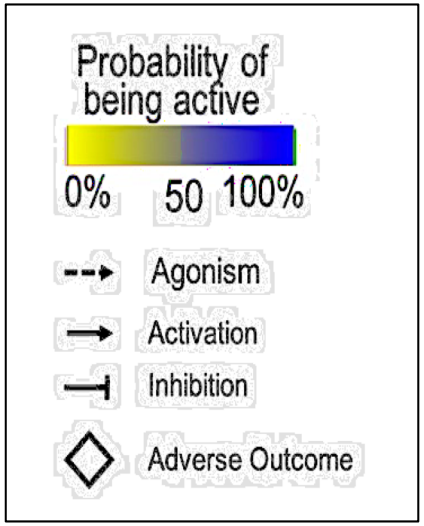
>0.5 uM B(k)F Inhibits HSD17b4 activity

inactive

HSD17b4

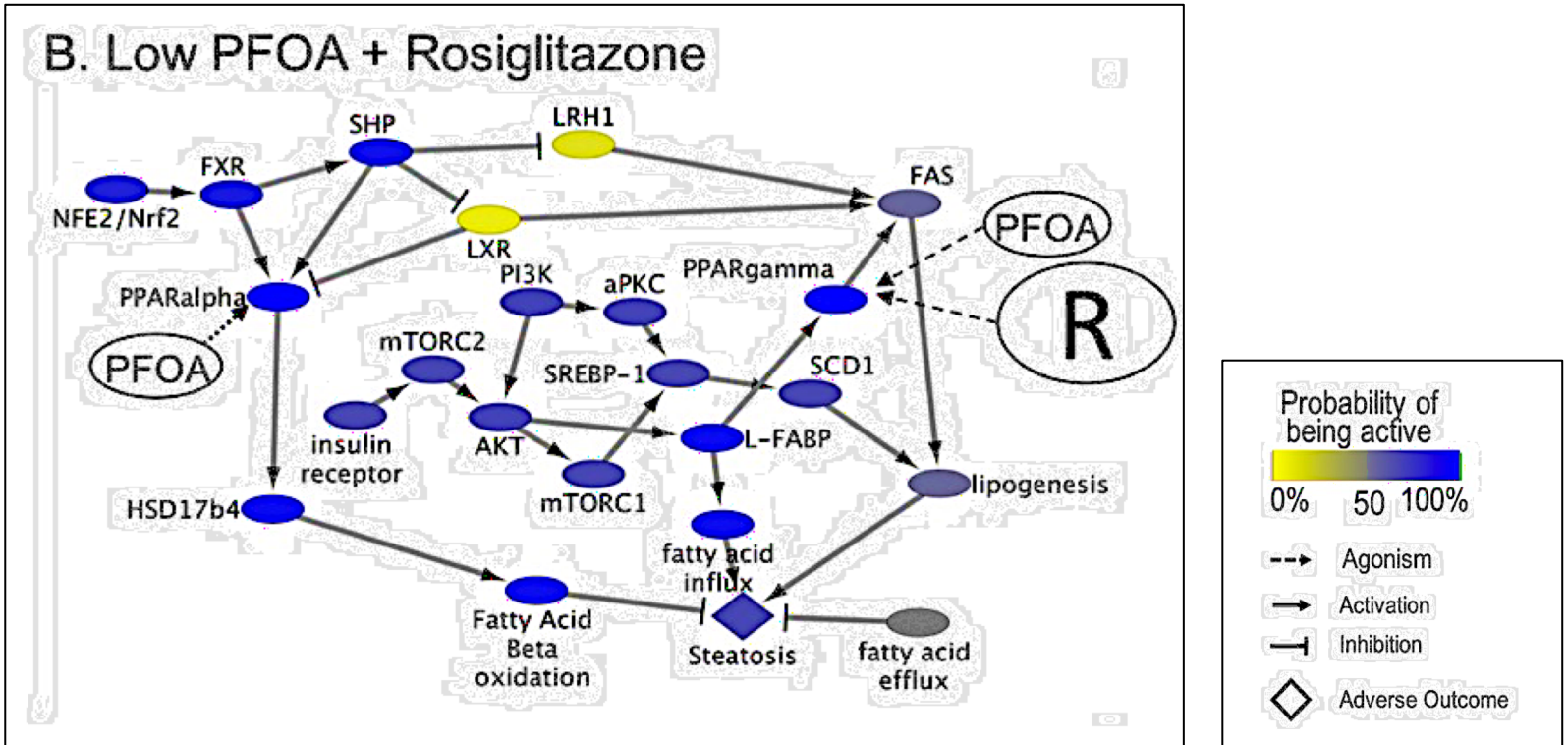
Set probability of being active=0





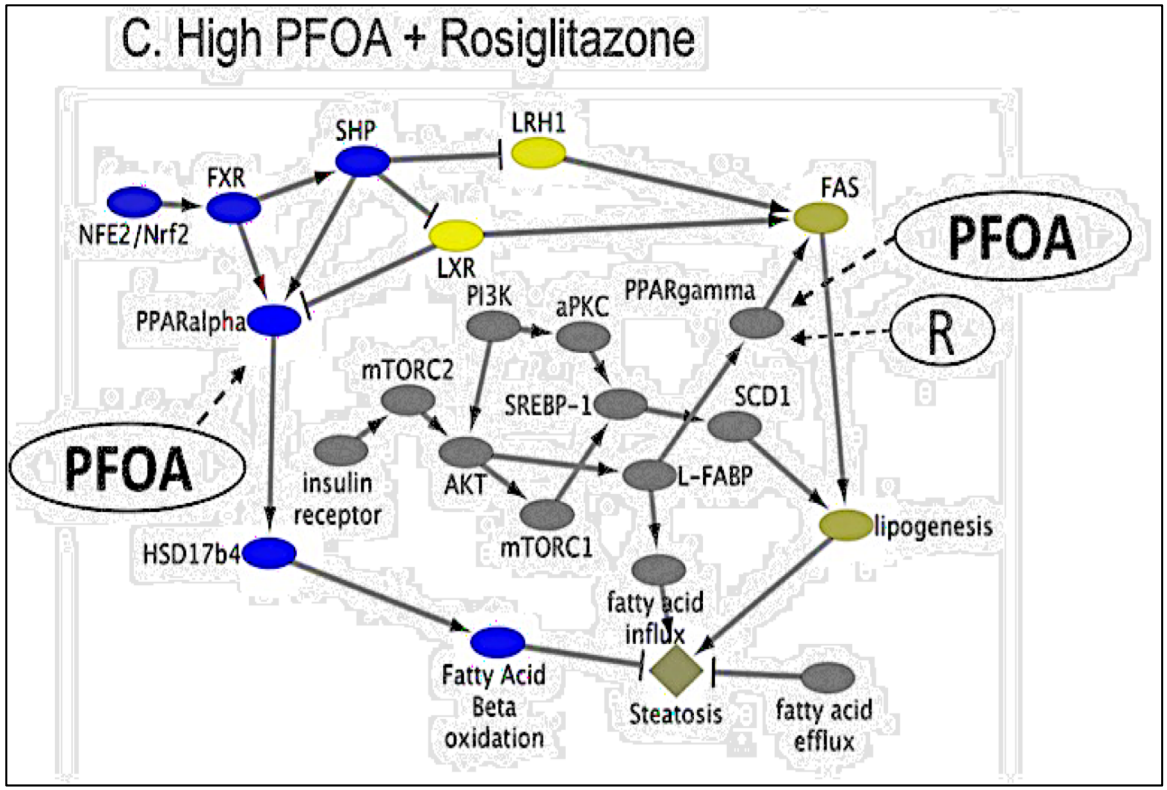
Examining hazards of chemical mixtures with Quantitative AOP networks case 1: water contamination &medicine

- Obesity can lead to type 2 diabetes which can be treated with rosiglitazone, an antidiabetic drug that is a full agonist of PPARγ (Lehmann et al., 1995).
- PFOA is a partial agonist for PPARγ and a full agonist of PPARα (Vanden Heuvel et al., 2003).
- Can interact in cases where diabetics using rosiglitazone drink water contaminated with PFOA
- PPARγ probability of being active was determined by the relative concentrations of full and partial agonists.
- In the presence of therapeutic levels of rosiglitazone and environmental concentrations of PFOA, the PPARγ probability of activity is expected to be 100% since rosiglitazone will outcompete PFOA to occupy PPARγ binding sites.
- This is consistent with observations of increased steatosis in clinical studies of rosiglitazone in obese patients (Massart et al., 2017) and manifestation of steatosis in mice fed a high fat diet in combination with rosiglitazone (Gao et al., 2016).



Examining hazards of chemical mixtures with Quantitative AOP networks case 2. Water contamination

- In the case where healthy people are exposed to both PFOA and rosiglitazone through contaminated water, PFOA is likely to be at much higher concentrations than rosiglitazone which would result in PFOA outcompeting rosiglitazone for occupancy of the PPARγ receptor
- Because of the low binding potential of PFOA for PPARγ (Vanden Heuvel et al., 2003) and presence of the strong binding rosiglitazone, the probability of activation of PPARγ can reasonably be set to 50%.
- Predictions are consistent with experimental studies in obese mice where reduction of PPARγ activity, via antagonism or gene knockout, decreases steatosis (Shiomi et al., 2015; Zhang et al., 2014; Morán-Salvador et al., 2011).



Conclusion/Recommendations

- AOPs provide a standardized approach relevant to regulatory needs.
- AOPs are conceptual models that **inform** design of qAOP models
- Define a **specific** question **before** developing a qAOP
- The complexity and type of qAOP model used is driven by resources and uncertainty:

  Time constraints, Data availability

  Extensive response-response data

  Biological fidelity

  Application
- Many different types of qAOP models can be made
- Toxicokinetics+qAOP -> internal exposure at MIE+Hazard
- Exposure+Toxicokinetics+qAOP-> external exposure+Hazard
- qAOPs can be used to to answer a wide range of questions, but developers should be transparent and document everything (e.g. TRACE)

Acknowledgements

ERDC

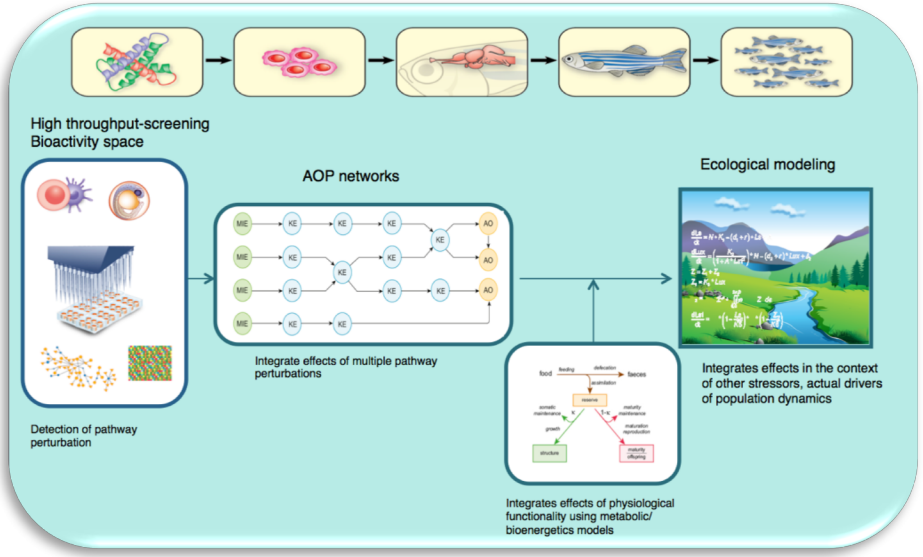
- Lyle Burgoon
- Ed Perkins
- Taylor Rycroft
- Christy Foran

EPA

- Michelle Angrish
- Nathan Pollesch

EAWAG

- Anze Zupanic



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