Advantages and implications of using Adverse Outcome Pathways (AOPs) for disease outcomes resulting from temporal exposures

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This talk does not necessarily reflect the views of the U.S. Environmental Protection Agency.

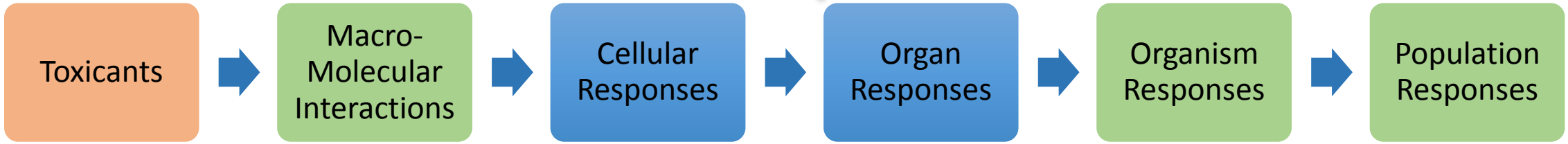
Outline

- Adverse Outcome Pathway Background
- AOPs and Temporal Exposure Issues
- Making It Happen

Goal: Bridge the Gap

- Tox21/SEURAT-1
  - High-throughput, *in vitro*
  - Capable of directly screening many chemicals
- Regulatory Decisions
  - Toxicity must relate to the impact on human health, endangered species, or wildlife populations

AOP Components



- Key Events (KEs) - nodes
  - Change in biological state
  - Measurable and essential for progression
  - MIE - initial point of chemical interaction
  - AO – adverse outcome of regulatory significance
- Key Event Relationships (KERs) - edges
  - Connections between two key events
  - Critical for assembling evidence in support of the AOP

Five Principles of AOP Development

1. AOPs Are Not Chemical Specific
2. AOPs Are Modular (consisting of KEs and KERs)
3. An Individual AOP Is a Pragmatic Unit of Development and Evaluation
4. For Most Real-World Applications, AOP Networks Are the Functional Unit of Prediction
5. AOPs Are Living Documents

Villeneuve, et al. *Tox Sci.,* **2014, 142:**312-320

OECD AOP Development Programme

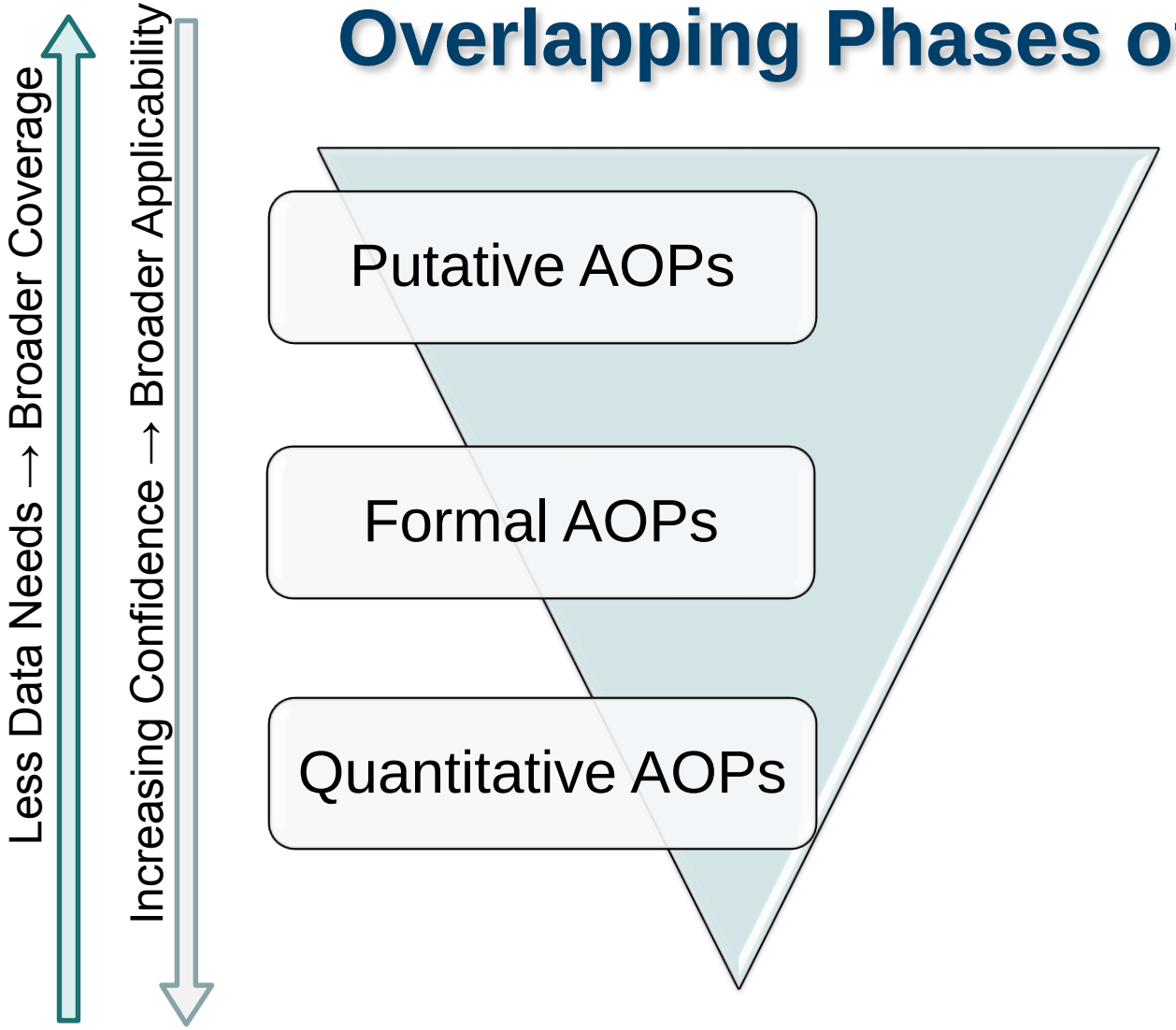
What is an Adverse Outcome Pathway (AOP)

In 2012, the OECD launched a new programme on the development of Adverse Outcome Pathways. An Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (see figure). AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

- Extended Advisory Group for Molecular Screening & Toxicogenomics (EAGMST)
- Guidance & Training
  - Guidance, User Handbook, many training options
- International Knowledgebase to capture information
  - >100 AOPs at various stages of development

http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

Overlapping Phases of AOP Development



- Define AOP
- Evaluate the AOP
- Quantitatively describe the AOP

OECD Handbook

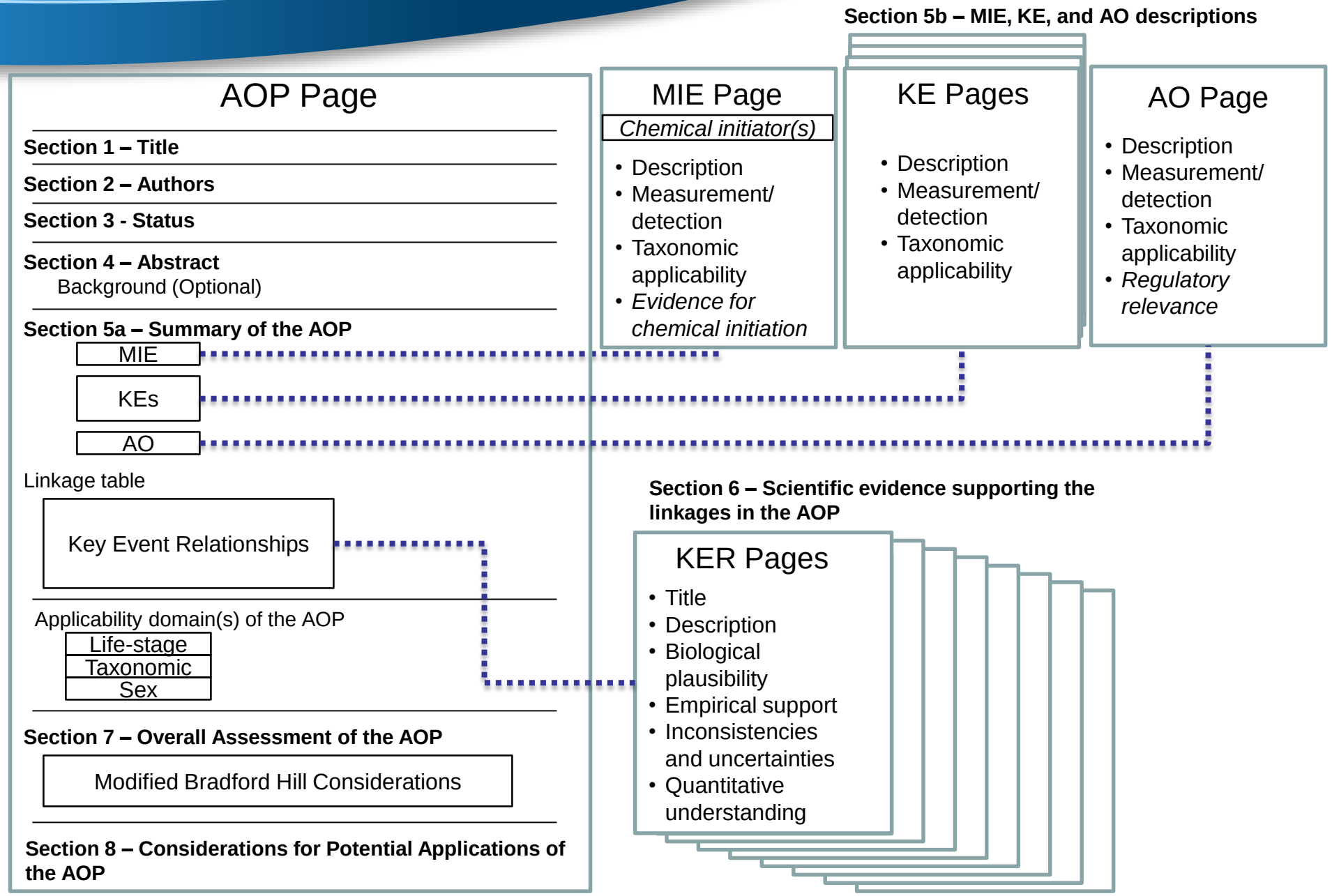
Step by step guide to AOP development

https://aopkb.org/common/AOP_Handbook.pdf

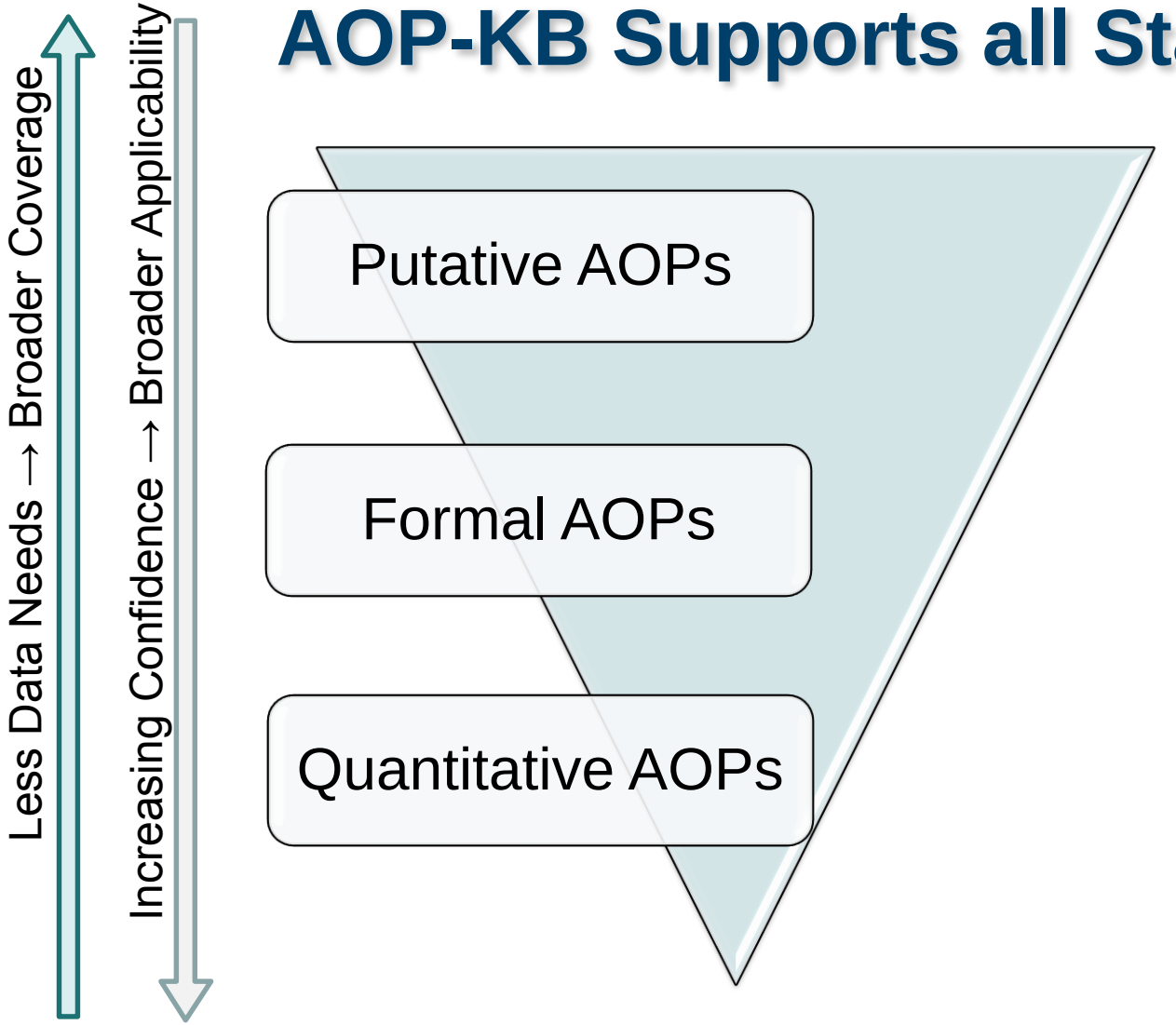
AOP-Wiki

Provides consistent structure based on the OECD handbook and facilitates collaborative AOP development

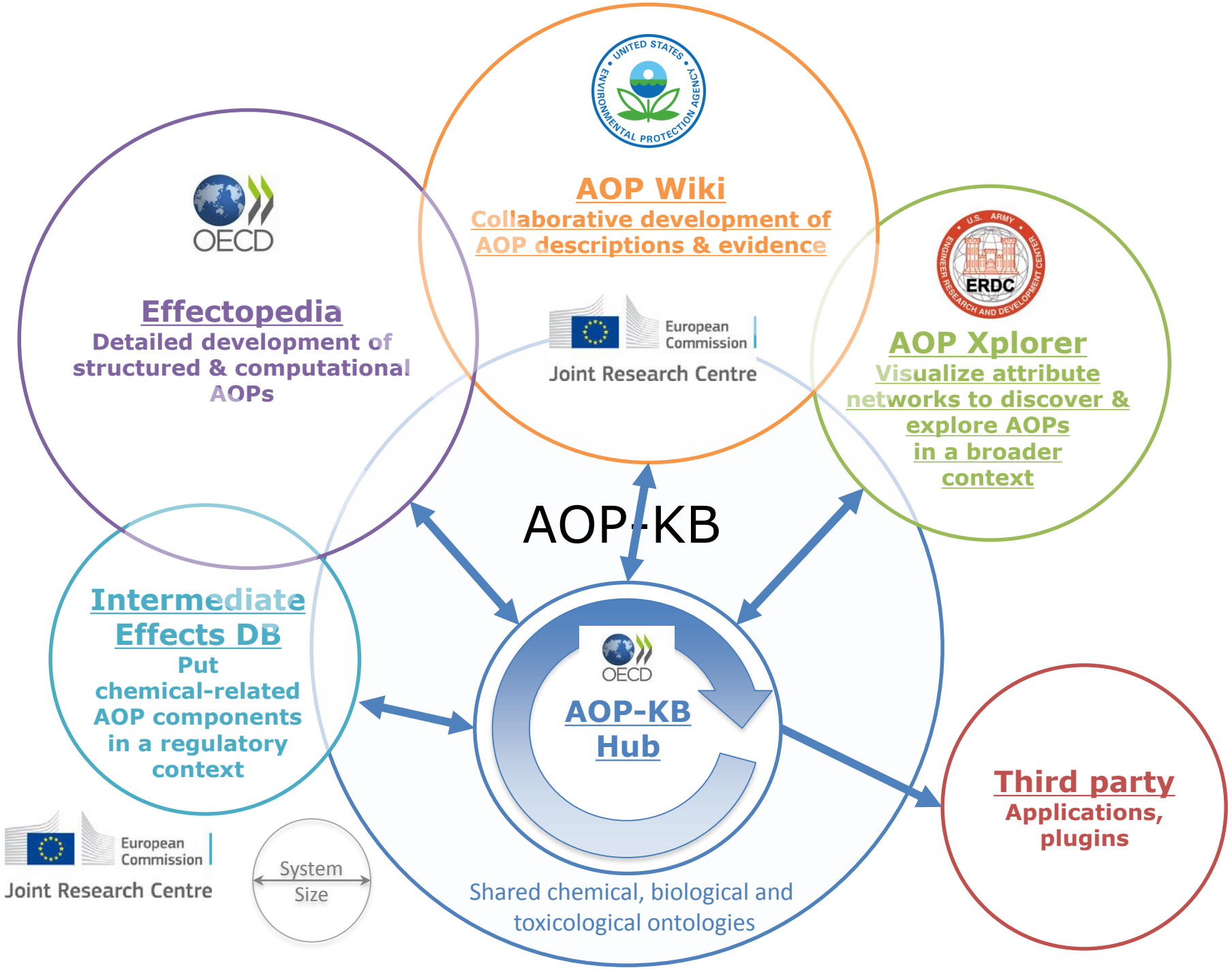
http://aopwiki.org/



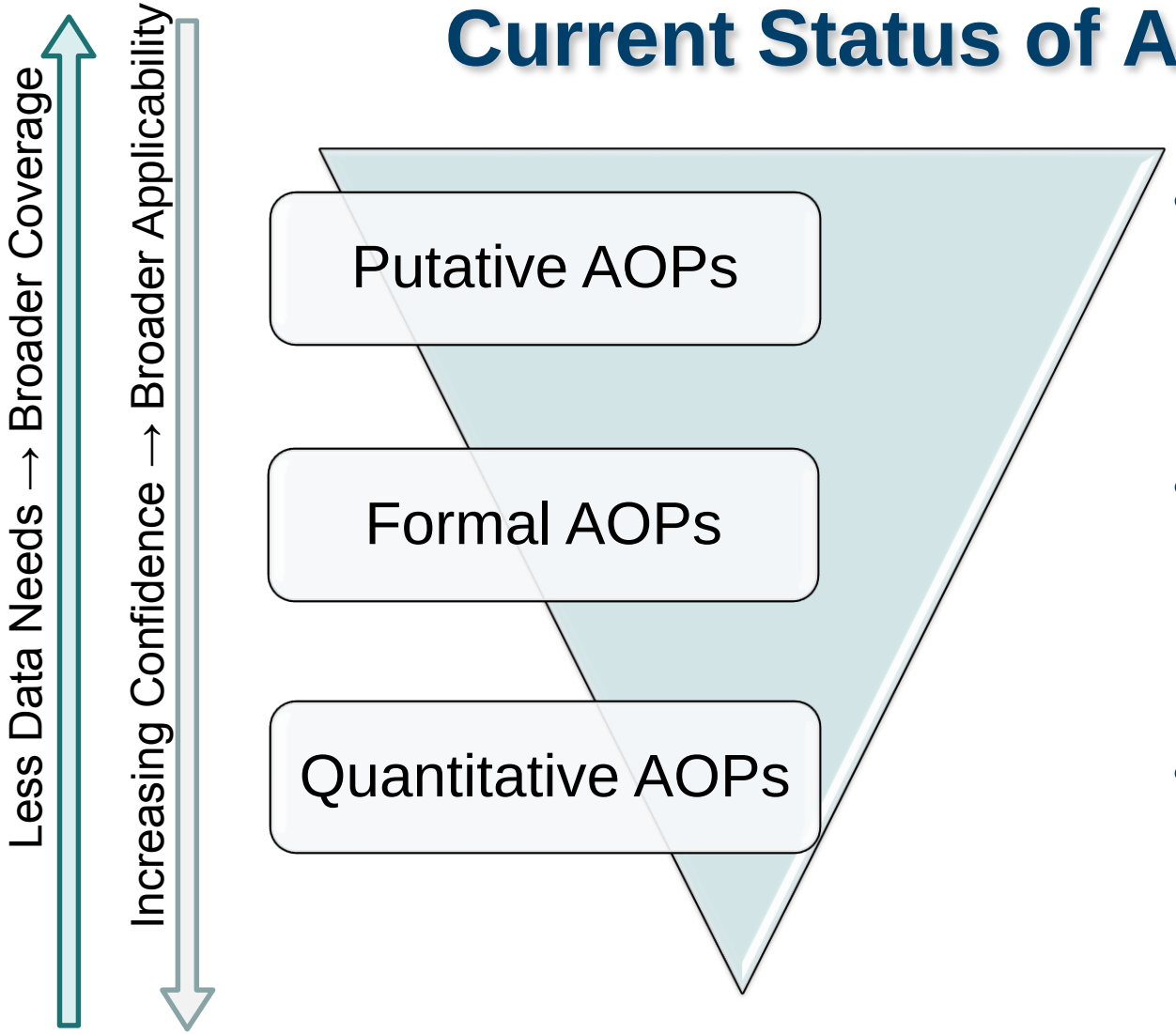
AOP-KB Supports all Stages of Development



- AOP-Xplorer – Helps assemble putative AOPs
- AOP-Wiki – Provides structured forms for evaluating AOPs
- Effectopedia – Provides tools for quantitatively describing AOPs


AOP Wiki
Collaborative development of AOP descriptions & evidence
Effectopedia
Detailed development of structured & computational AOPs
AOP Xplorer
Visualize attribute networks to discover & explore AOPs in a broader context
AOP-KB
Intermediate Effects DB
Put chemical-related AOP components in a regulatory context
AOP-KB Hub
Third party
Applications, plugins
Shared chemical, biological and toxicological ontologies
System
Size
European Commission
Joint Research Centre
OECD
ERDC

Current Status of AOP Development



- > 100 putative AOPs in the AOP-Wiki (most not under active development)
- 14 formal AOPs undergoing OECD review
- < 5 quantitative AOPs under development

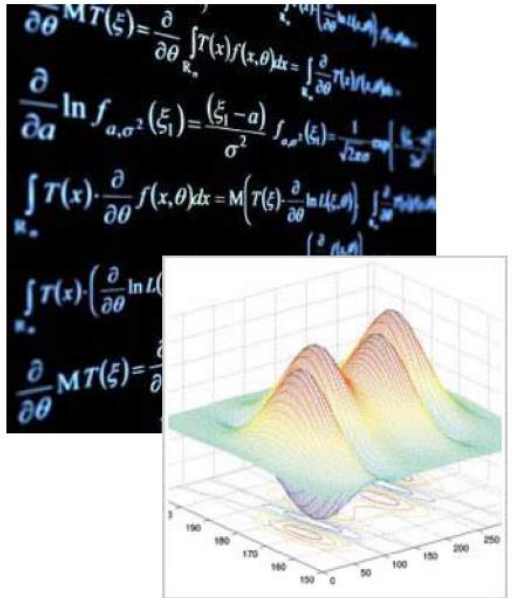
What do AOPs provide that can help when dealing with temporal exposures?

http://nas-sites.org/emergingscience/files/2012/06/Whelan.pdf

Understanding

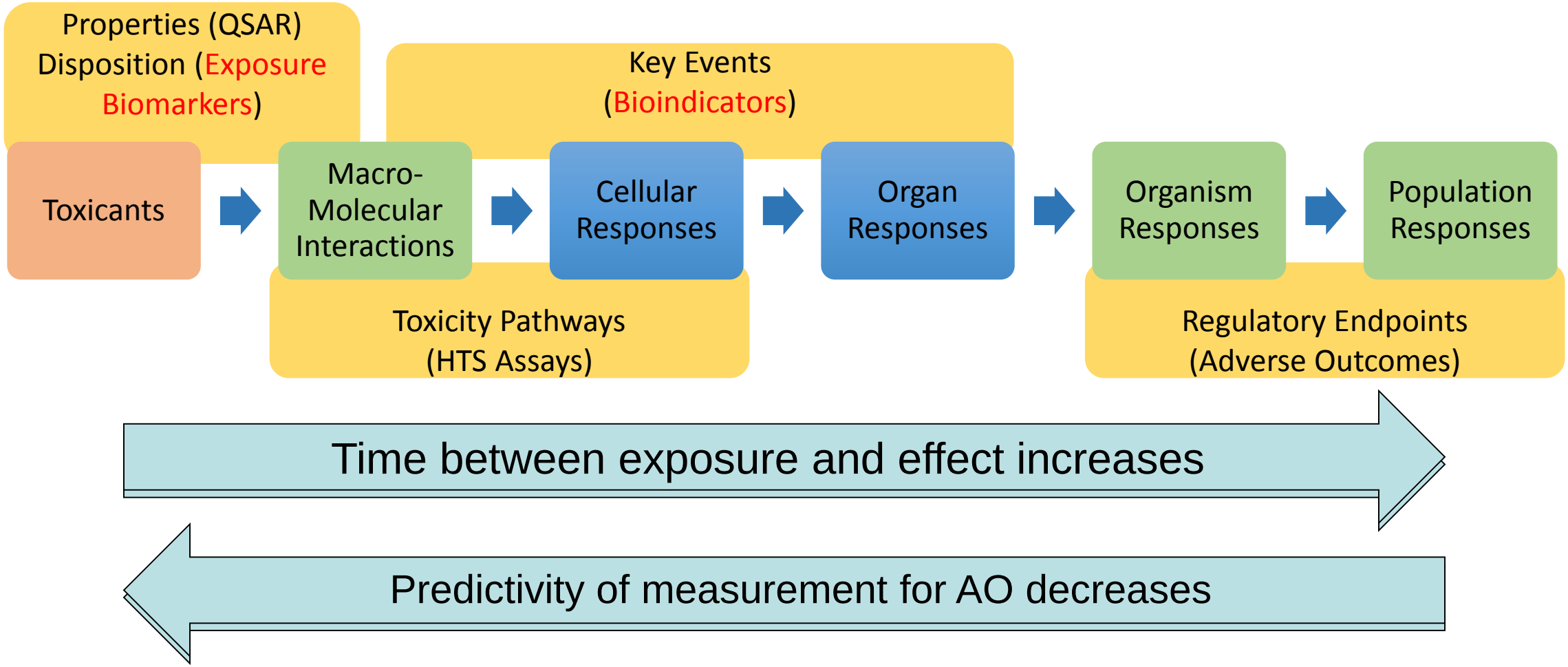
- Maurice Whelan (OECD EAGMST Co-chair)
- NAS Systems Biology-Informed Risk Assessment
  - June 14-15, 2012

But we need to go further – AOPs are a step on the pathway to more formal representation of our knowledge on system composition and behaviour.



- Emphasise the need to *understand*, and not just measure - position *modelling* at the centre.

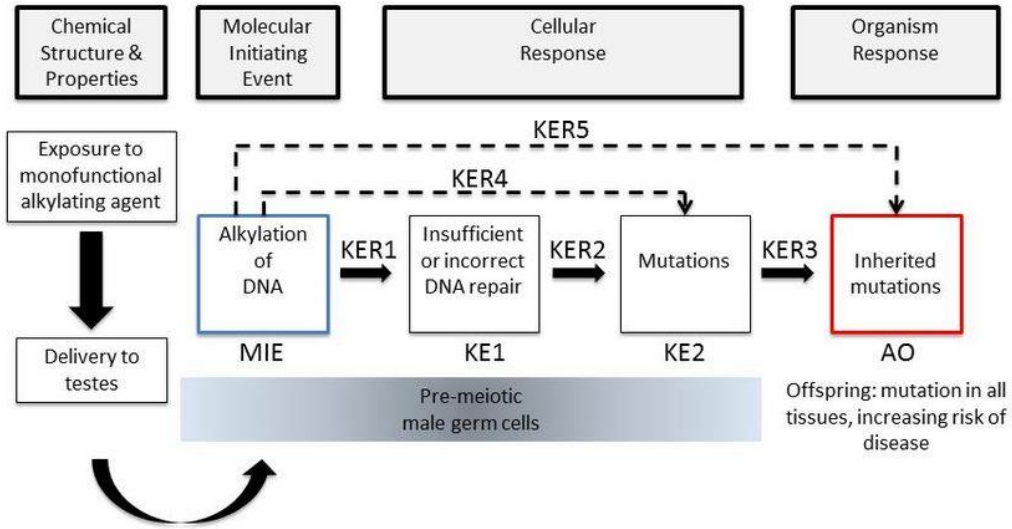
Match Measurements with Time Frame of Exposure

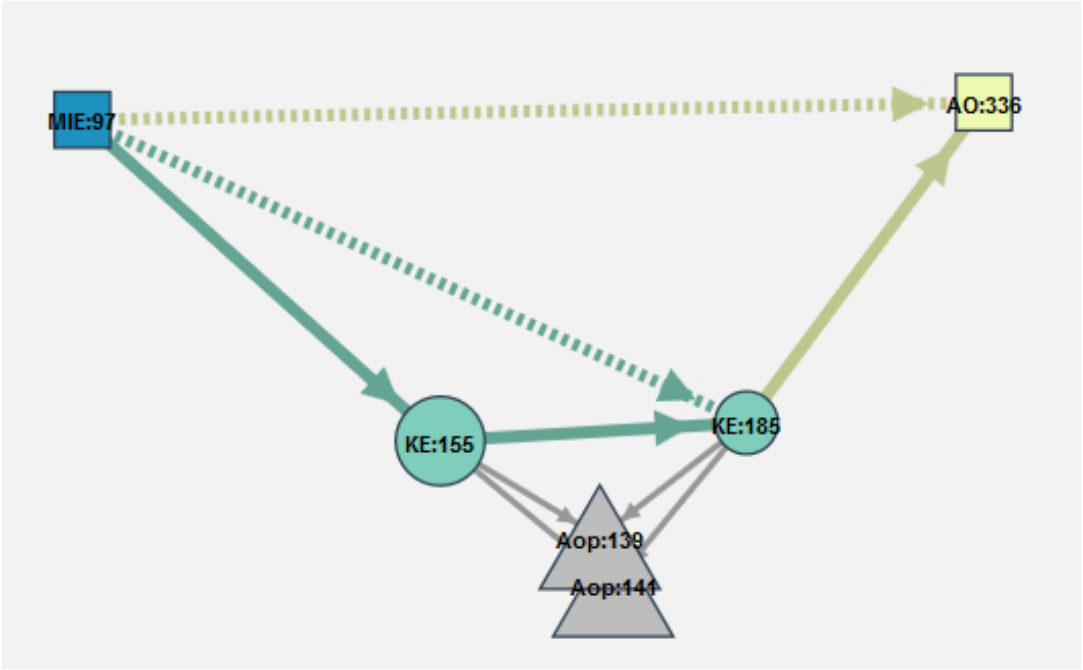


AOP Title

Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations

Short name: Alkylation of DNA leading to heritable mutations



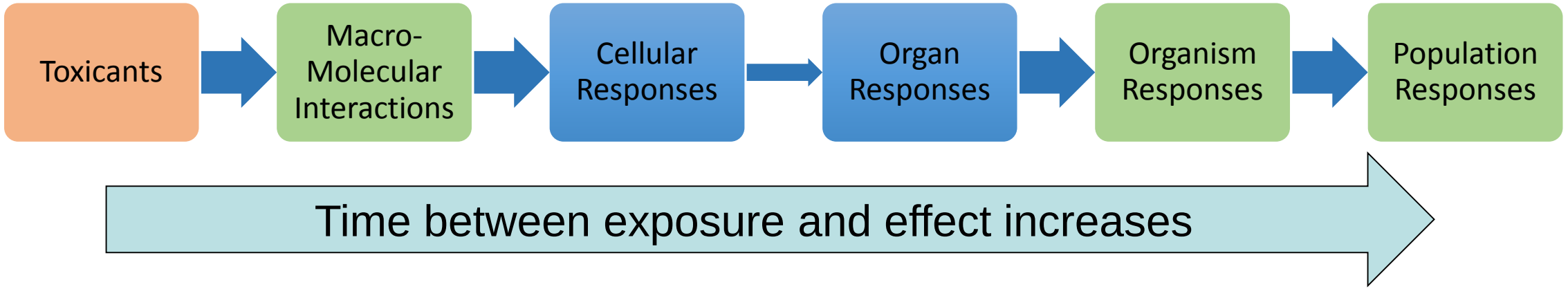


Carole Yauk – https://aopwiki.org/wiki/index.php/Aop:15

Relationships Among Key Events and the Adverse Outcome

Event	Description	Triggers	Weight of Evidence	Quantitative Understanding
DNA, Alkylation	Directly Leads to	Insufficient or incorrect DNA repair, N/A	Strong	Moderate
Insufficient or incorrect DNA repair, N/A	Directly Leads to	Mutations, Increase	Strong	Moderate
DNA, Alkylation	Indirectly Leads to	Mutations, Increase	Strong	Moderate
DNA, Alkylation	Indirectly Leads to	Heritable mutations in offspring, Increase	Strong	Moderate
Mutations, Increase	Directly Leads to	Heritable mutations in offspring, Increase	Strong	Moderate

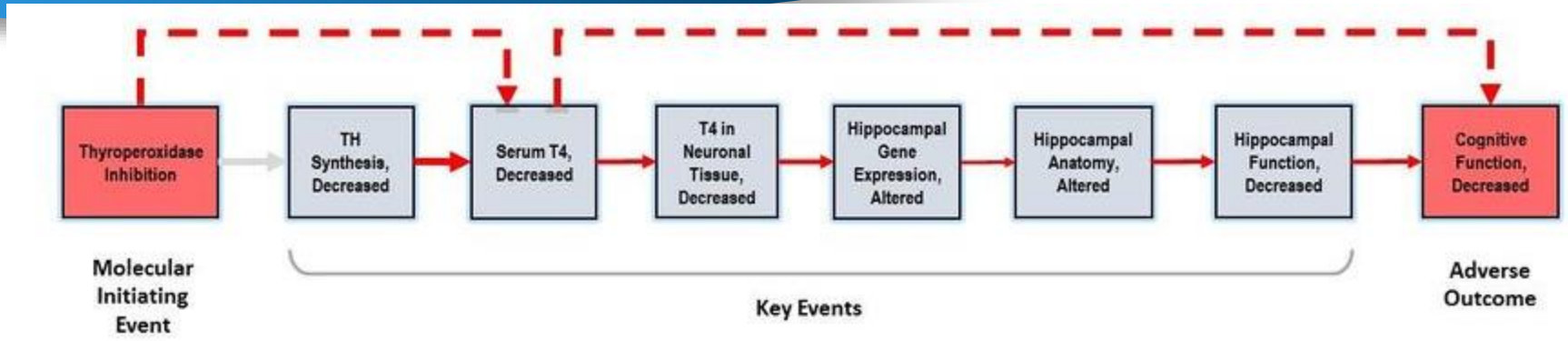
Factors Determining Predictivity of Early Key Events

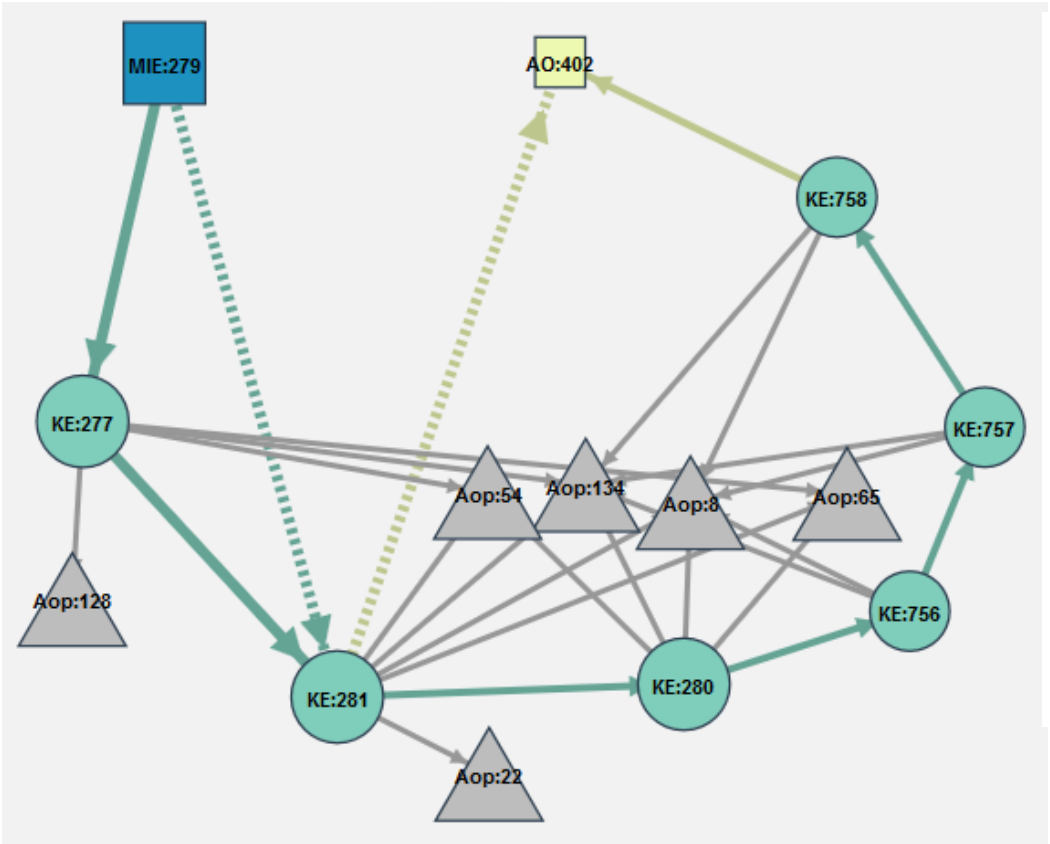


- Evidence supporting the KERs between that KE and the AO
- Quantitative understanding of the downstream KERs
- Modifying factors that influence downstream KEs & KERs

AOP Title

Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals





Relationships Among Key Events and the Adverse Outcome

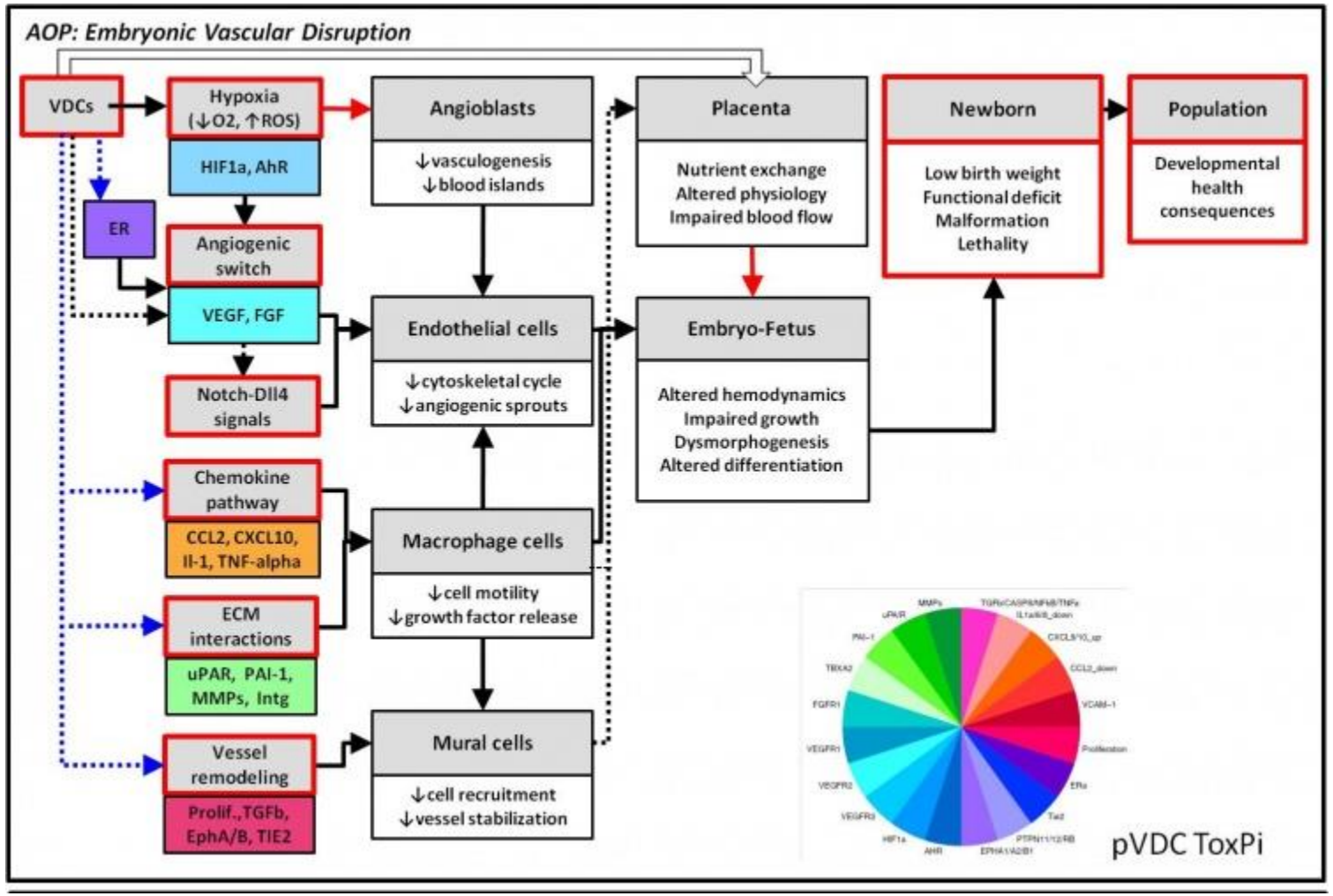
Event	Description	Triggers	Weight of Evidence	Quantitative Understanding
Thyroperoxidase, Inhibition	Directly Leads to	Thyroid hormone synthesis, Decreased	Strong	Weak
Thyroid hormone synthesis, Decreased	Directly Leads to	Thyroxin (T4) in serum, Decreased	Strong	Weak
Thyroxin (T4) in serum, Decreased	Directly Leads to	Thyroxin (T4) in neuronal tissue, Decreased	Moderate	Weak
Thyroxin (T4) in serum, Decreased	Indirectly Leads to	Cognitive Function, Decreased	Strong	Moderate
Thyroxin (T4) in neuronal tissue, Decreased	Directly Leads to	Hippocampal gene expression, Altered	Moderate	Weak
Hippocampal gene expression, Altered	Directly Leads to	Hippocampal anatomy, Altered	Moderate	Weak
Hippocampal anatomy, Altered	Directly Leads to	Hippocampal function, Decreased	Moderate	Weak
Hippocampal function, Decreased	Directly Leads to	Cognitive Function, Decreased	Moderate	Weak
Thyroperoxidase, Inhibition	Indirectly Leads to	Thyroxin (T4) in serum, Decreased	Strong	Weak

Gilbert, Paul, Crofton - https://aopwiki.org/wiki/index.php/Aop:42

AOP Title

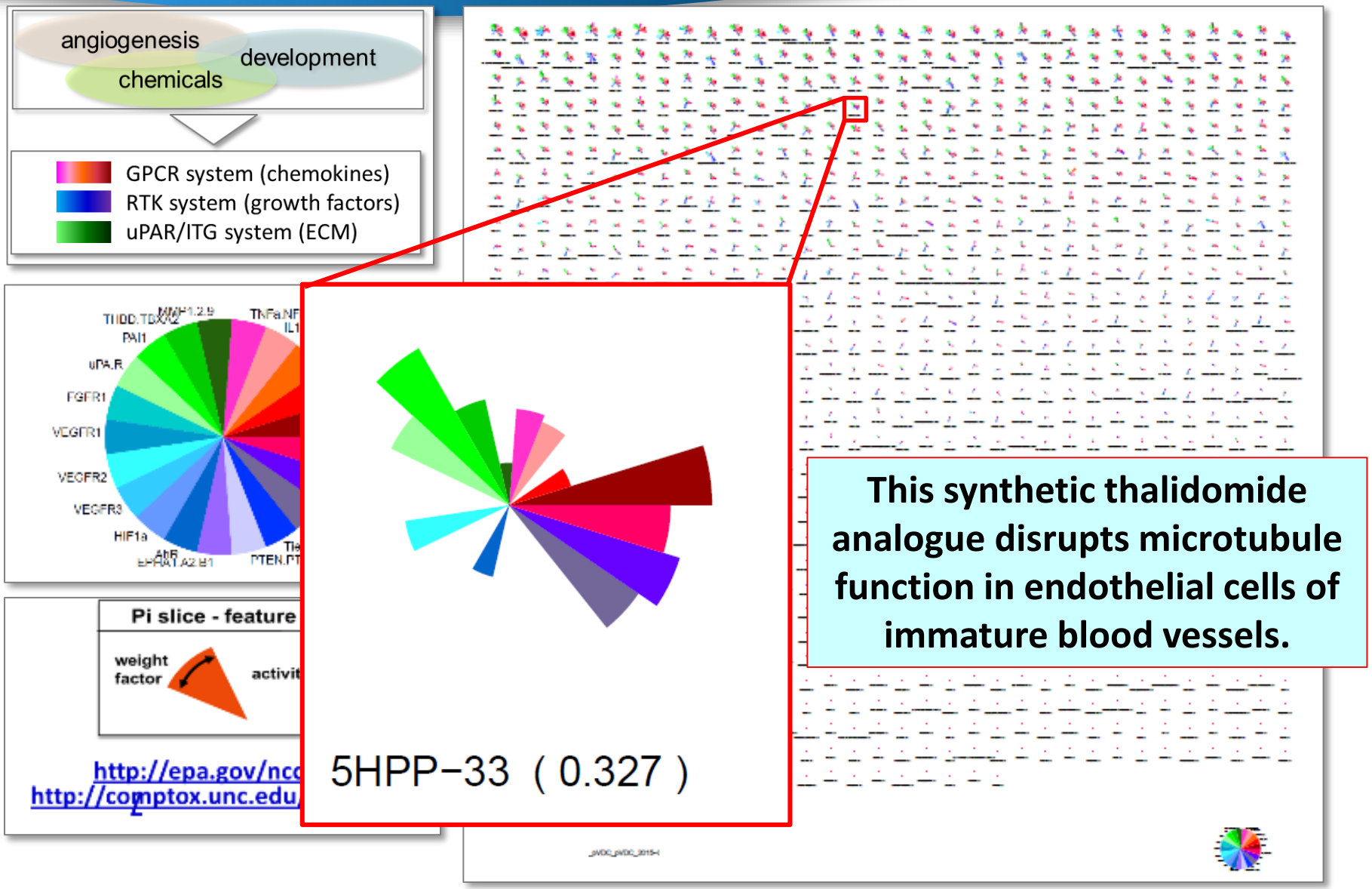
VEGF Signaling and Vascular Disruption Leading to Adverse Developmental Outcomes

Short name: Developmental Vascular Toxicity



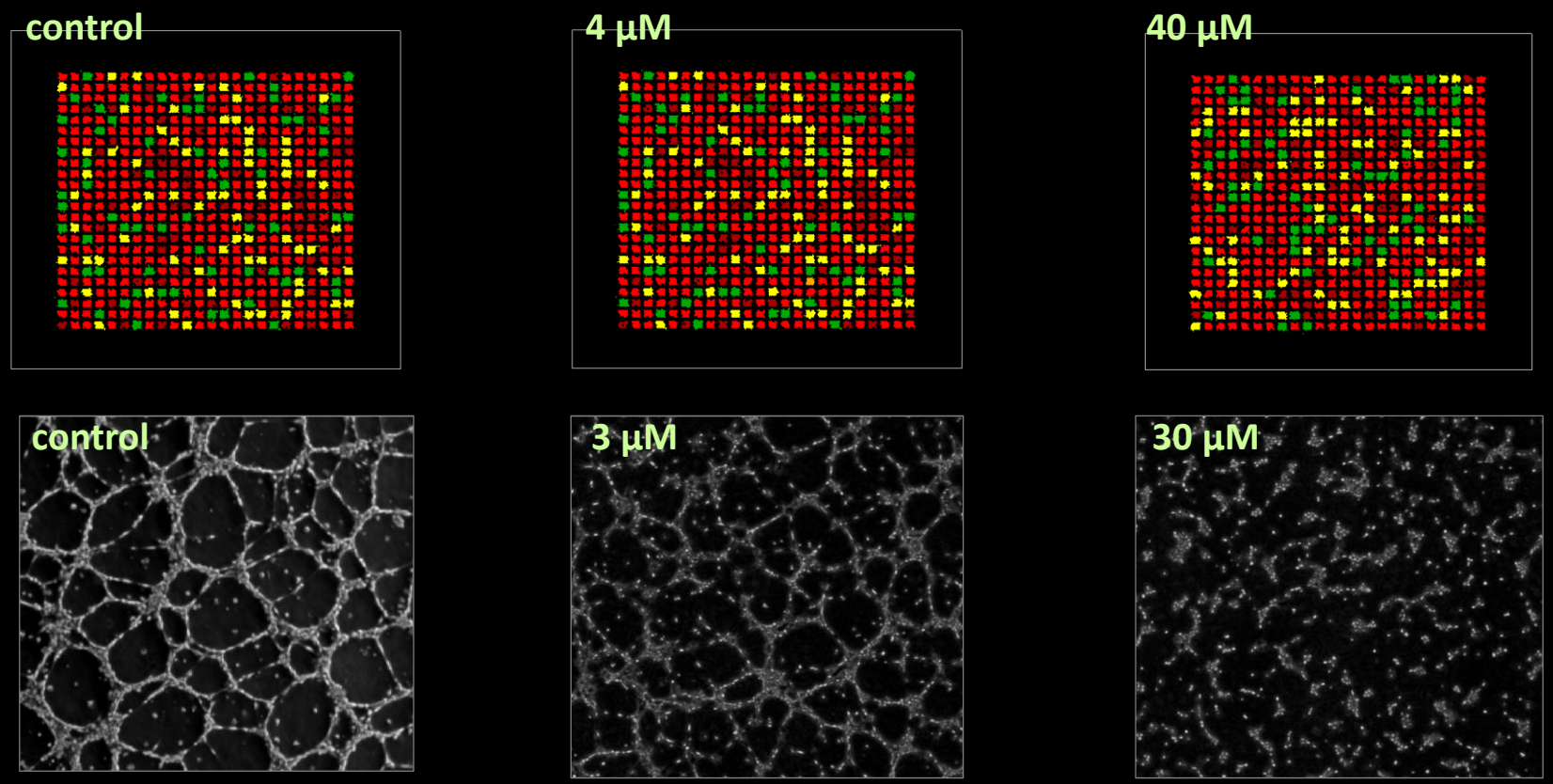
Kleinstreuer, Knudsen - https://aopwiki.org/wiki/index.php/Aop:43

ToxCast: chemicals sorted by predicted vascular disruption (pVDCs)



Slide courtesy of Tom Knudsen

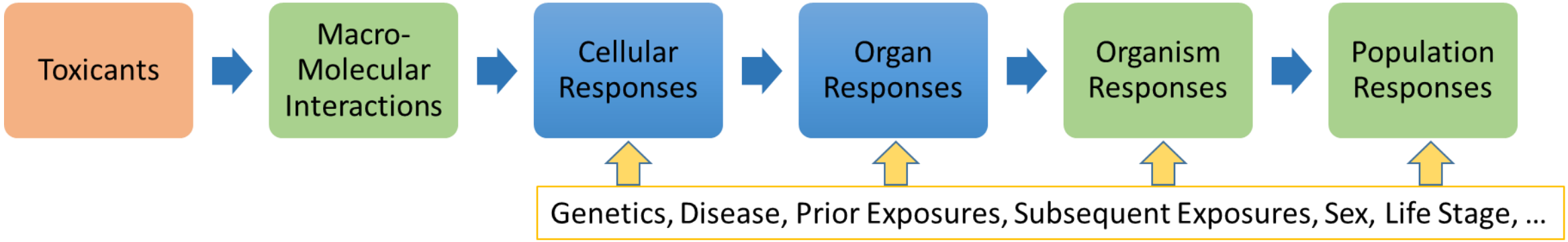
5HPP-33 concentration response predicted *in silico from* ToxCast and demonstrated *in vitro* with a human endothelial cell assay



Slide courtesy of Tom Knudsen

SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol

Factors Determining Predictivity of Early Key Events



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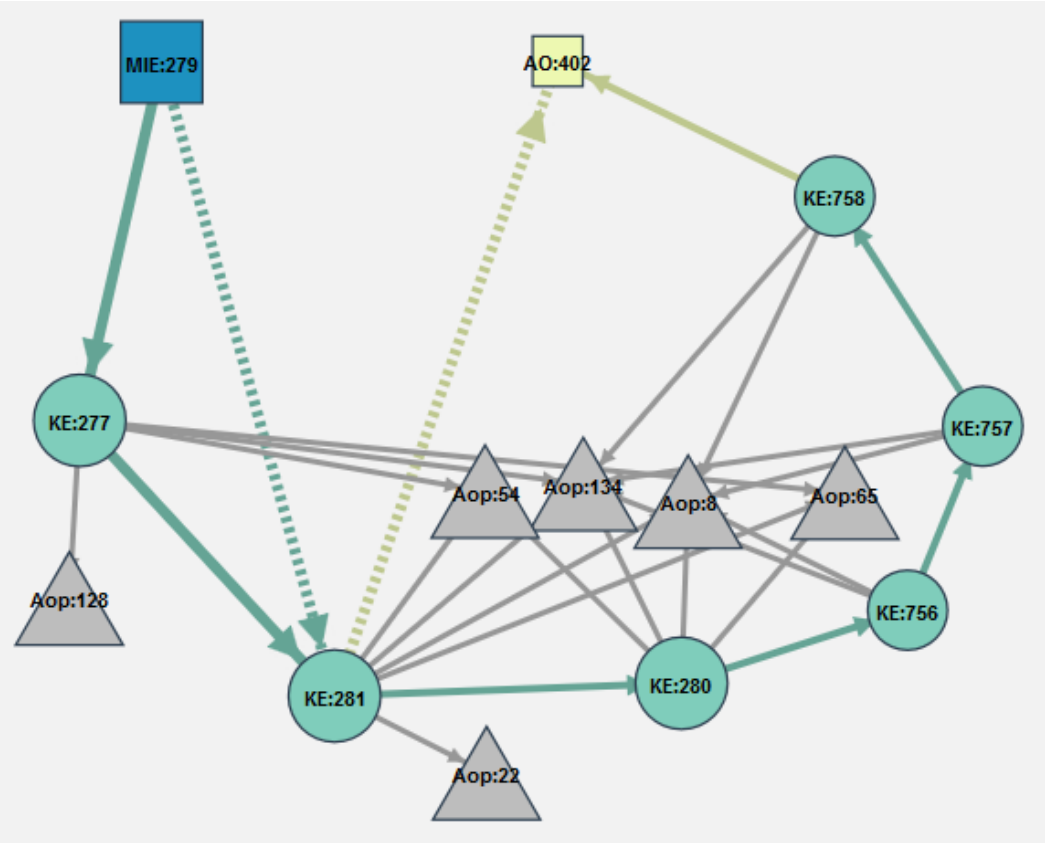
- Quantitative understanding of the downstream KERs
- Modifying factors that influence downstream KEs & KERs

Modifying Factors Emerge Naturally from AOP Networks



Gilbert, Paul, Crofton, et al. –
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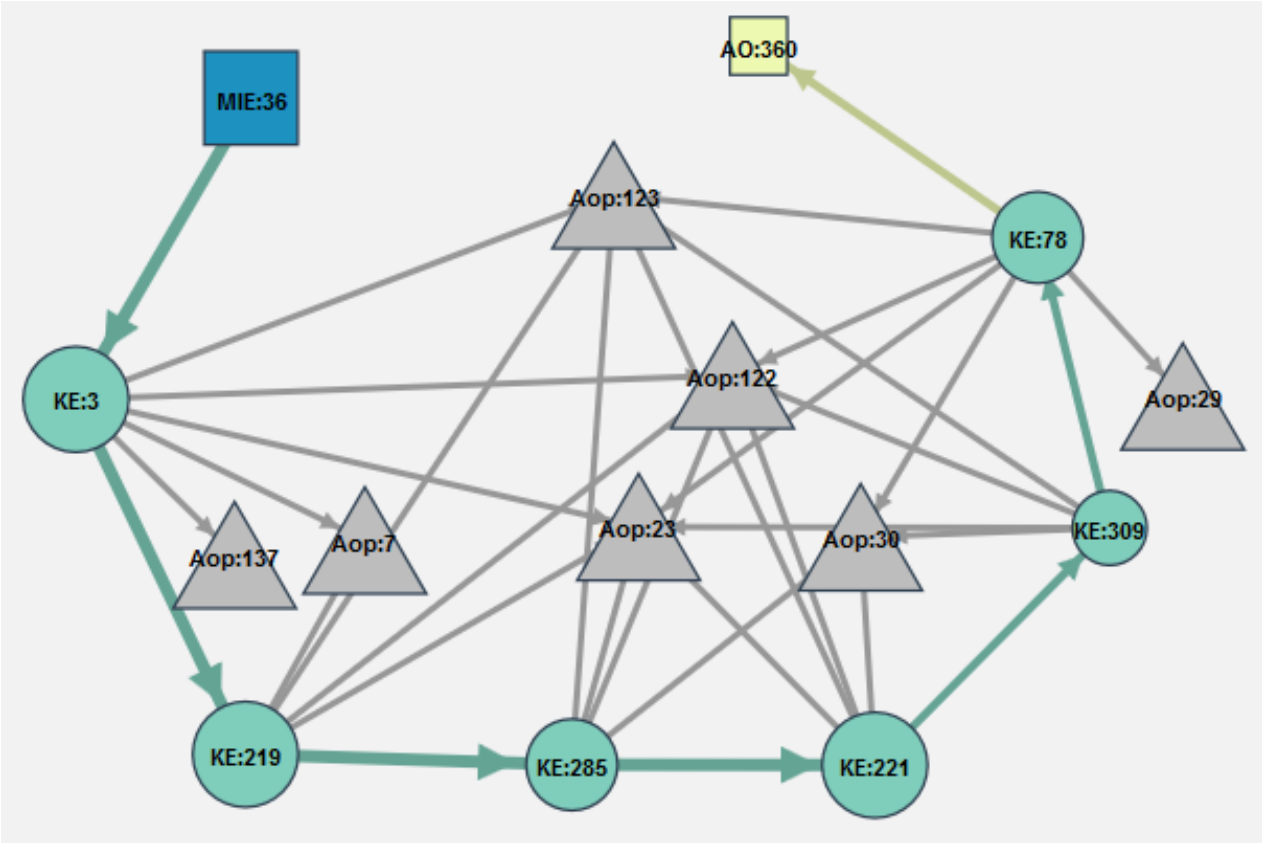
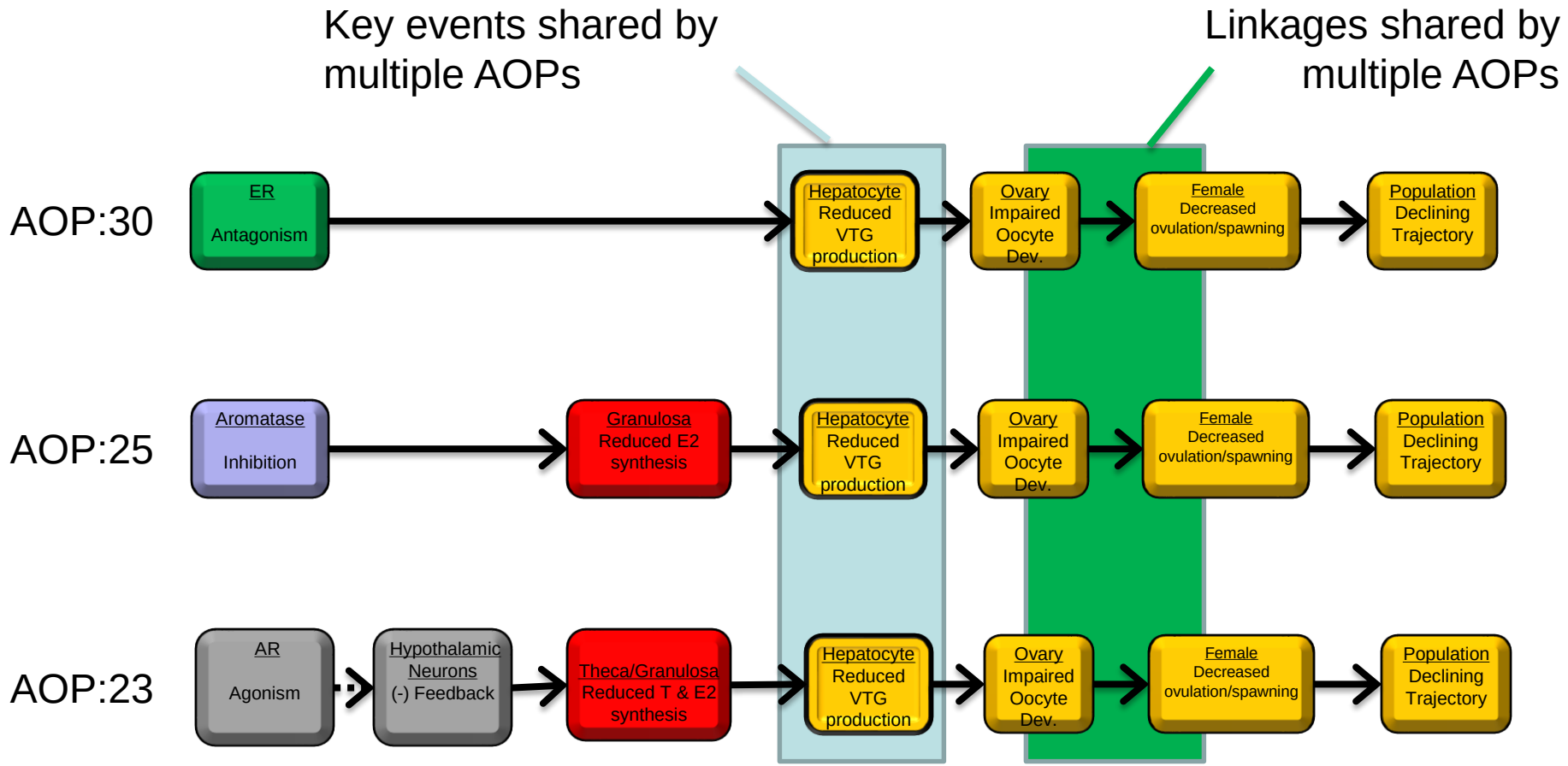
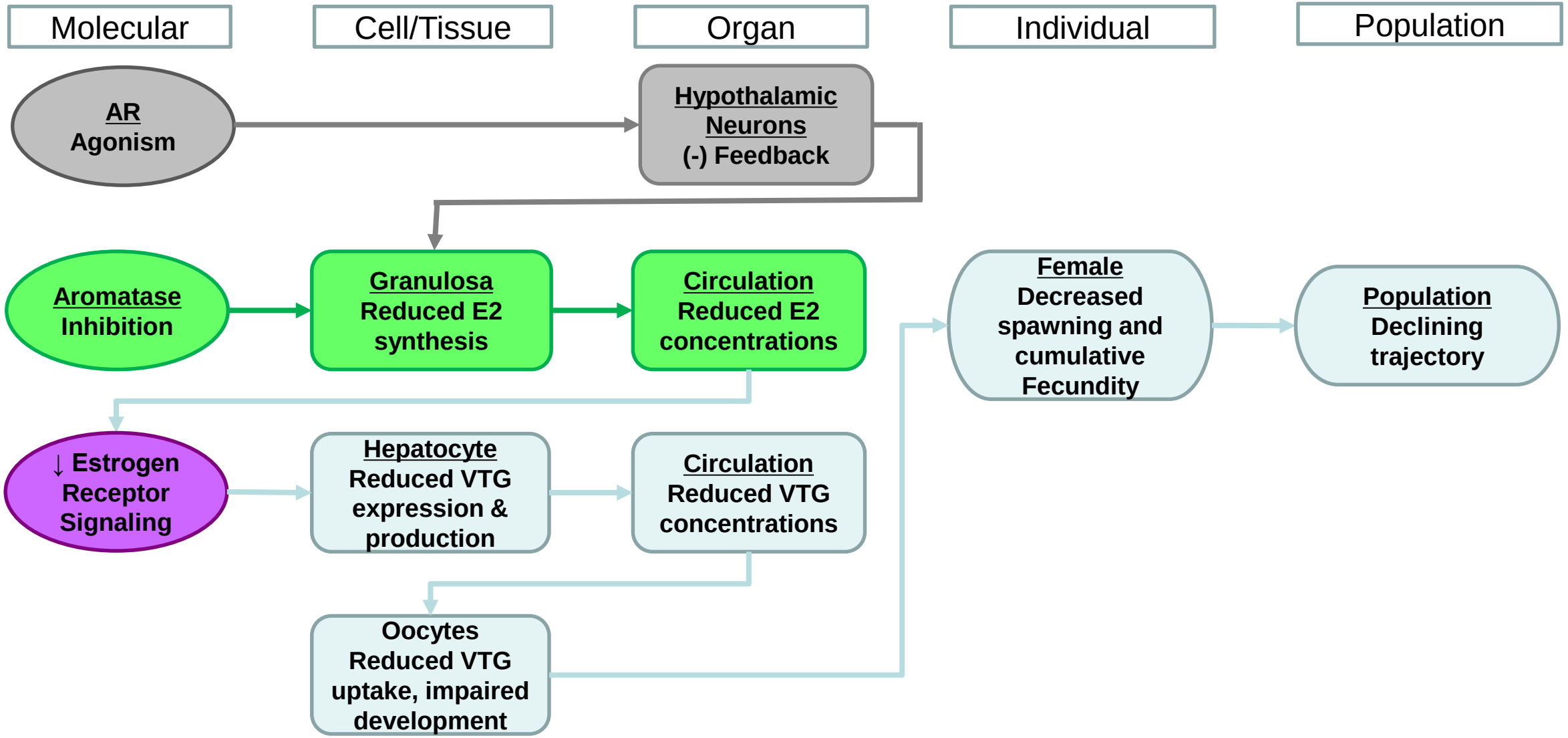
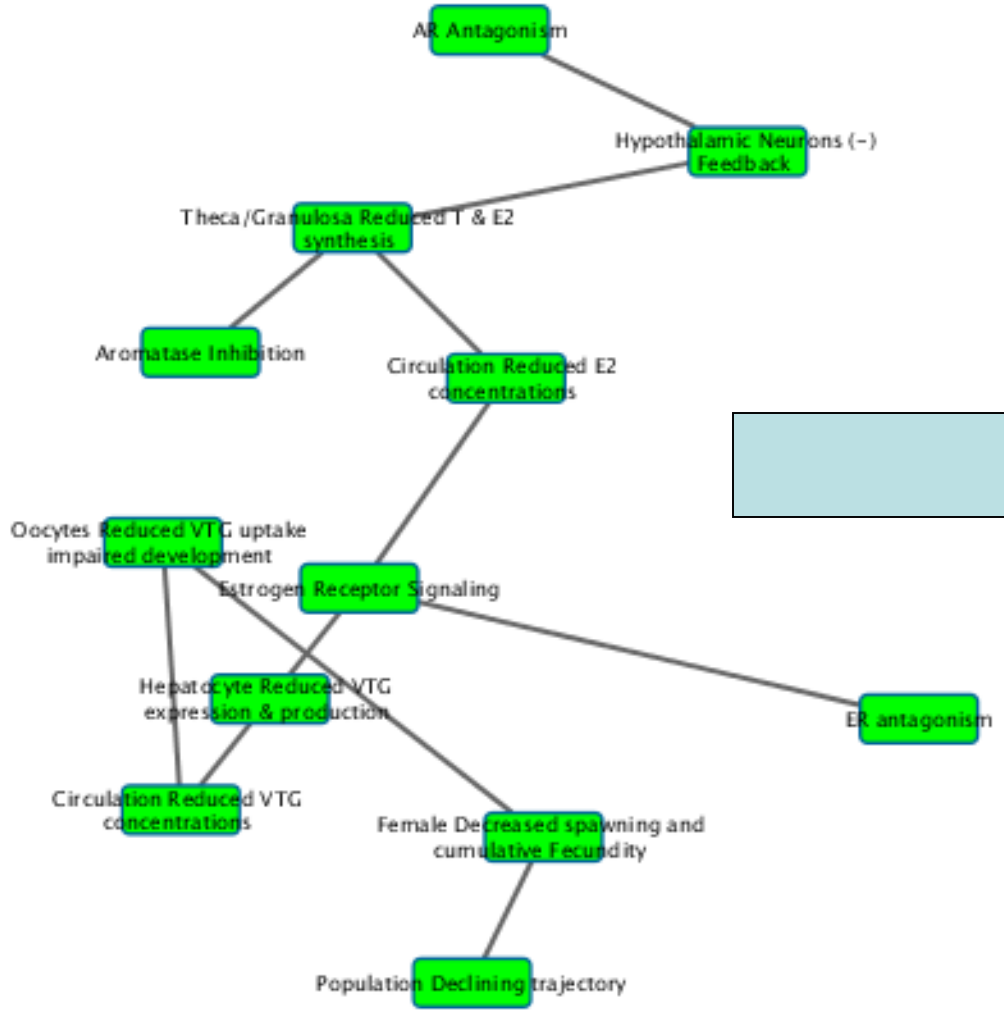


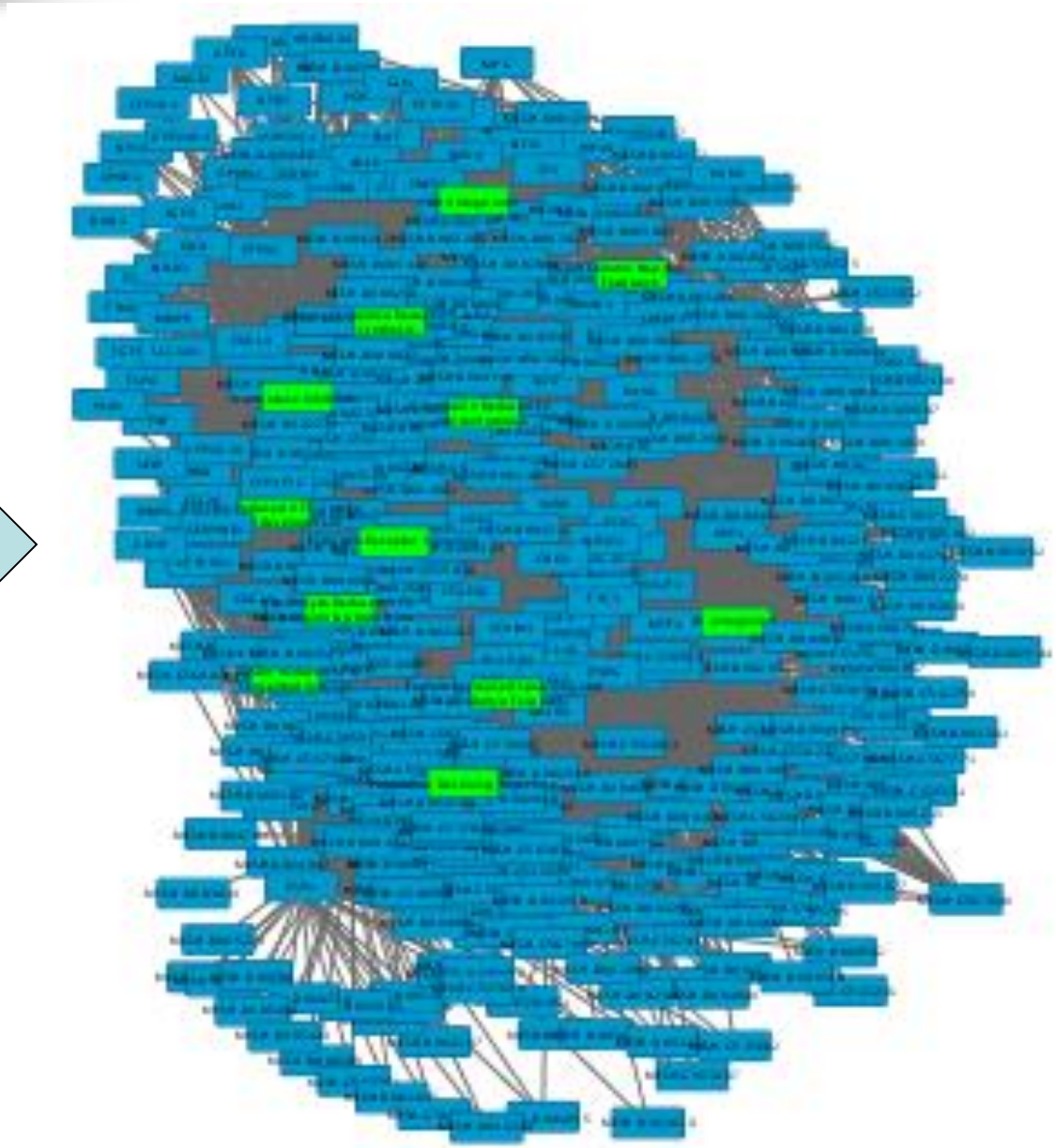
Villeneuve, et al. –
https://aopwiki.org/wiki/index.php/Aop:25



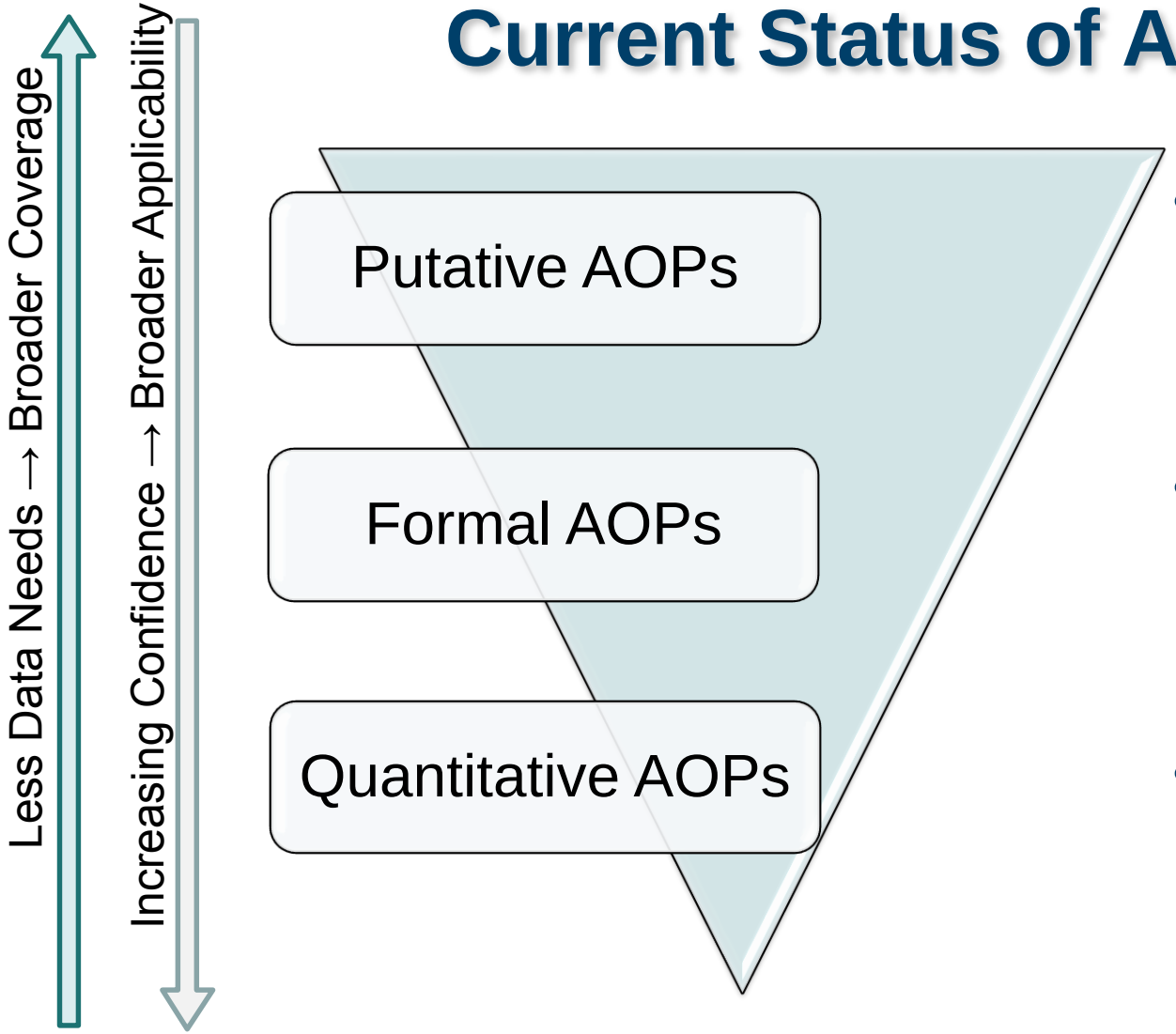
Dan Villeneuve


Molecular
Cell/Tissue
Organ
Individual
Population
AR
Agonism
Hypothalamic
Neurons
(-) Feedback
Aromatase
Inhibition
Granulosa
Reduced E2
synthesis
Circulation
Reduced E2
concentrations
Female
Decreased
spawning and
cumulative
Fecundity
Population
Declining
trajectory
↓ Estrogen
Receptor
Signaling
Hepatocyte
Reduced VTG
expression &
production
Circulation
Reduced VTG
concentrations
Oocytes
Reduced VTG
uptake, impaired
development


AR Antagonism
Hypothalamic Neurons (-) Feedback
Theca/Granulosa Reduced T & E2 synthesis
Aromatase Inhibition
Circulation Reduced E2 concentrations
Oocytes Reduced VTG uptake impaired development
Estrogen Receptor Signaling
Hepatocyte Reduced VTG expression & production
ER antagonism
Circulation Reduced VTG concentrations
Female Decreased spawning and cumulative Fecundity
Population Declining trajectory



Current Status of AOP Development



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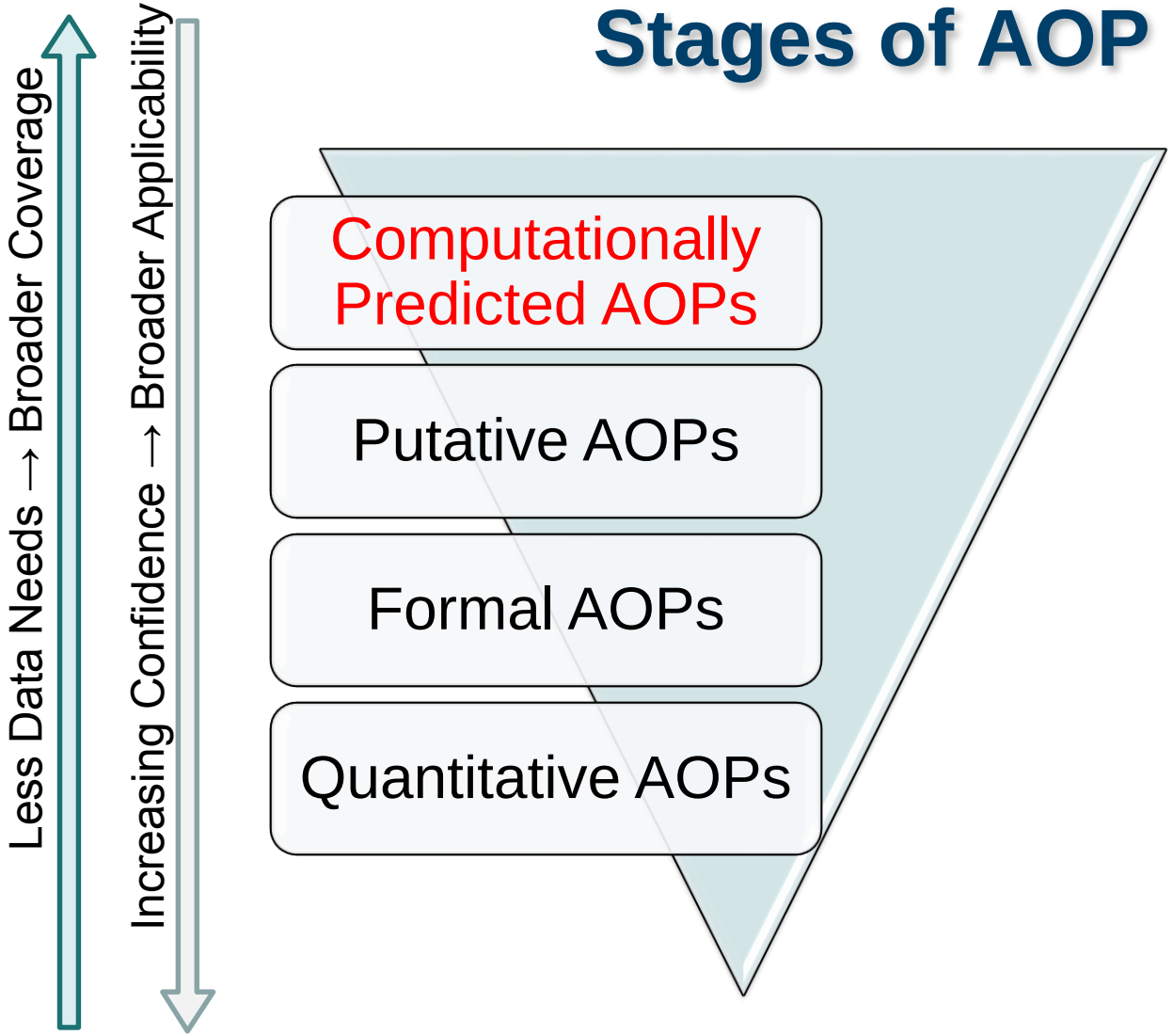
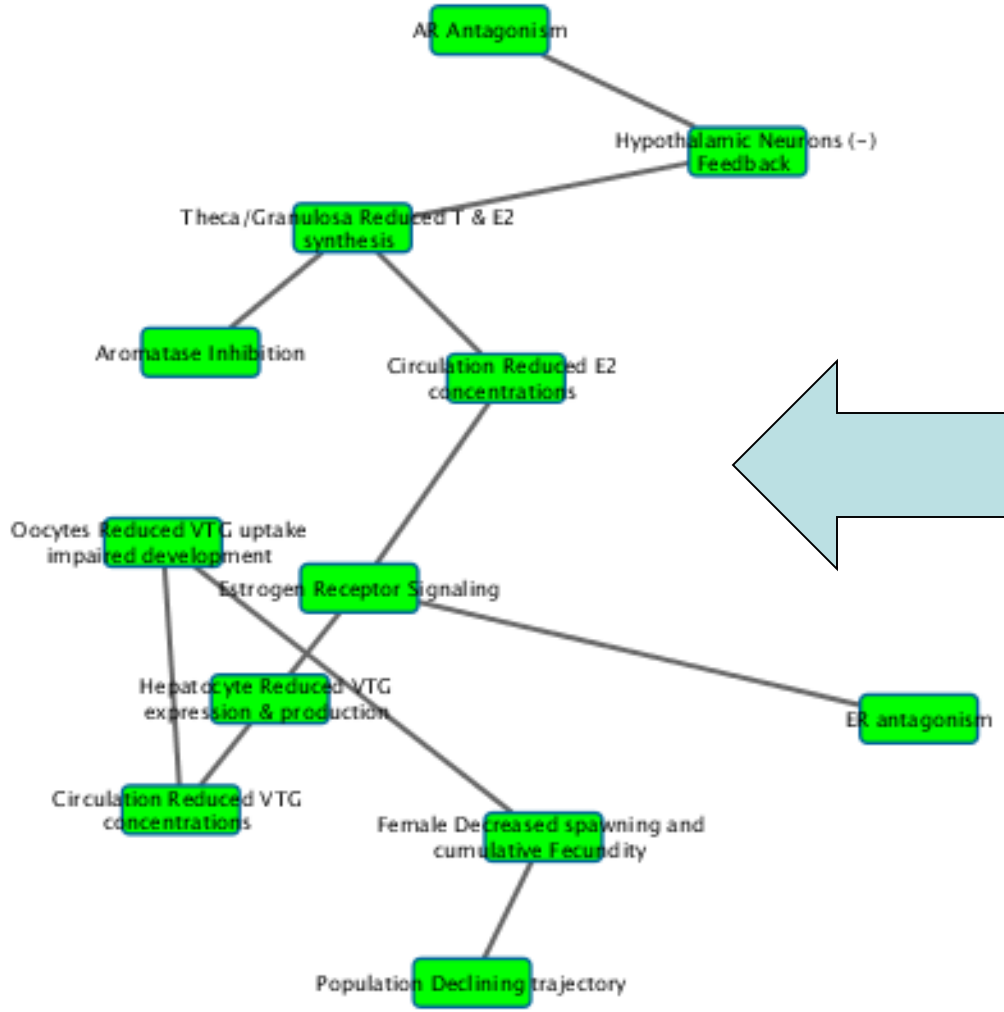
- 14 formal AOPs undergoing OECD review
- < 5 quantitative AOPs under development

Stages of AOP Development



- Still have many assays without AOPs
  - EPA ToxCast: >400 targets
  - ~40 have AOPs
- Also need AOPs -> targets for new assays
- Automated data-mining can create hypothetical AOPs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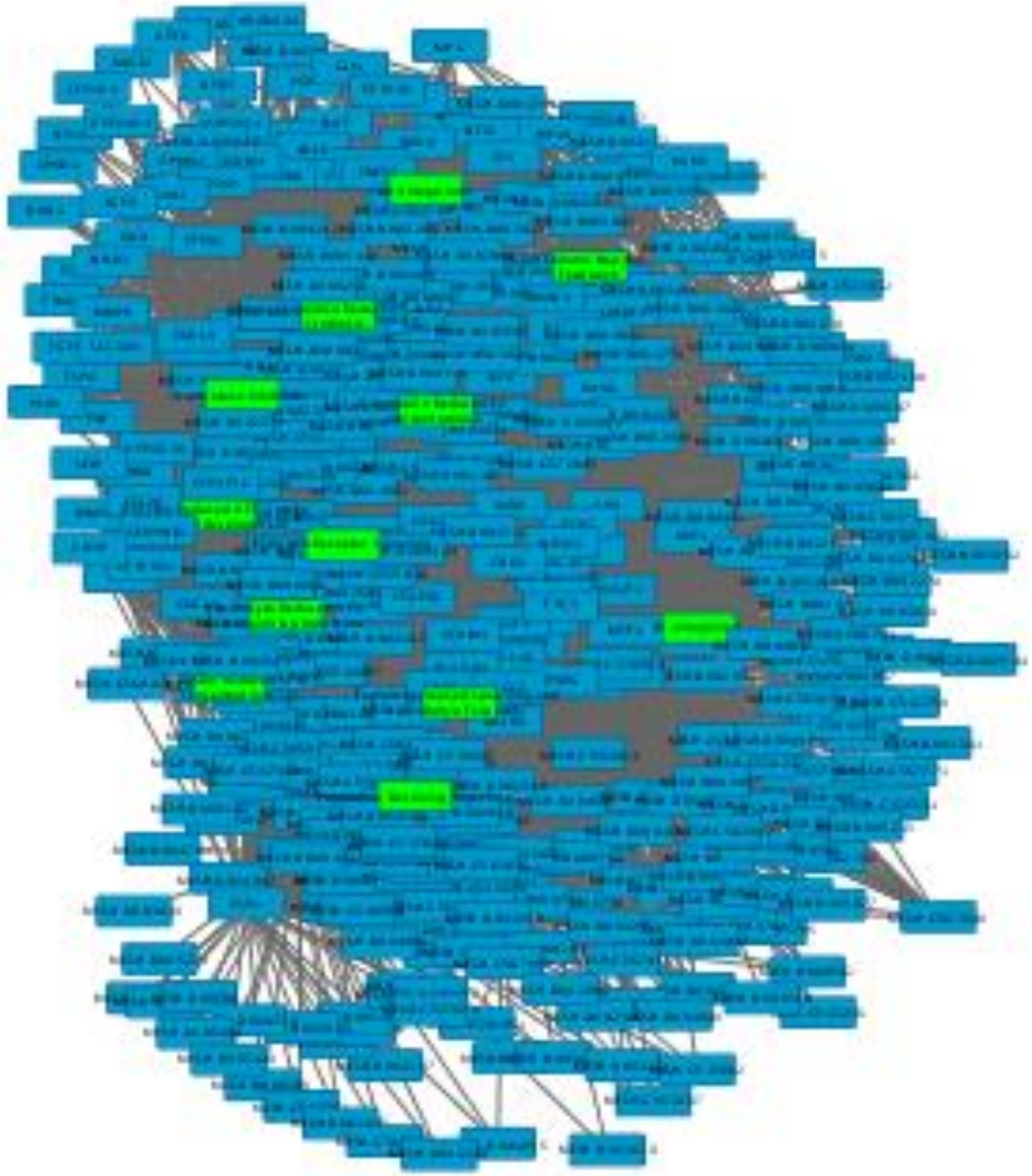
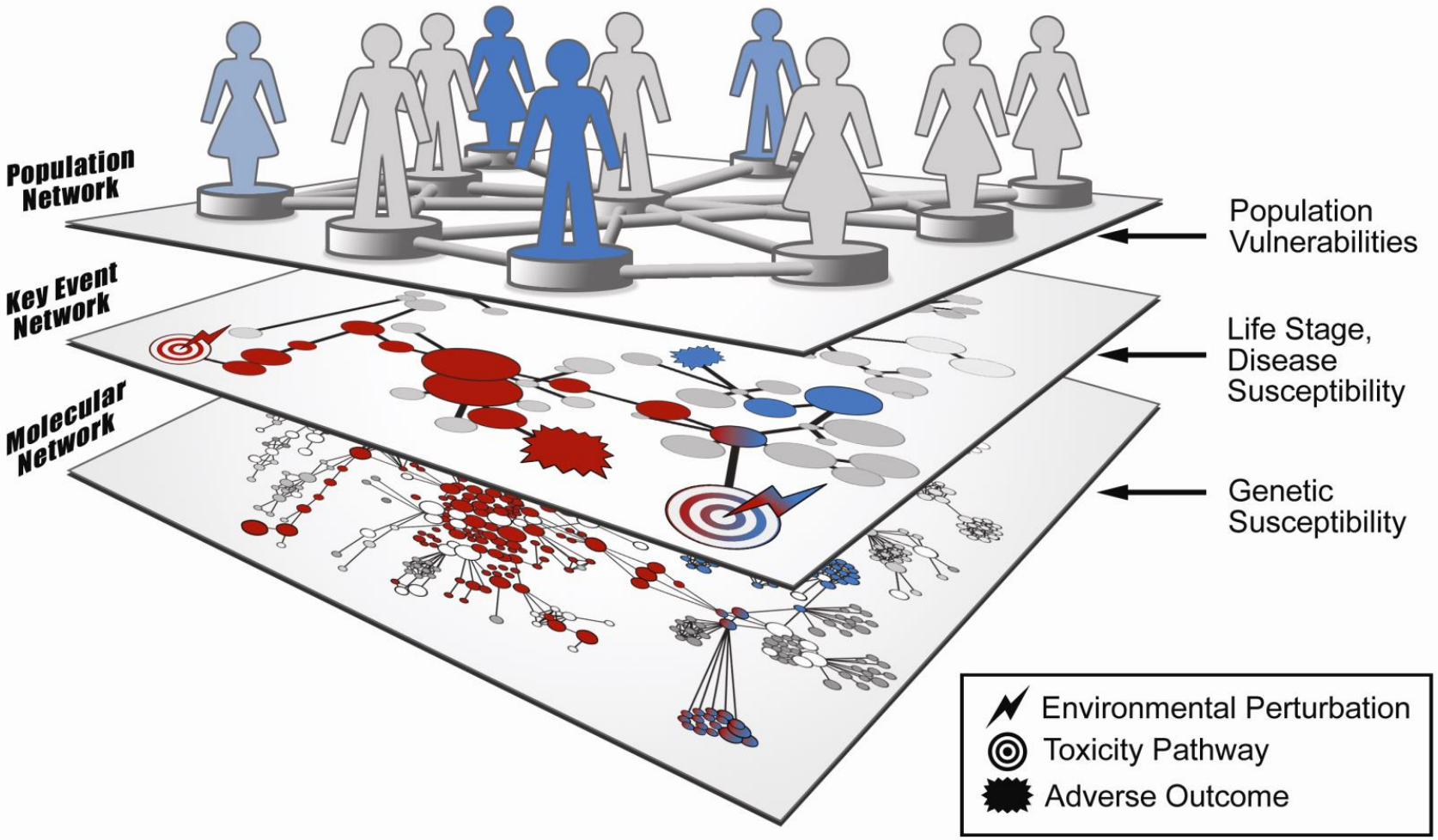


Non-chemical Factors Also Emerge from AOP Networks



Acknowledgements

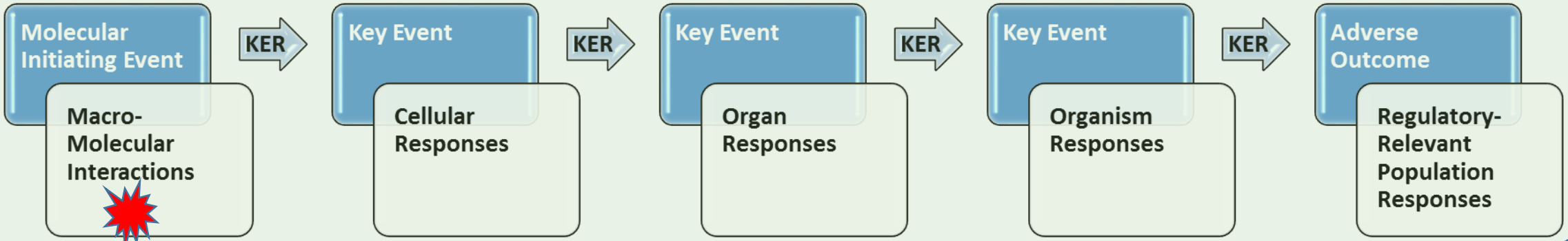
- AOP-Wiki Authors
- AOP-KB Development Team
- CSS AOP Discovery & Development Project Team
- OECD External Advisory Group on Molecular Screening & Toxicogenomics
- IPCS/WHO Mode of Action Steering Committee

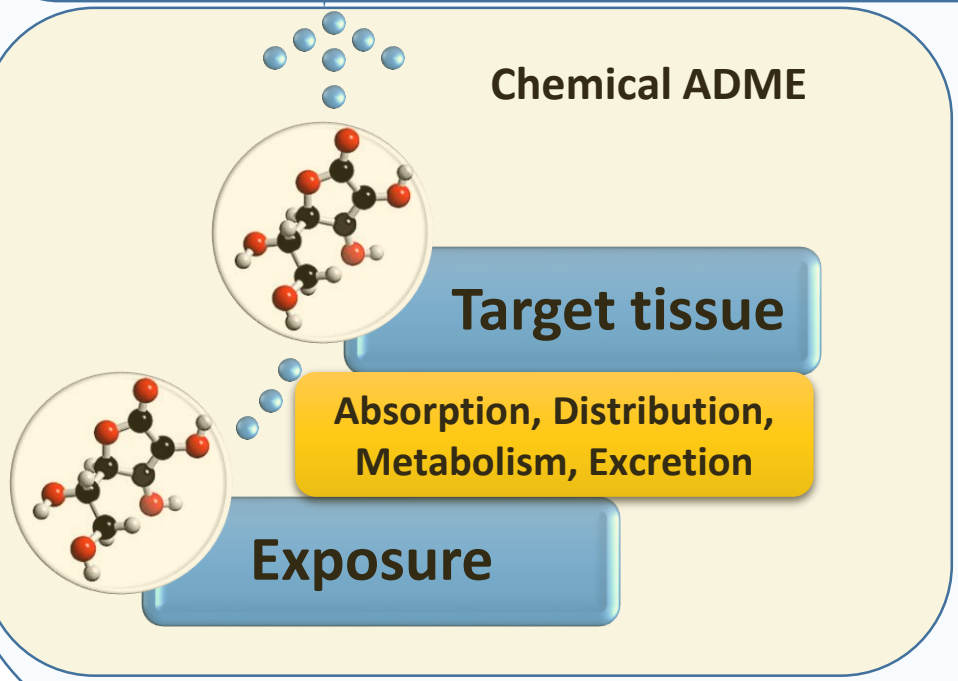
Questions?


Population Network
Key Event Network
Molecular Network
SYSTEMS TOXICOLOGY

Mode of Action

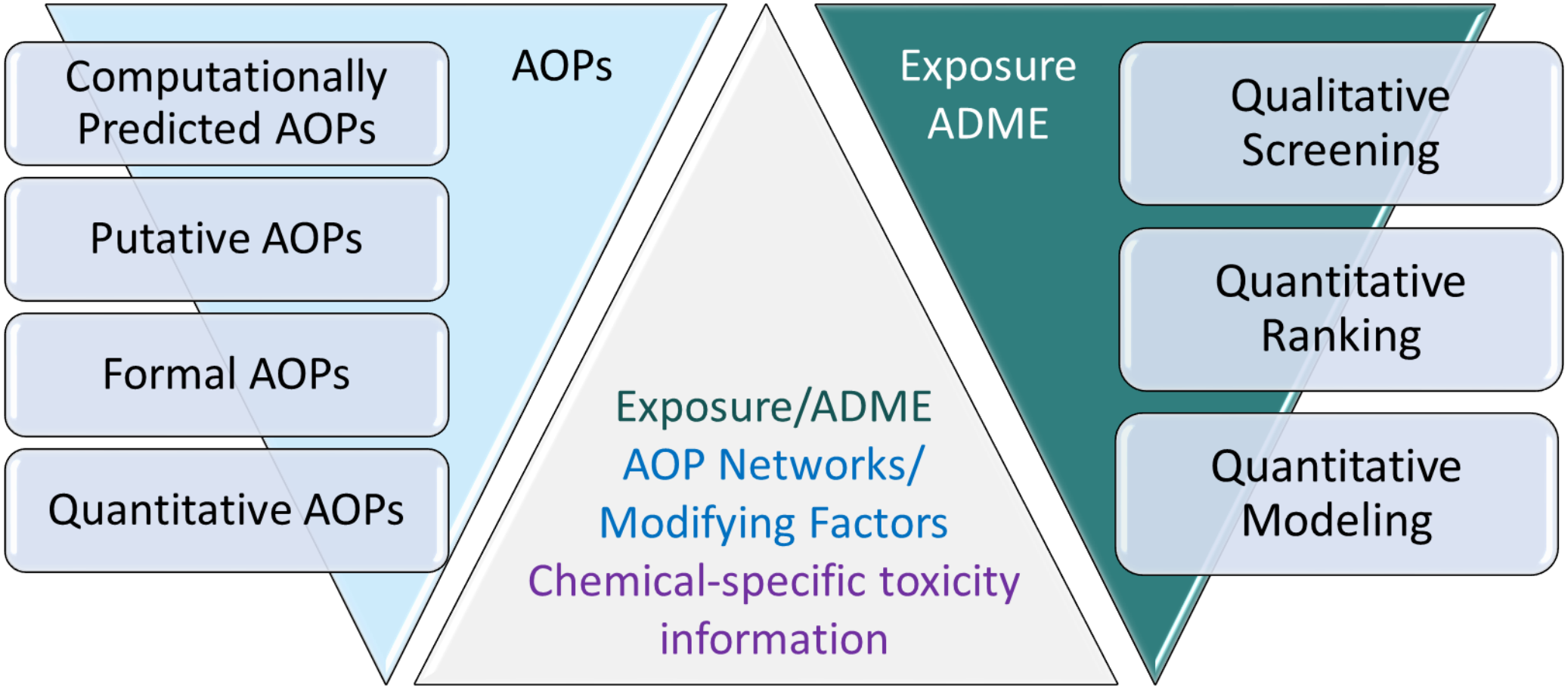
Adverse Outcome Pathway



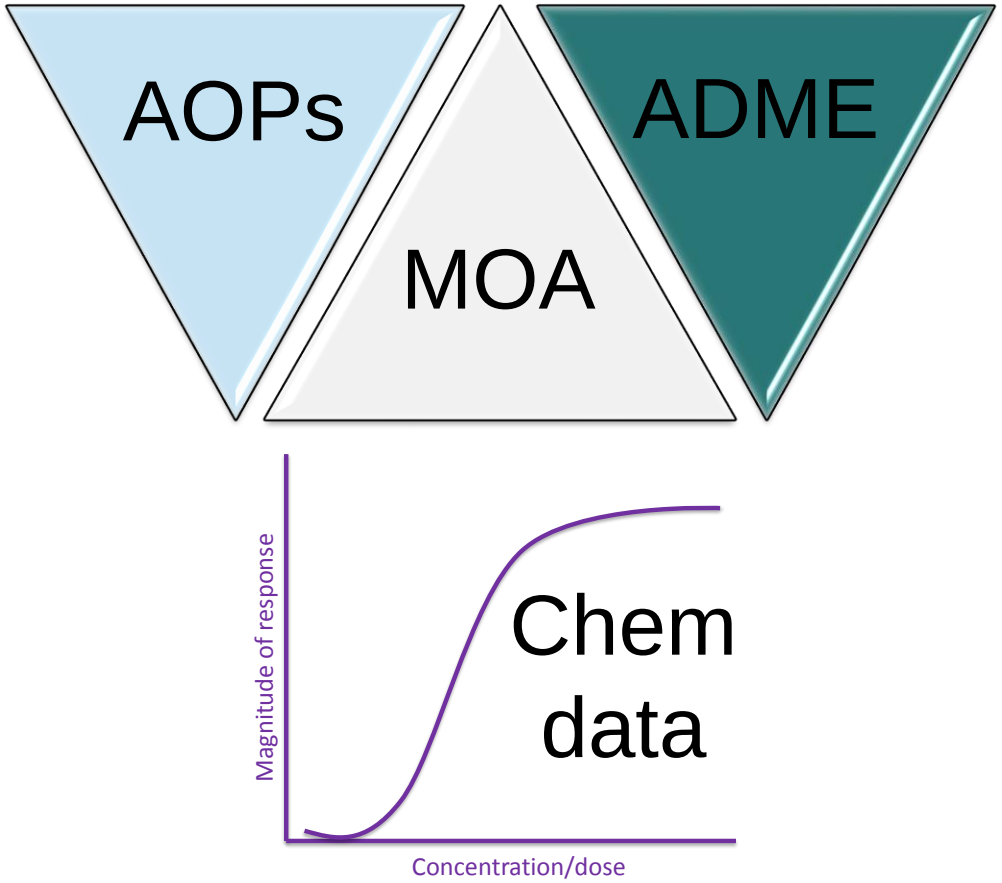


Edwards, et al., JPET, 2016 356:170-181.

Tiered Approach to ADME Complements AOP Info



Integrated Approaches to Testing and Assessment (IATA)



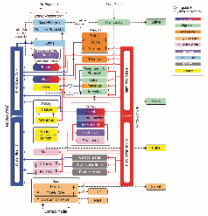
- AOP Confidence
- ADME Confidence
- Chemical Data
  - QSAR
  - Read across
  - HTS
  - In vivo laboratory
  - Epidemiology/field studies
- Exposure Predictions

Source to Outcome Continuum
Tox21, MOA, AOP, IATA

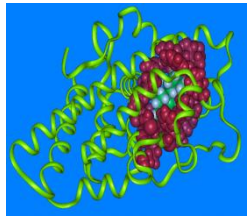
Source

Environmental Contaminant

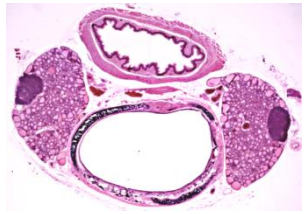
External Exposure



Internal Dose



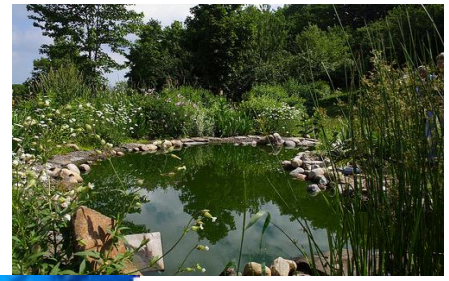
Key Event



Cellular Effects

Individual

Population



Community

What is regulated

Toxicity Pathway, NRC 2007

Criteria for regulation

Mode of Action, IPCS/EPA/ILSI 2001-2008

Exposure Predictions

Adverse Outcome Pathway, Ankley 2010, Villeneuve 2014

Acknowledgements

Chemical Safety for Sustainability cpAOP Team

- Core Team
  - Cataia Ives
  - Chris Grant
  - Holly Mortensen
  - Mark Nelms
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- EPA Collaborators
  - Joachim Pleil
  - Richard Judson
  - Matt Martin
  - Jimmy Phuong
  - Sean Watford
  - Keith Houck
  - Charles Wood
  - Michelle Angrish
- NCSU/MDIBL
  - Carolyn Mattingly
  - Benjamin King
  - Allan Peter Davis
- NIEHS
  - Scott Auerbach
  - Rebecca Boyles

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- Stephen Edwards
- David Lyons
- Dan Villeneuve
- Kevin Crofton
- Gary Ankley
- Robert Kavlock

- Clemens Wittwehr
- Brigitte Landesmann
- Marina Goumenou
- Sharon Munns
- Maurice Whelan

Hristo Aladjov
Joop DeKnecht

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  - Max Felsher
  - Rose Combs
  - Landon Grindheim
  - Cataia Ives

- Ed Perkins
- Lyle Burgoon
- Natalia Garcia Reyero
- Tanwir Habib

- OECD External Advisory Group on Molecular Screening & Toxicogenomics
- IPCS/WHO Mode of Action Steering Committee