



AOP Networks



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Outline

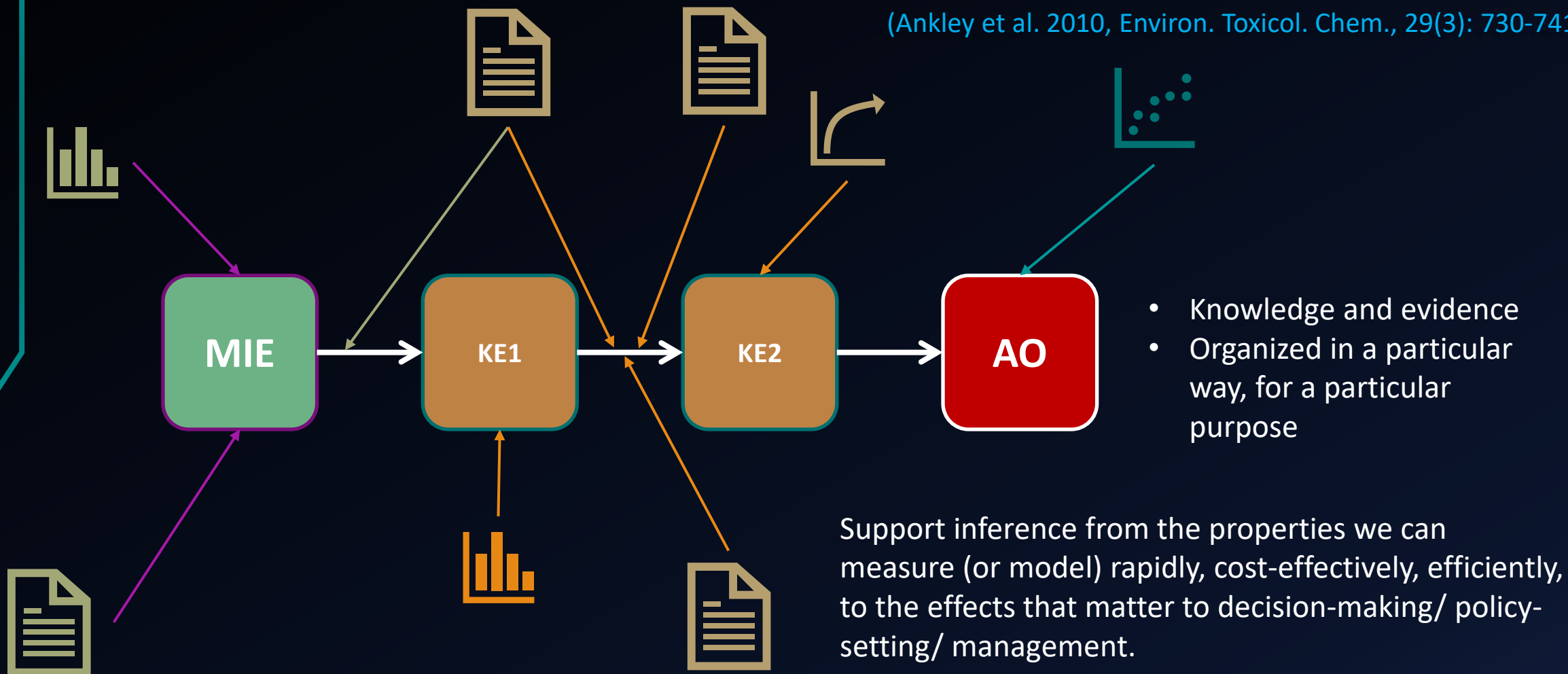
- What AOP networks are
- Navigating AOPN in AOP-Wiki
- Analysis of AOPN
- Example Applications



Adverse Outcome Pathways (AOPs)

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.)



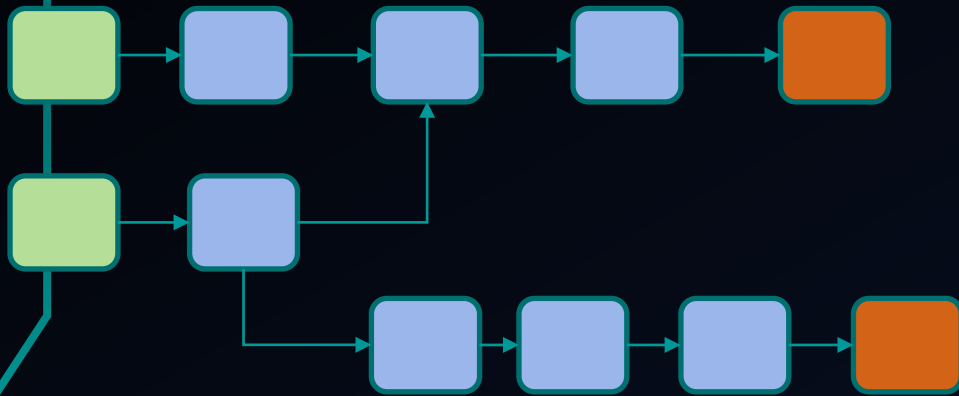
Principles of AOP development

1. AOPs are not stressor-specific – intended to capture generalizable biological phenomenon
2. AOPs are modular
3. An individual AOP is a pragmatic unit of development and evaluation – a single series of events linking one cause to one outcome of concern
4. Greater complexity is captured via networks of AOPs that share common KEs and/or KERs
5. AOPs are living documents and are expected to evolve over time (knowledge synthesis)



Definition

- An AOP network is an assembly of two or more AOPs that share one or more key events.



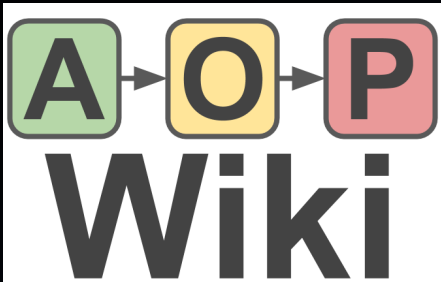
In the AOP-Wiki, AOPs that share one or more Event pages form a network

The image displays a smartphone screen showing the AOPWiki mobile interface. The URL bar shows 'test.aopwiki.org'. The page title is 'AOPWiki' and the subtitle is 'Welcome to the Adverse Outcome Pathway Wiki (AOP-Wiki)'. A red warning message states: 'This wiki is intended for testing purposes only. All content on this wiki may be lost at any time. To participate in the OECD AOP development effort, please visit <http://aopkb.org>.' Below this, a link to the HELP section is provided: 'Please refer to the HELP section on main wiki (https://aopwiki.org/info_pages/2) for help information.' The 'View Content' section includes buttons for 'AOPs', 'Key Events', and 'KE Relationships'. At the bottom, there are links for 'Contribute' and 'Cookie Policy'.

To the right of the smartphone, a diagram illustrates the structure of an AOP Page. It shows a central 'AOP Page' box with a header and a table of content. Dashed lines connect this box to two separate lists of page types:

- KE Pages**
 - Description
 - Measurement/detection
 - Domain of applicability
- KER Pages**
 - Title
 - Description
 - Biological plausibility
 - Empirical support
 - Inconsistencies and uncertainties
 - Quantitative understanding

The text 'Aopwiki.org' is written below the diagram.



AOP Networks in the AOP-Wiki

- Event pages:

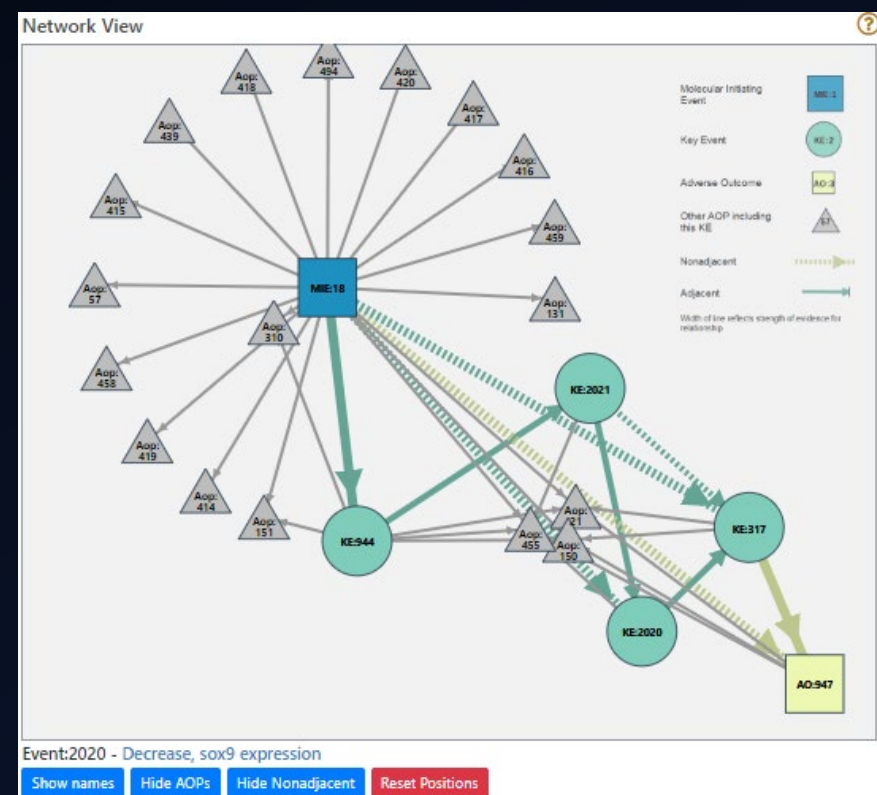
AOP Name	Role of event in AOP	Point of Contact	Author Status	OECD Status
AhR mediated mortality, via COX-2	MolecularInitiatingEvent	Markus Hecker (send email)	Open for citation & comment	WPHA/WNT Endorsed
AhR activation to steatosis	MolecularInitiatingEvent	Michelle Angrish (send email)	Under Development: Contributions and Comments Welcome	
AhR activation-uroporphyrin	MolecularInitiatingEvent	Amani Farhat (send email)	Open for citation & comment	WPHA/WNT Endorsed
AhR activation to ELS mortality, via VEGF	MolecularInitiatingEvent	Amani Farhat (send email)	Open for citation & comment	WPHA/WNT Endorsed

Activation, AhR
19 AOPs

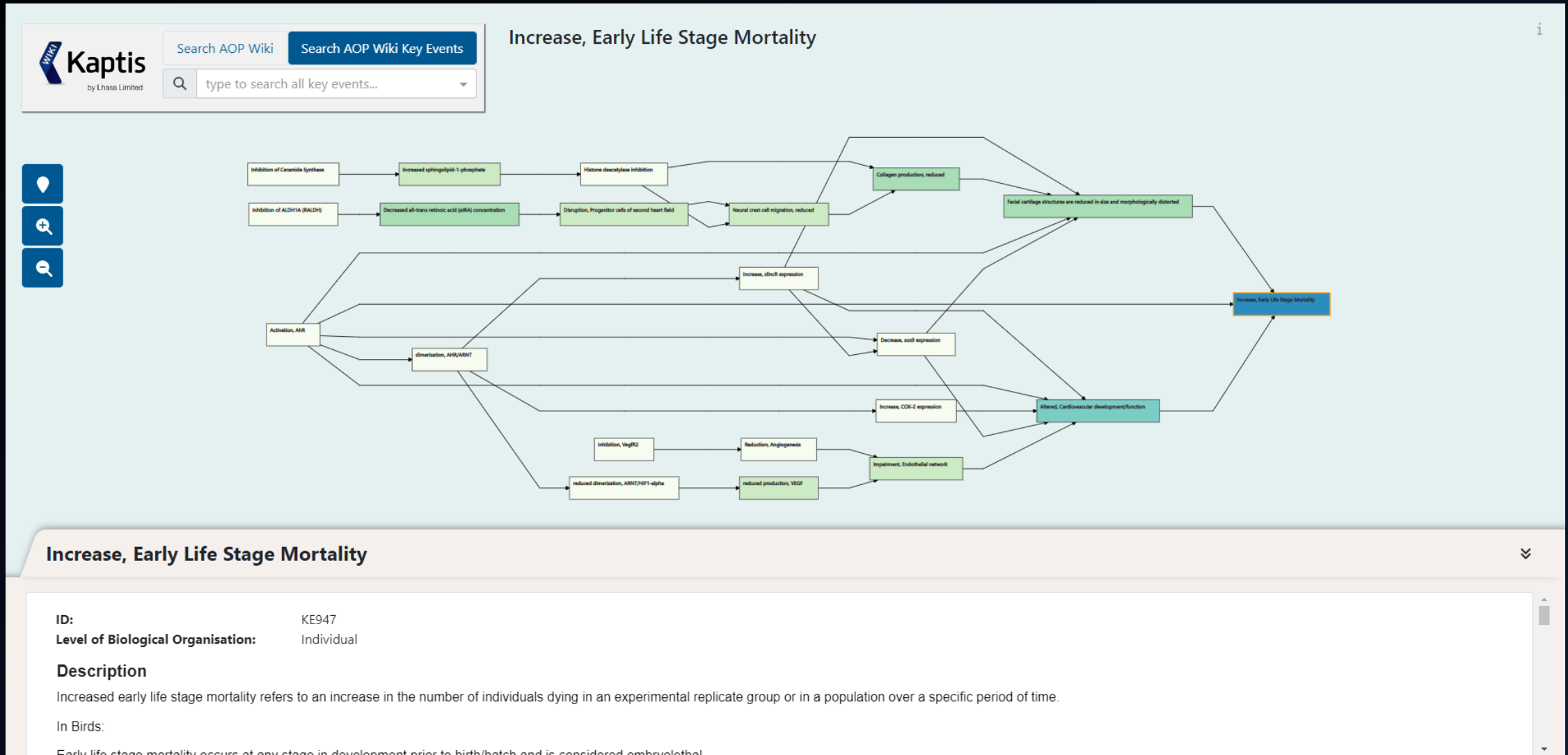
AOP Name	Role of event in AOP	Point of Contact	Author Status	OECD Status
Ahr mediated early stage mortality via craniofacial malformations	KeyEvent	Prarthana Shankar (send email)	Under development: Not open for comment. Do not cite	
Ahr mediated early stage mortality via cardiovascular toxicity	KeyEvent	Prarthana Shankar (send email)	Under development: Not open for comment. Do not cite	

Decrease, sox9 expression
2 AOPs

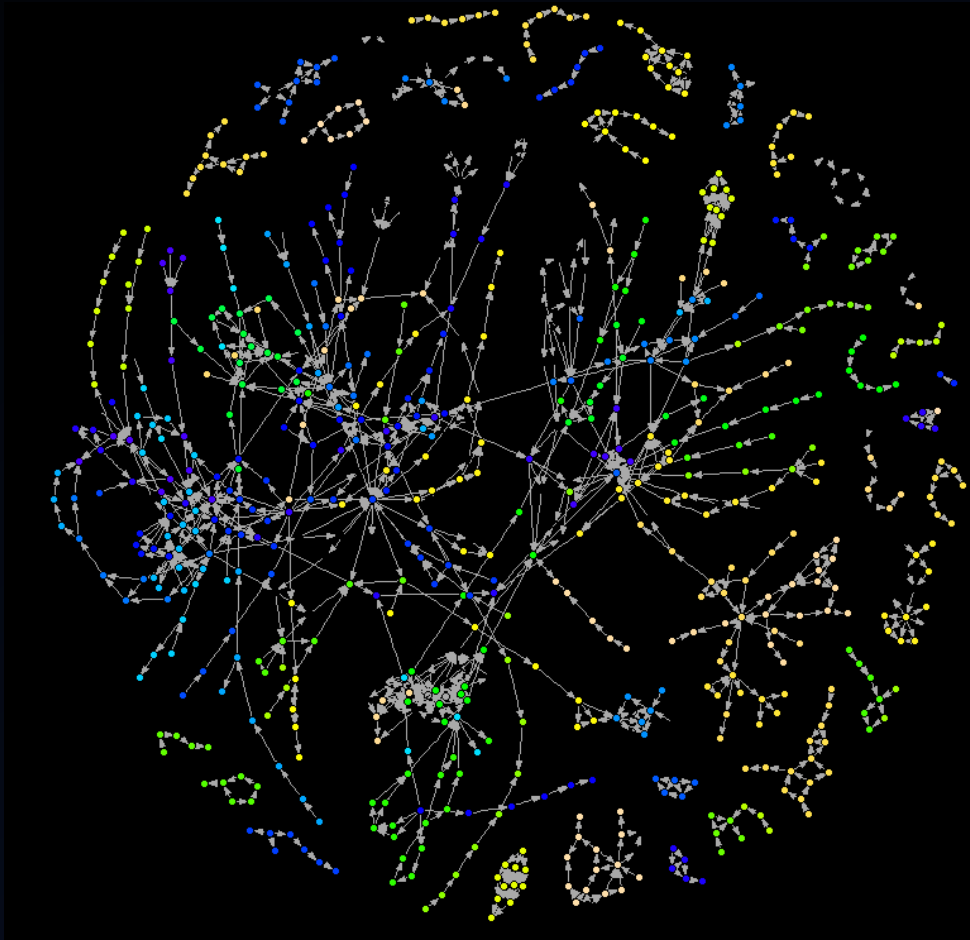
- AOP pages (network view):



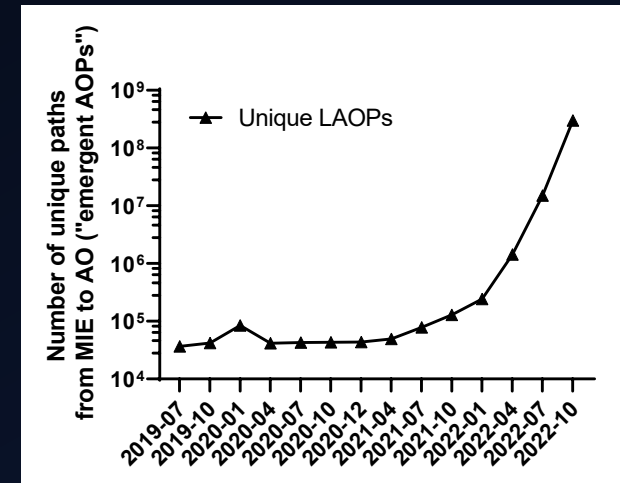
Wiki Kaptis – “3rd party tool” – can be used to visualize and interact with AOPN



Global AOP-network



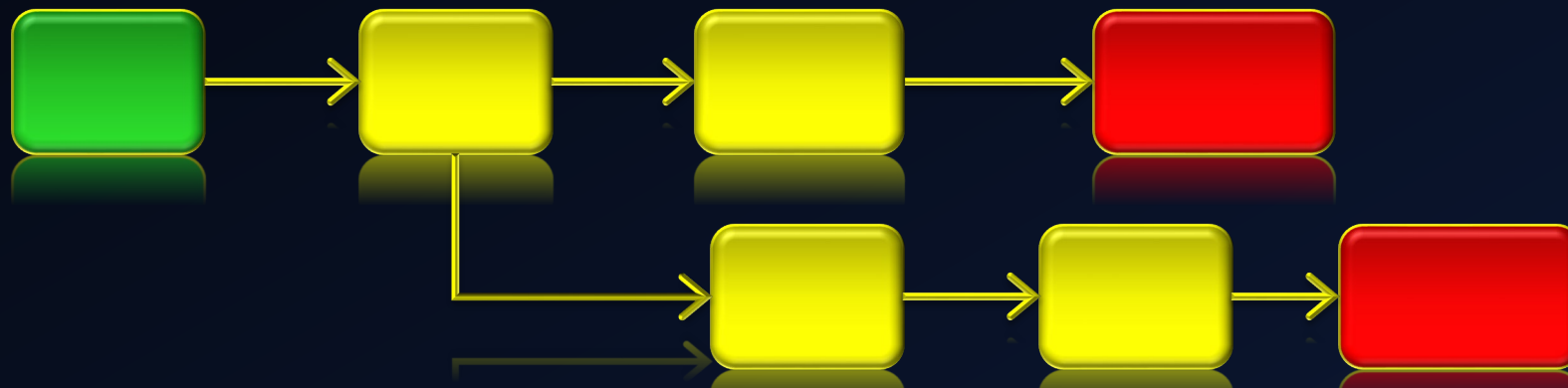
- October 2022
 - 416 user specified AOPs
 - 1197 Events
 - 1710 relationships
 - Over 1.4 million unique paths from MIE to AO – increasing exponentially

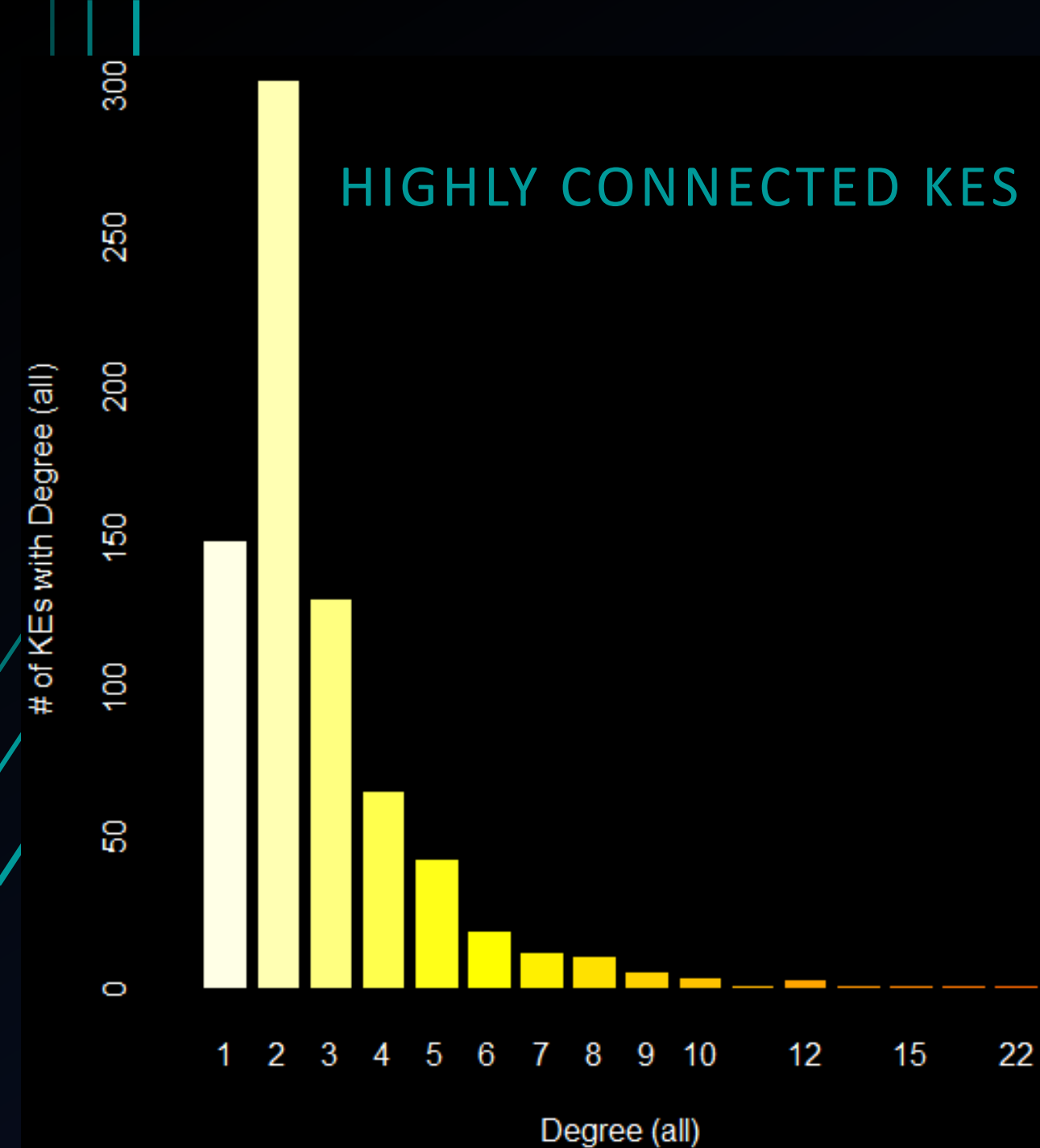


Analyzing AOP Networks

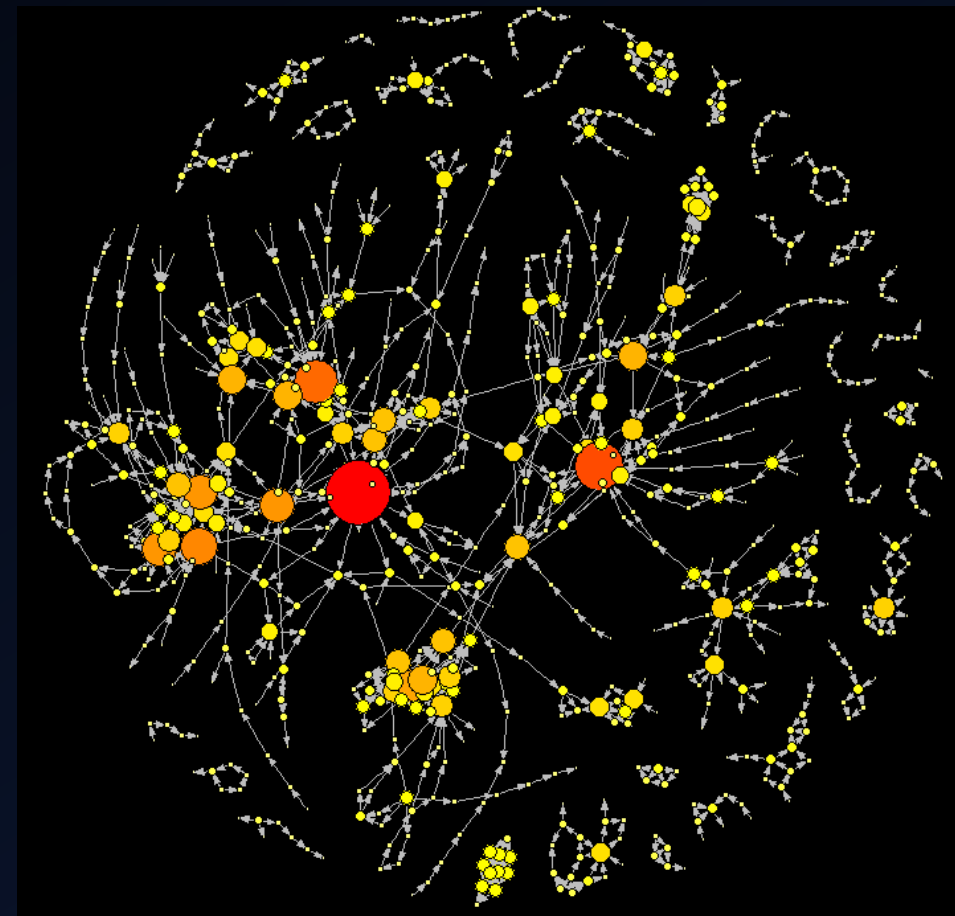
Network	Nodes	Edges
Transportation	Stations	Routes between stations
Computer	Computers and servers	Data transmission
Social networks	People	Relationships
Molecular	Genes, proteins	Interactions
Food web	Species	Energy flow
AOP	Key events	Key Event Relationships

- Particularly as AOP networks get larger, computational analyses of AOP networks may be helpful in identifying important features.
- Lots of approaches from graph theory and network science can be applied and adapted to analyze AOP networks.

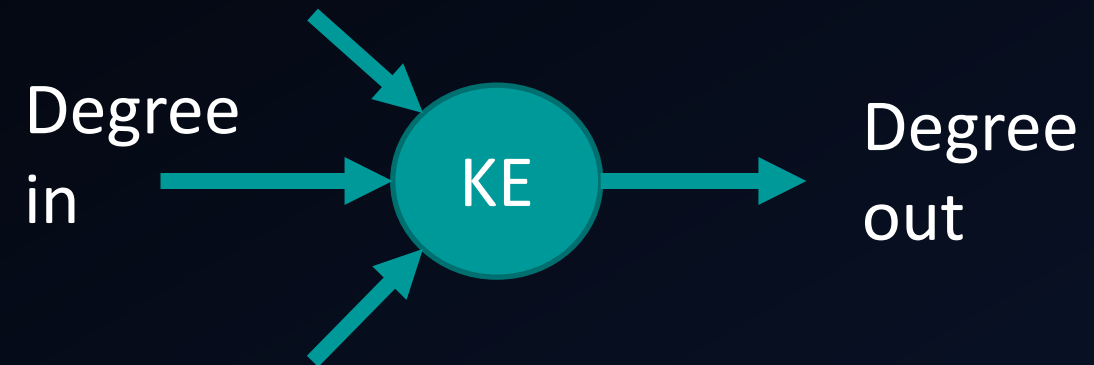
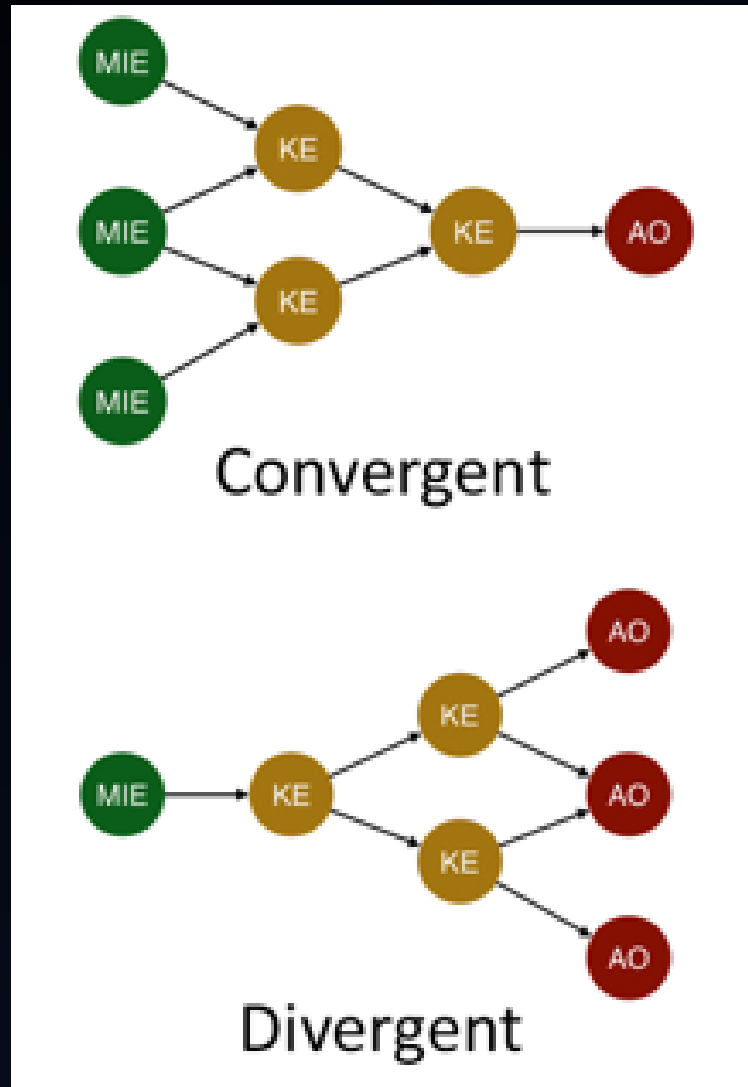




Increased oxidative stress, is a highly connected node in the AOPN.



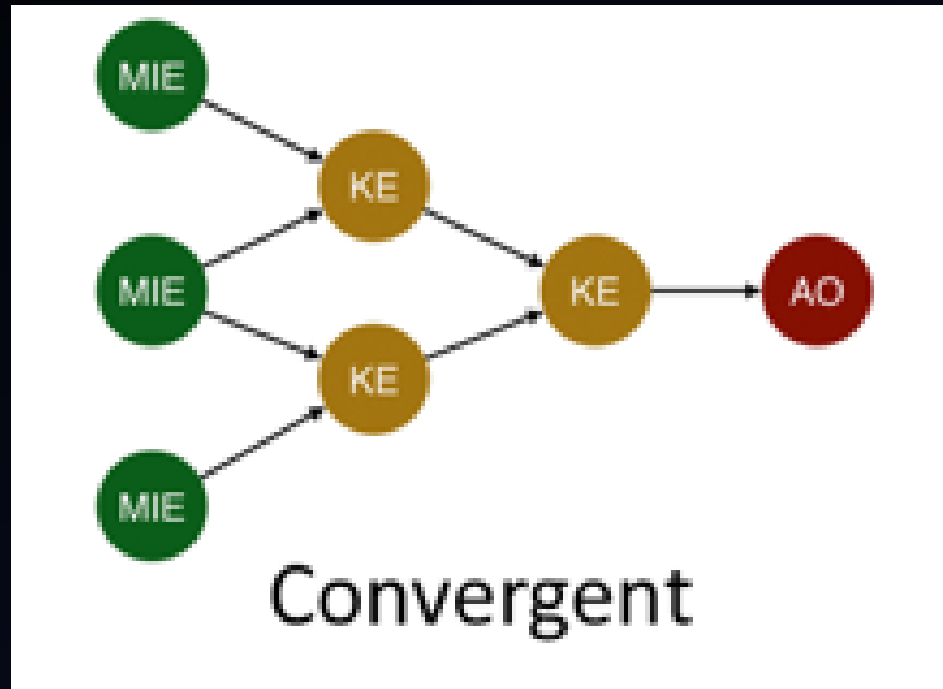
How can we identify important network features?



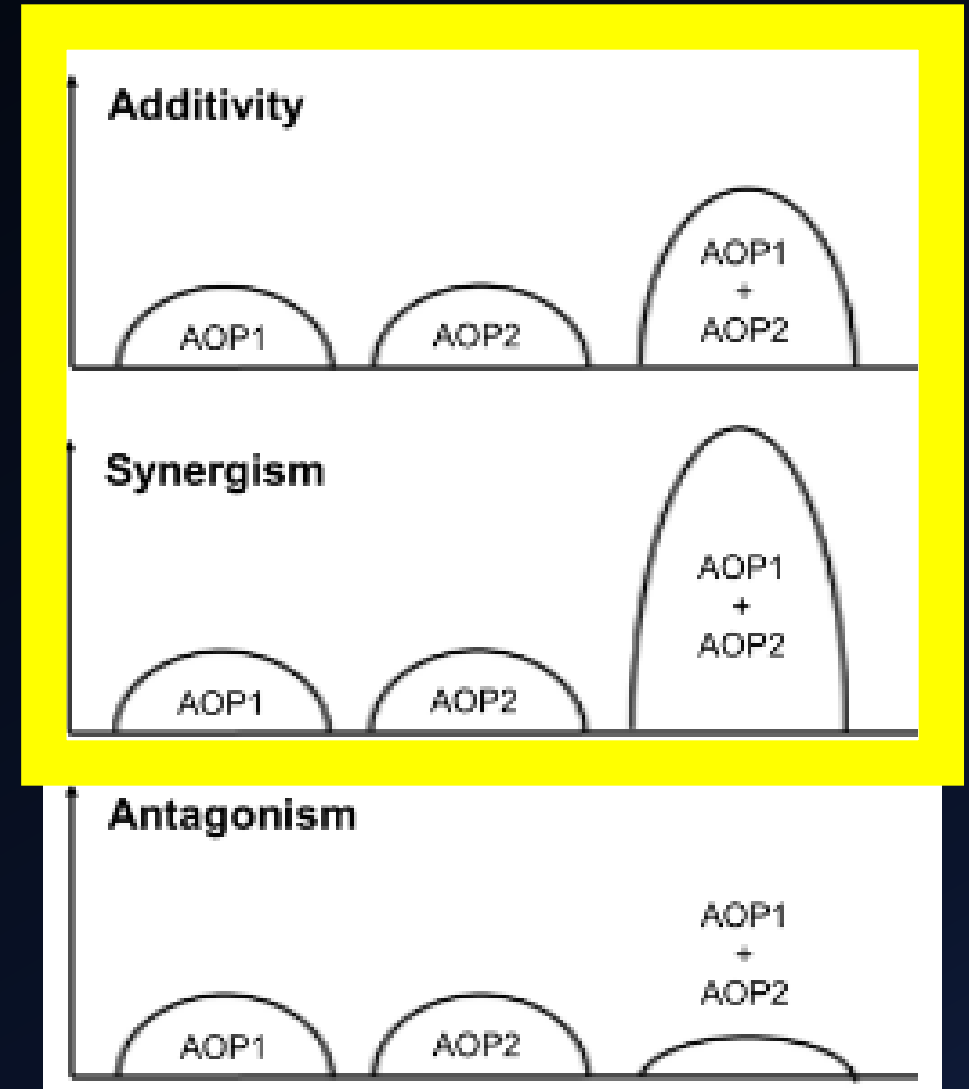
Degree in > Degree out = convergent

Degree in < degree out = divergent

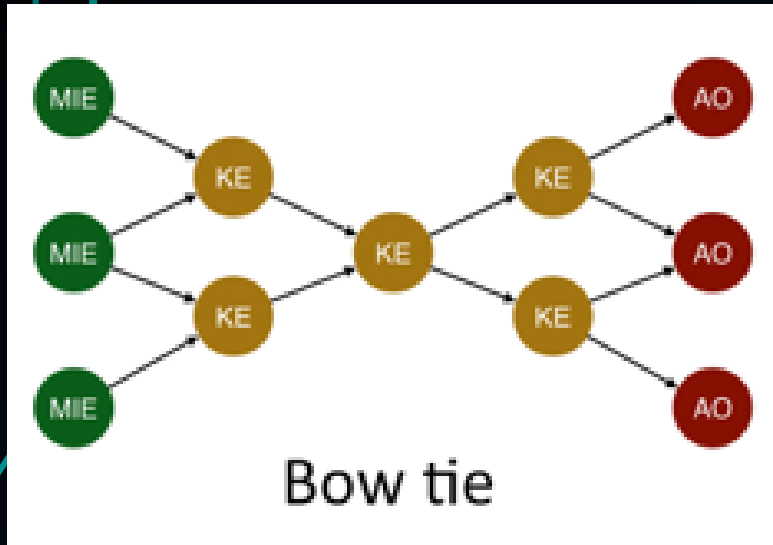
What do they reveal about interactive effects?



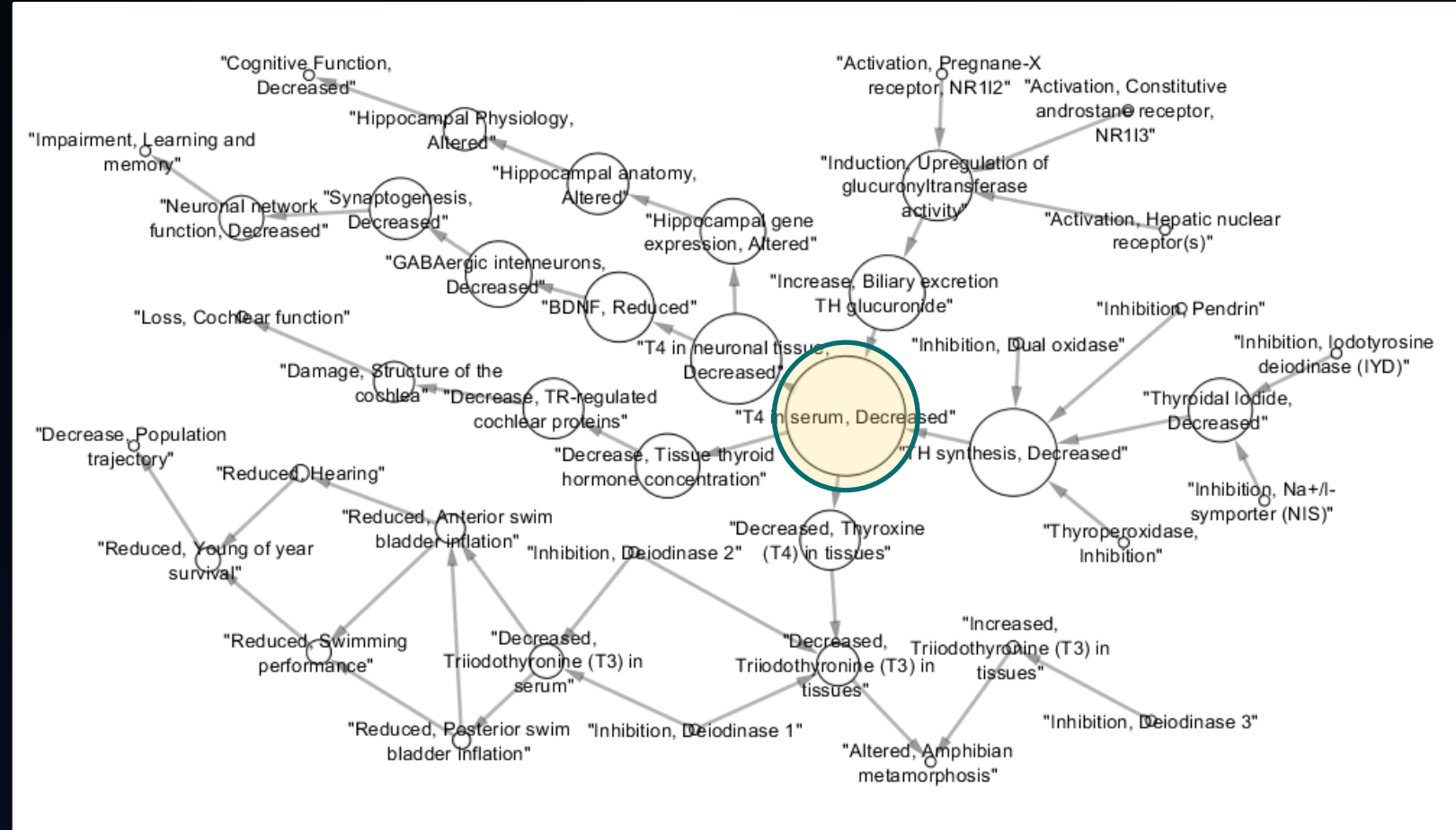
Perturbation Intensity



How can we identify important network features?



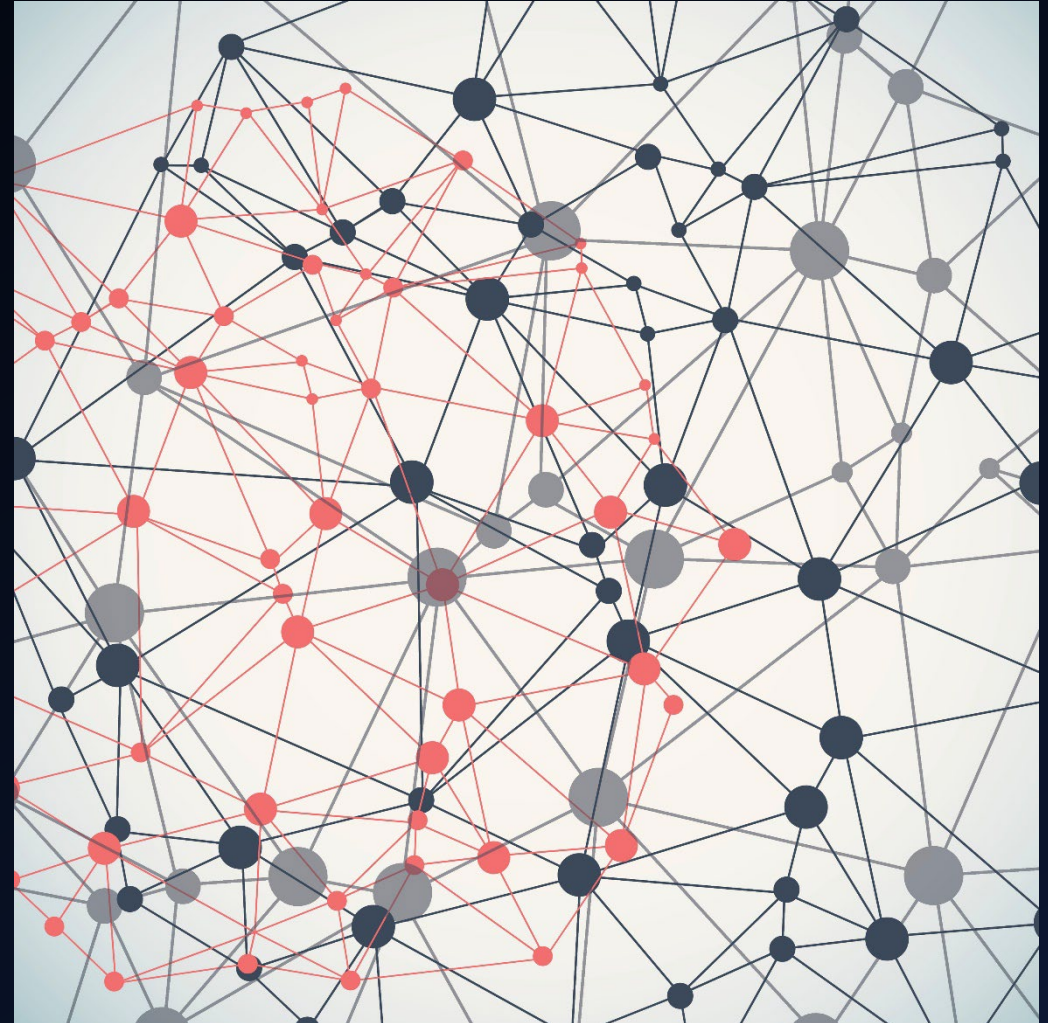
Betweenness centrality



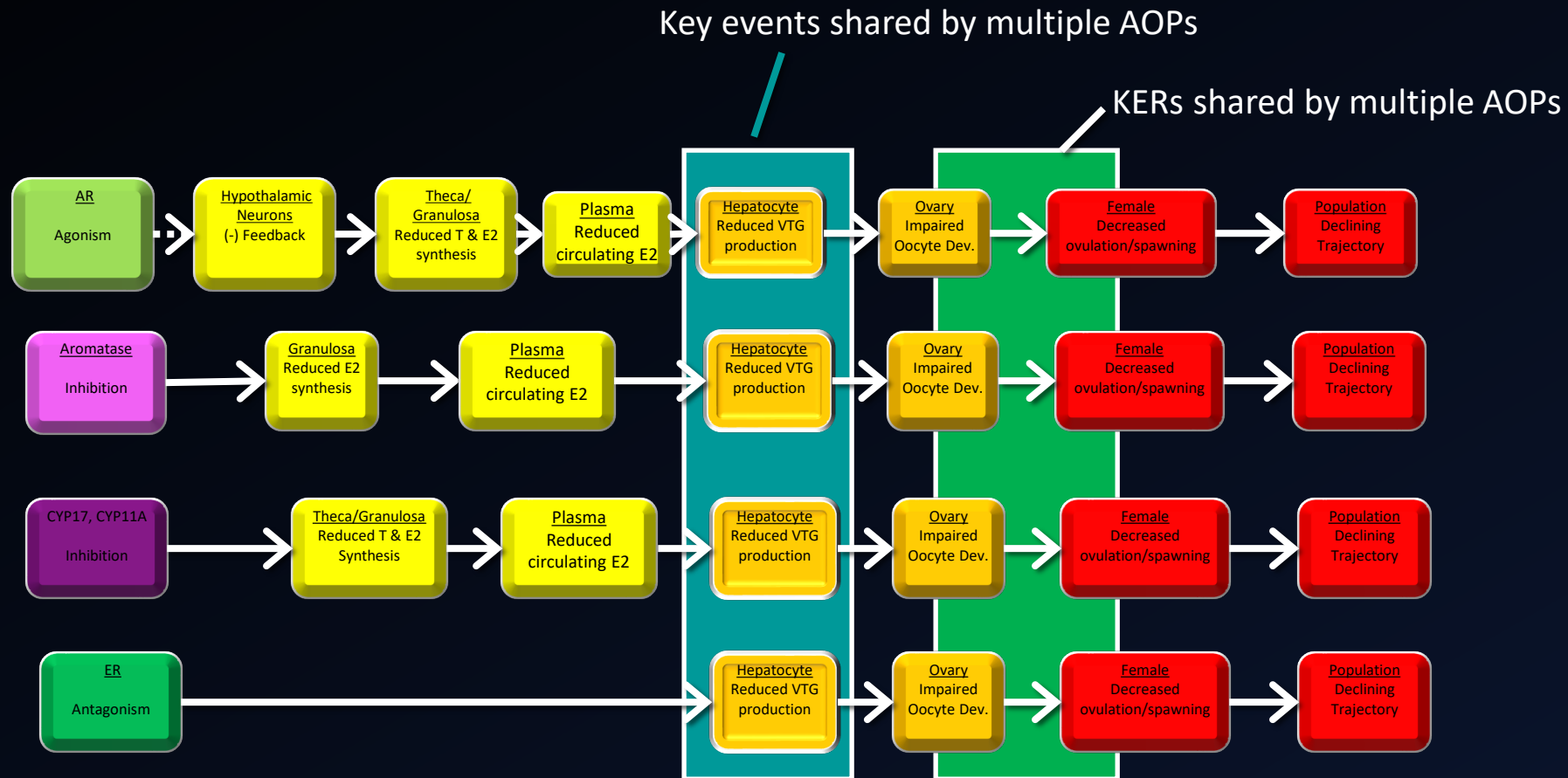
Measure of the number of *shortest paths* between any two nodes (j, k) in the network that pass through the node of interest (i) (Kitsak et al. 2007).

How are AOP Networks Useful?

- Pragmatic development
- Design of tiered testing frameworks
- Multiple stressors
- Single stressors – multiple modes of action

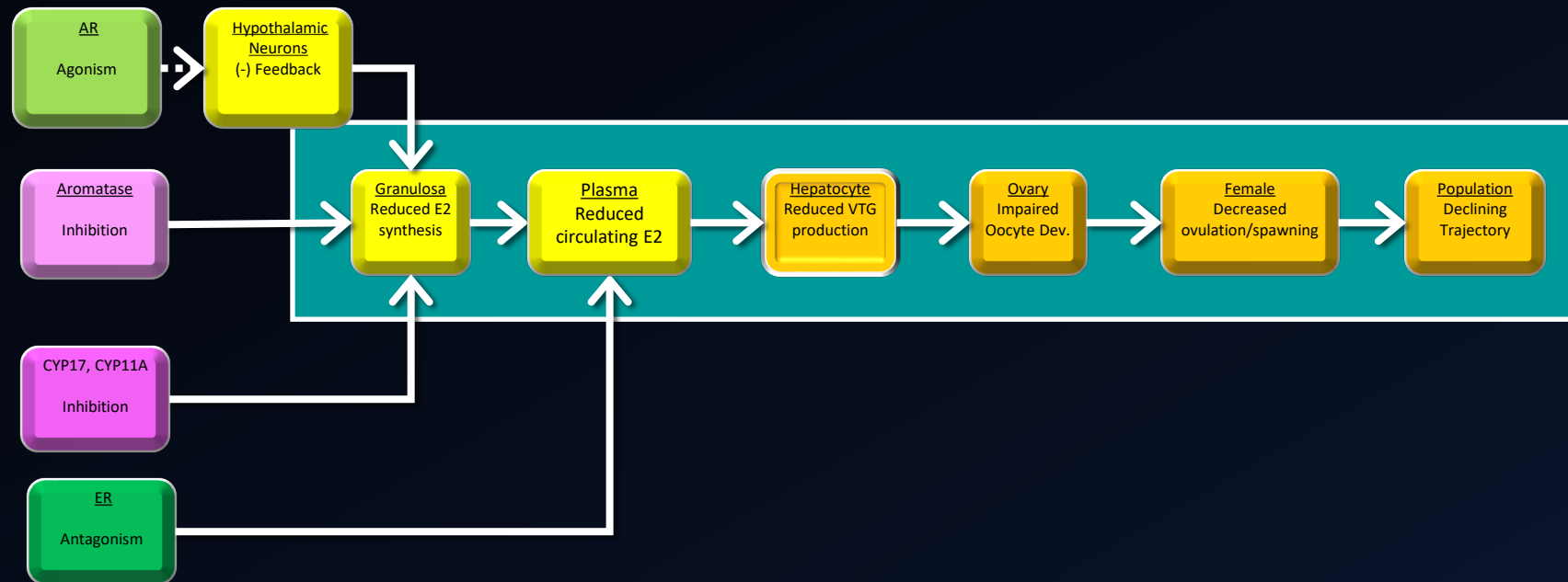


Pragmatic AOP Development



Pragmatic AOP Development

- No need to create page de novo each time it is used
- Can simply use or add to existing pages in the AOP-Wiki



ET&C PATHWAYS AND PREDICTIONS

Pathways and Predictions articles are summaries of multi-process biological responses to chemicals described by extensive datasets. Adverse outcome pathways (AOPs) are one example of this where comprehensive compilations of concepts and evidence comprising a given AOP can be obtained from an open-source AOP Wiki (aopwiki.org).

AOP Report: Adverse Outcome Pathways for Aromatase Inhibition or Androgen Receptor Agonism Leading to Male-Biased Sex Ratio and Population Decline in Fish

Gerald T. Ankley,^{a,*} Kelvin Santana-Rodriguez,^b Kathleen M. Jensen,^a David H. Miller,^c and Daniel L. Villeneuve^a

^aGreat Lakes Toxicology and Ecology Division, US Environmental Protection Agency, Duluth, Minnesota

^bOak Ridge Institute for Science and Education, Research Participant at Great Lakes Toxicology and Ecology Division, US Environmental Protection Agency, Duluth, Minnesota

^cGreat Lakes Toxicology and Ecology Division, US Environmental Protection Agency, Ann Arbor, Michigan

Modular organization of the information supports more efficient development of AOPs within an AOP network.

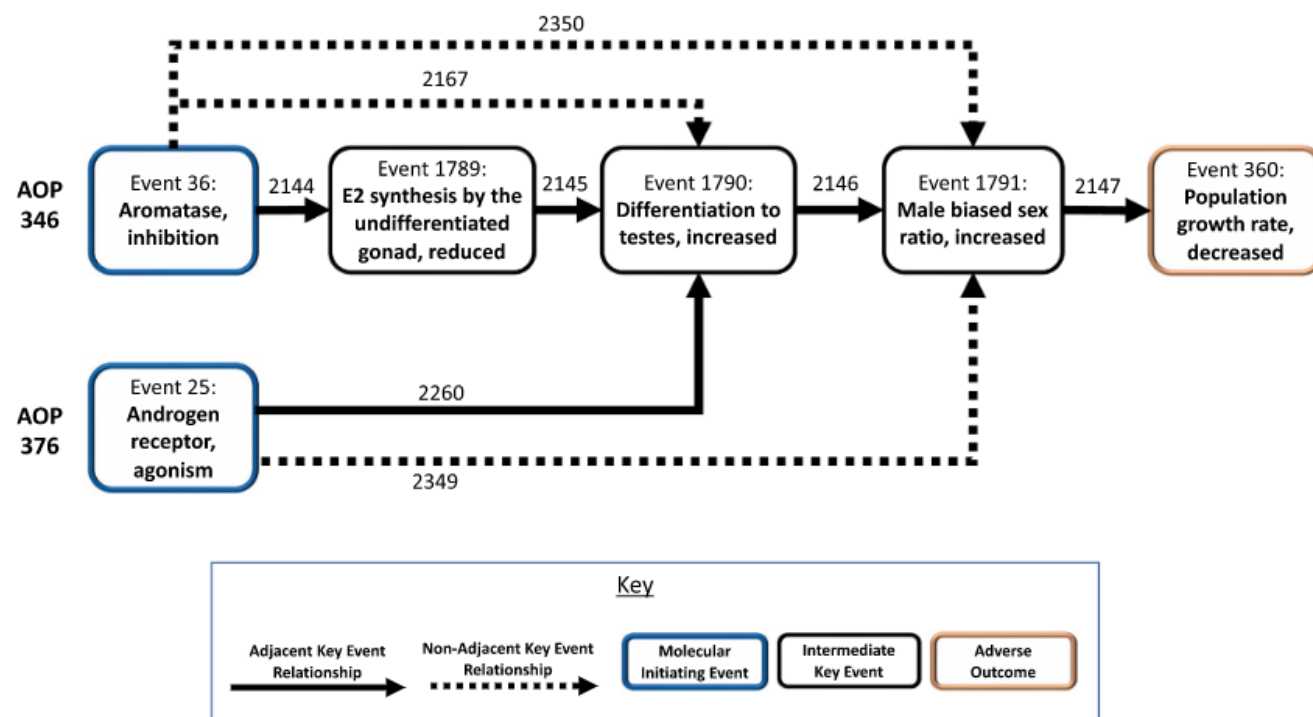


FIGURE 1: Graphical representation of adverse outcome pathways 346 and 376 (Society for the Advancement of Adverse Outcome Pathways, n.d.). AOP = adverse outcome pathway; E2 = 17 β -estradiol.

AOP 346

- Creation of 3 new KEs
- 6 new KERs

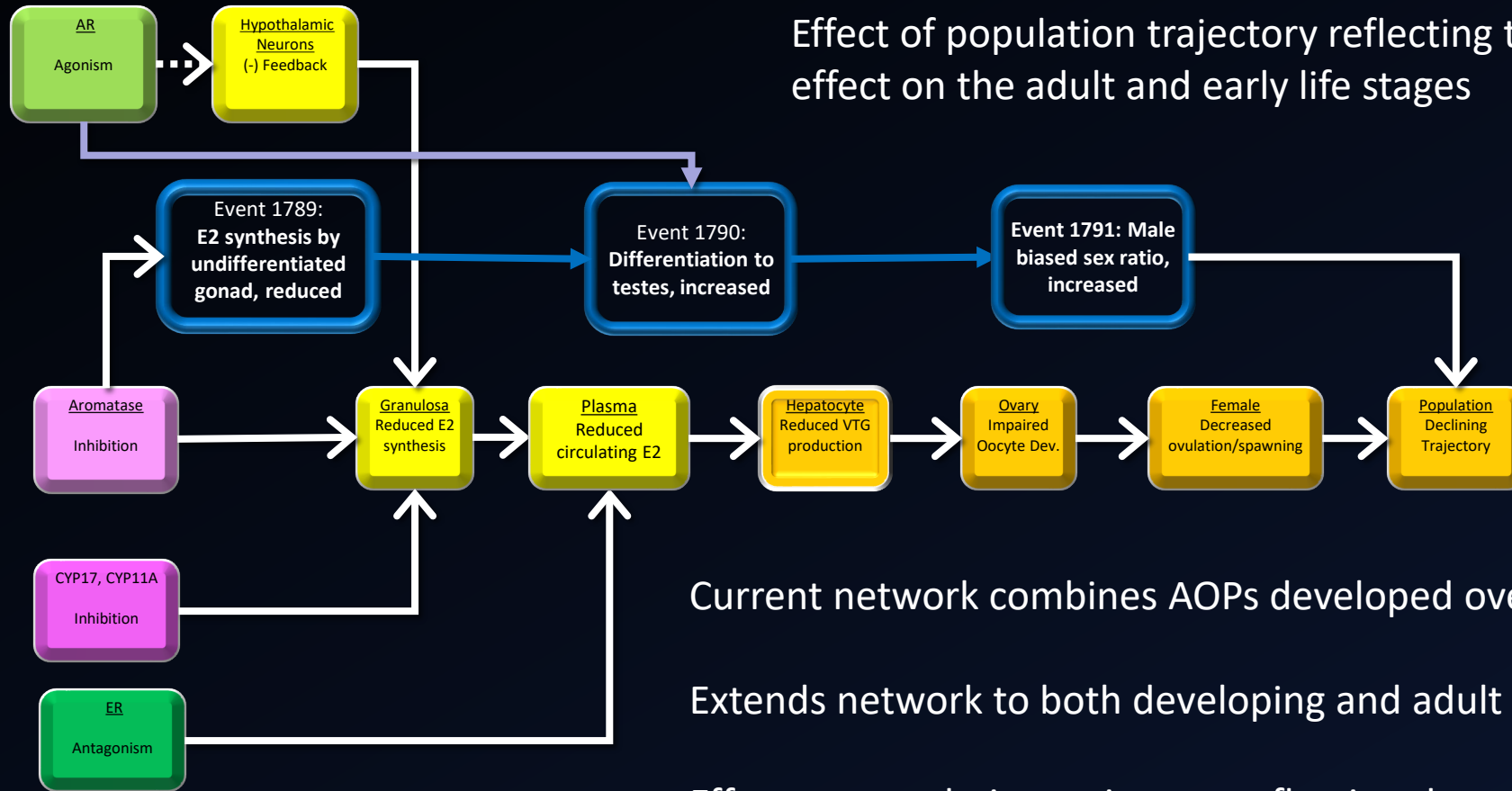
AOP 376

- 0 new KEs
- 2 new KERs

Practical Implication

Extends network to both developing and adult life stages

Effect of population trajectory reflecting the combined effect on the adult and early life stages



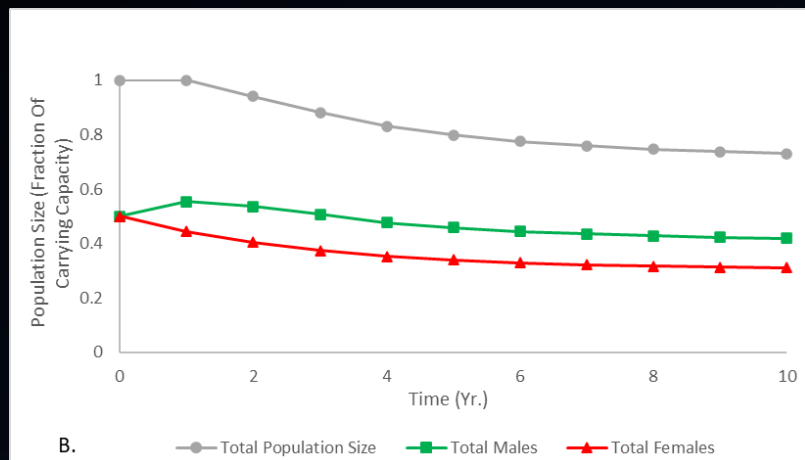
Current network combines AOPs developed over a 10 year span

Extends network to both developing and adult life stages

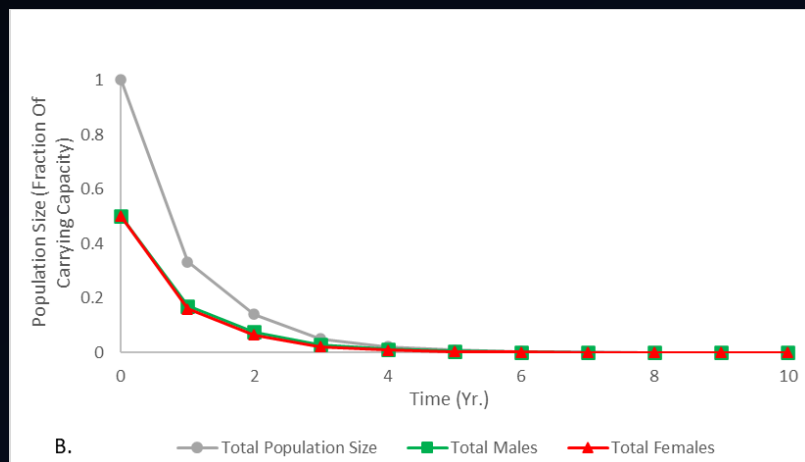
Effect on population trajectory reflecting the combined effect on the adult and early life stages

Practical Application

Population at carrying capacity subsequently exposed to 100 μg prochloraz/L.

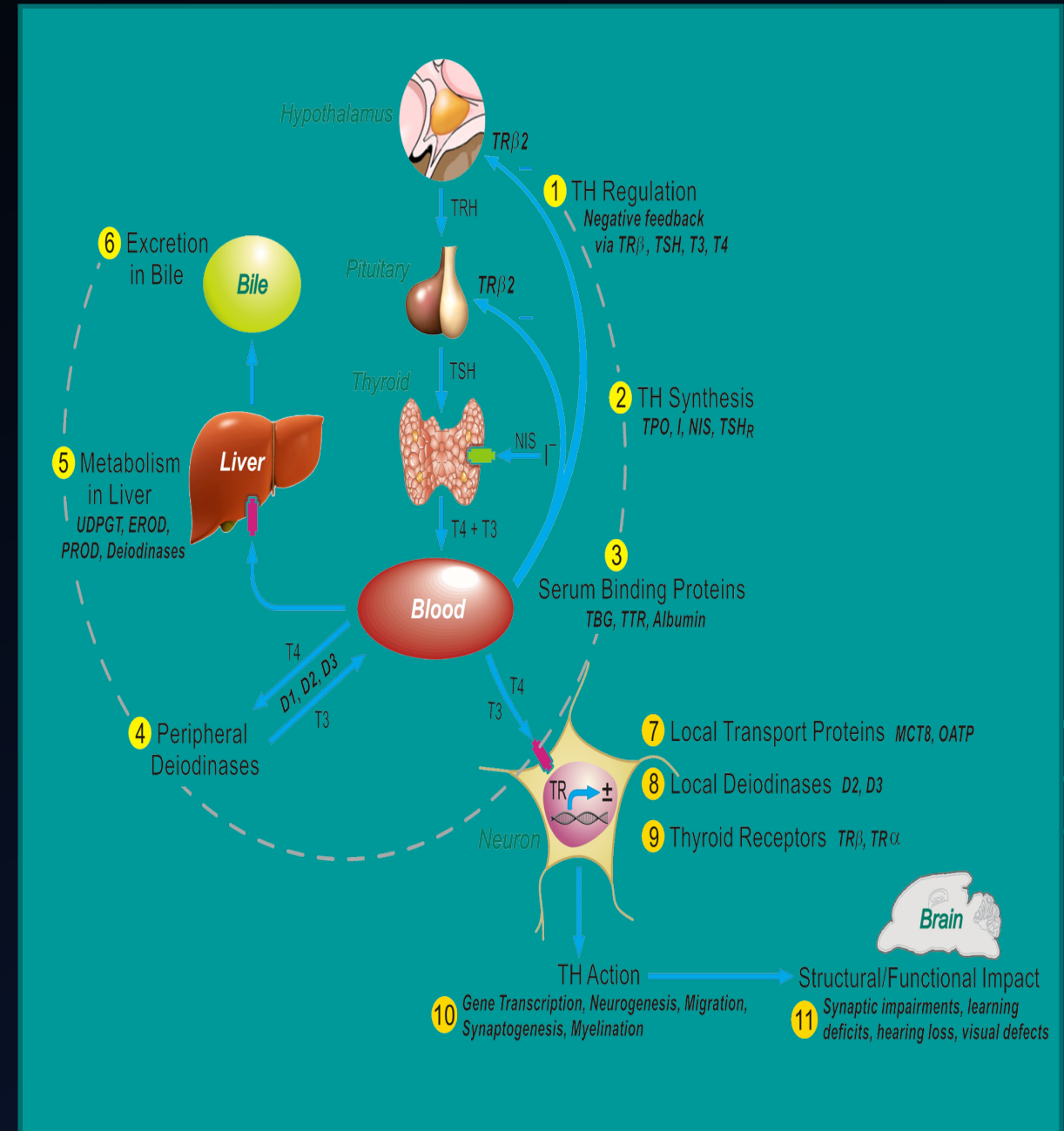
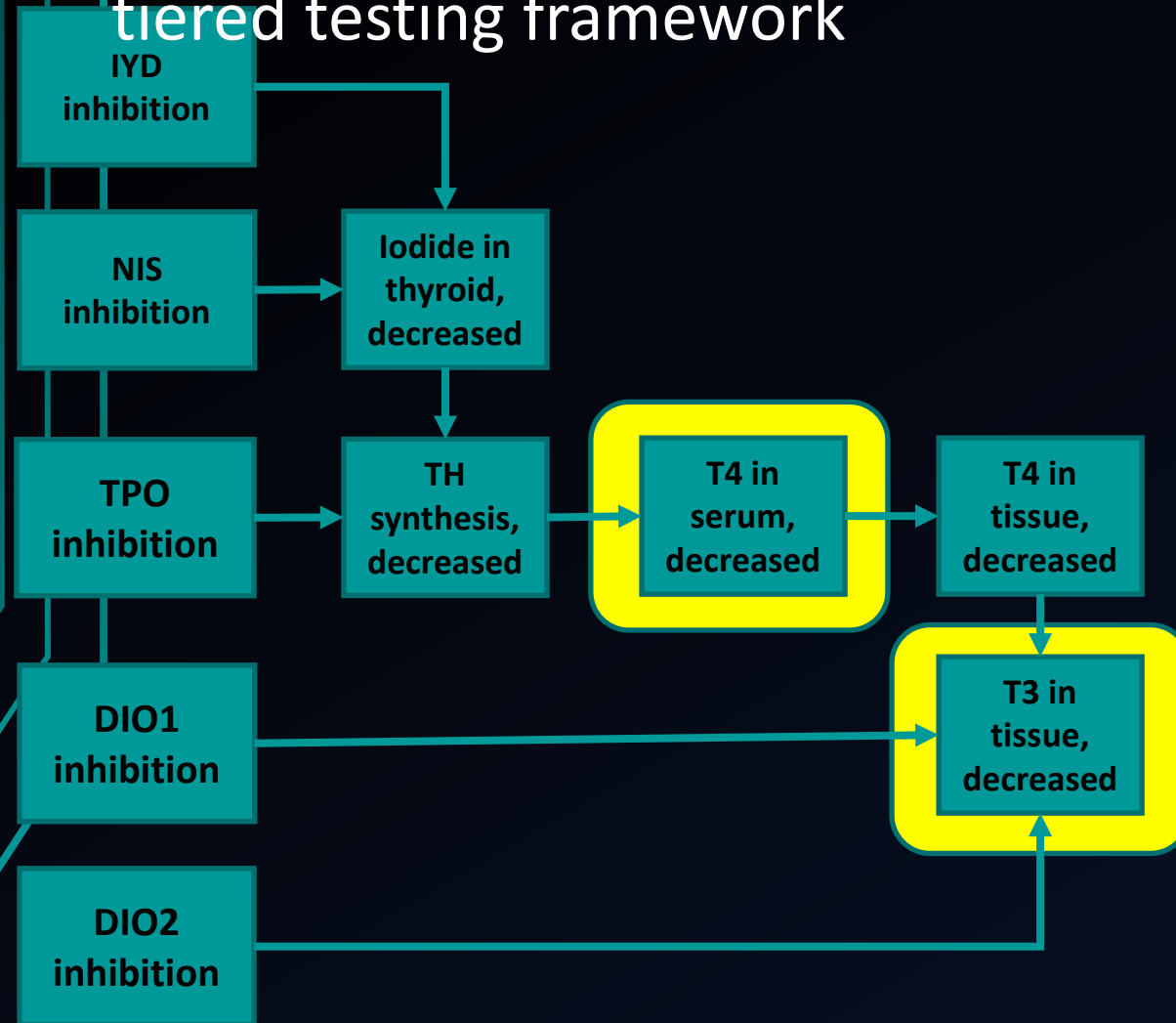


Considering only effects on sex ratio (AOP 346)

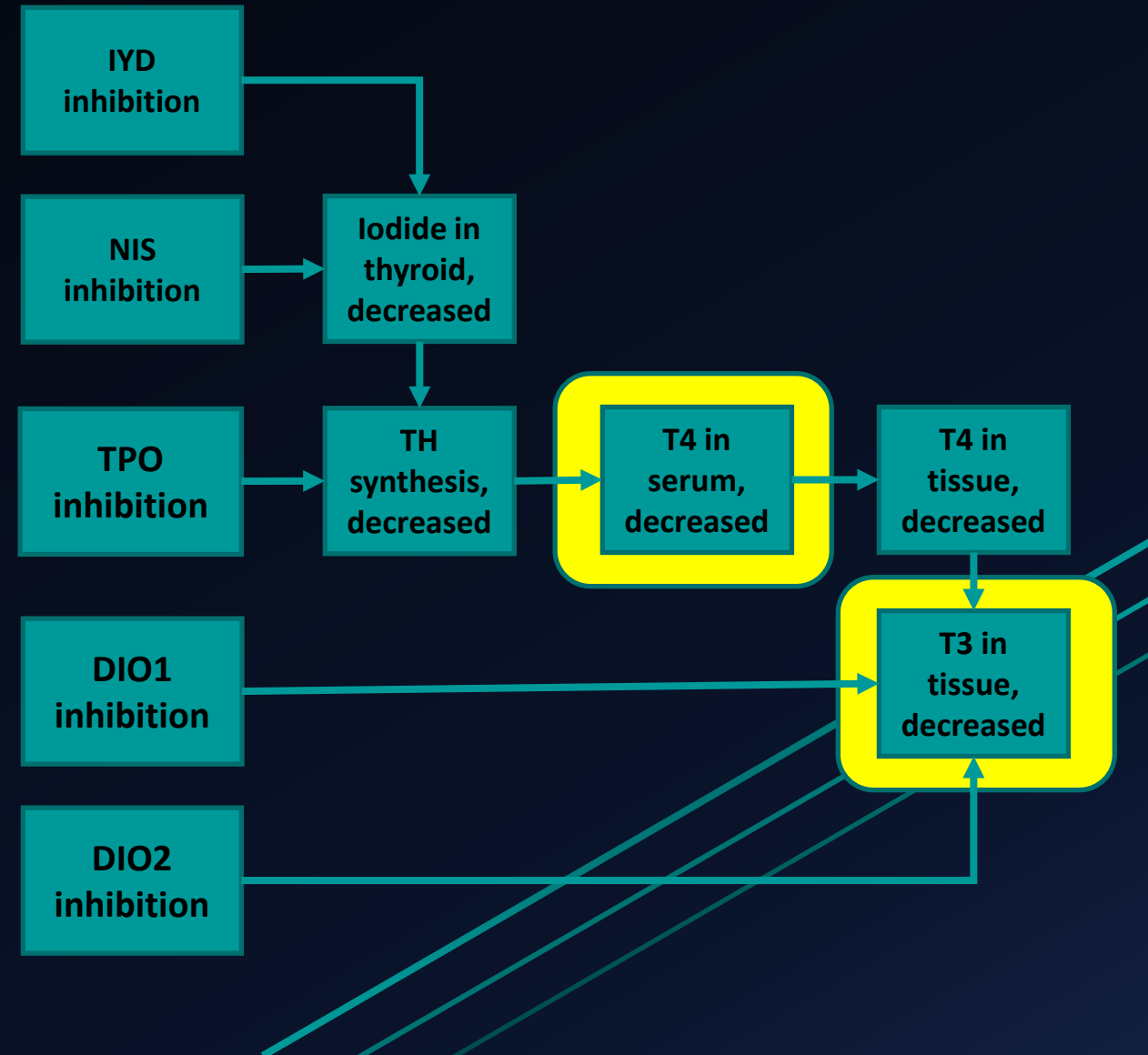
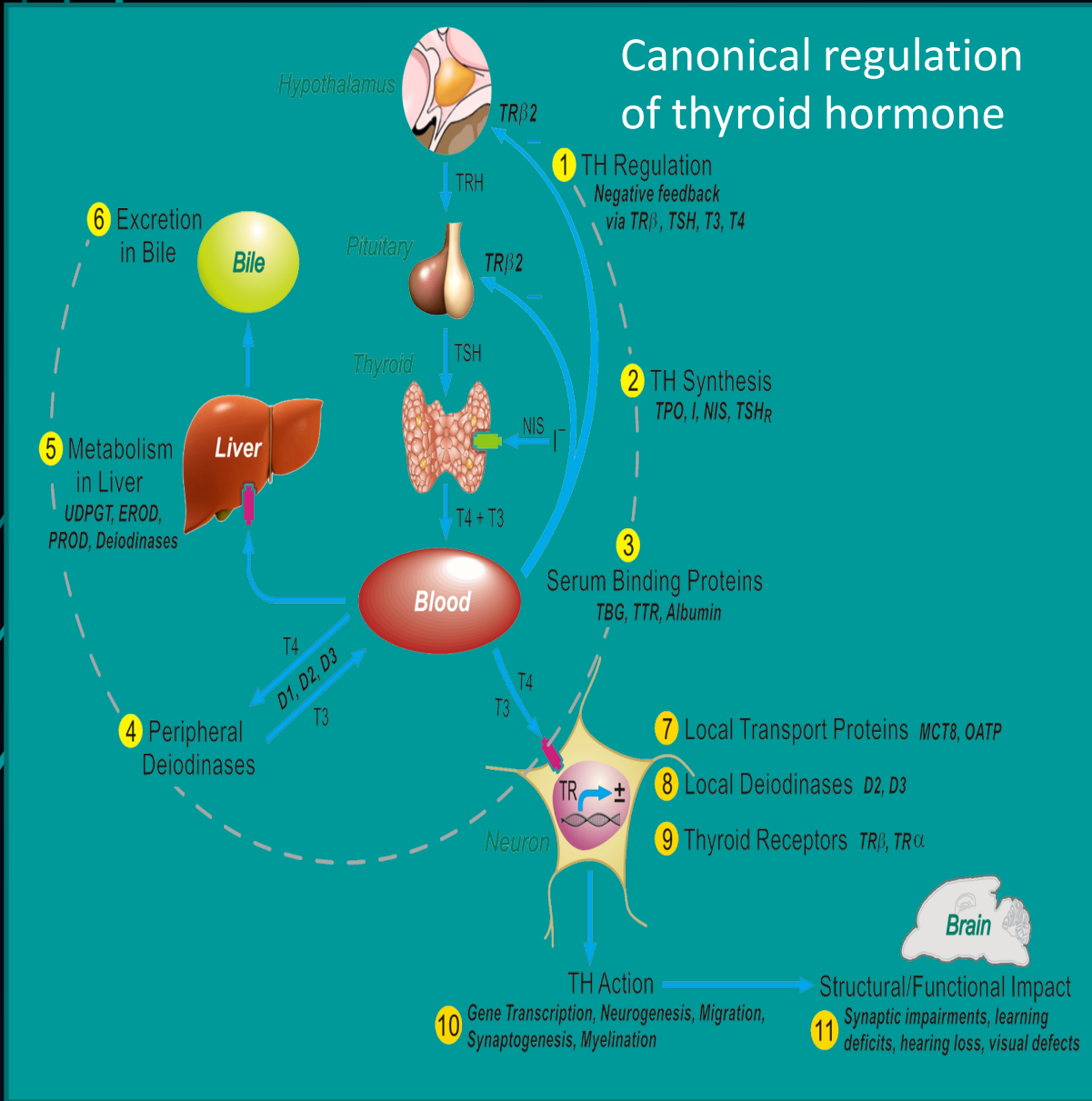


Considering both effects on sex ratio (AOP 346) and female vitellogenin production (AOP 25)

AOP Network to inform tiered testing framework



AOP Network to inform tiered testing framework



IYD
inhibition

HT assay developed
>150 chemicals screened

NIS
inhibition

HT assay developed
>150 chemicals screened

TPO
inhibition

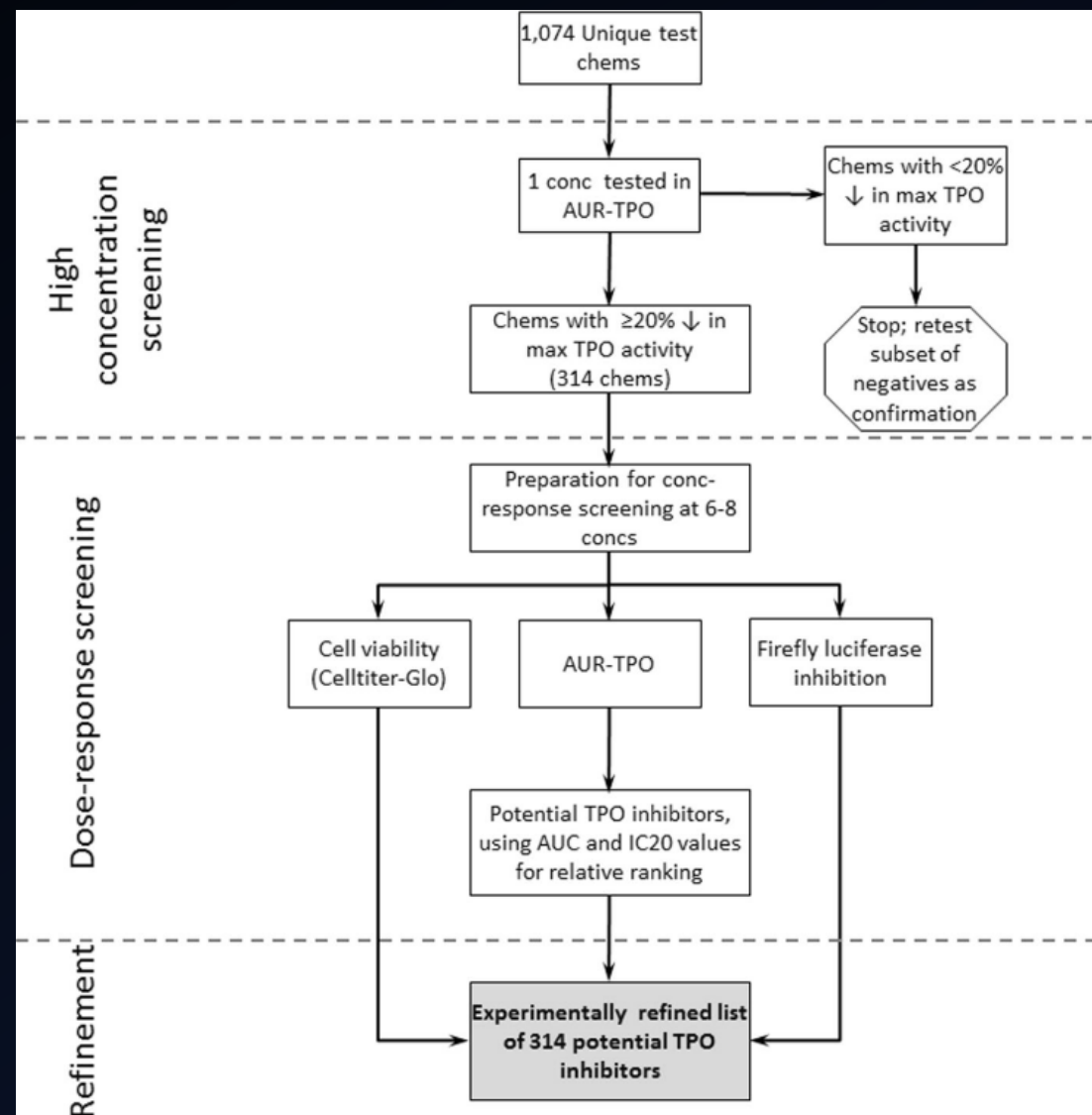
HT assay developed
>1000 chemicals
screened

DIO1
inhibition

HT assay developed
>150 chemicals screened

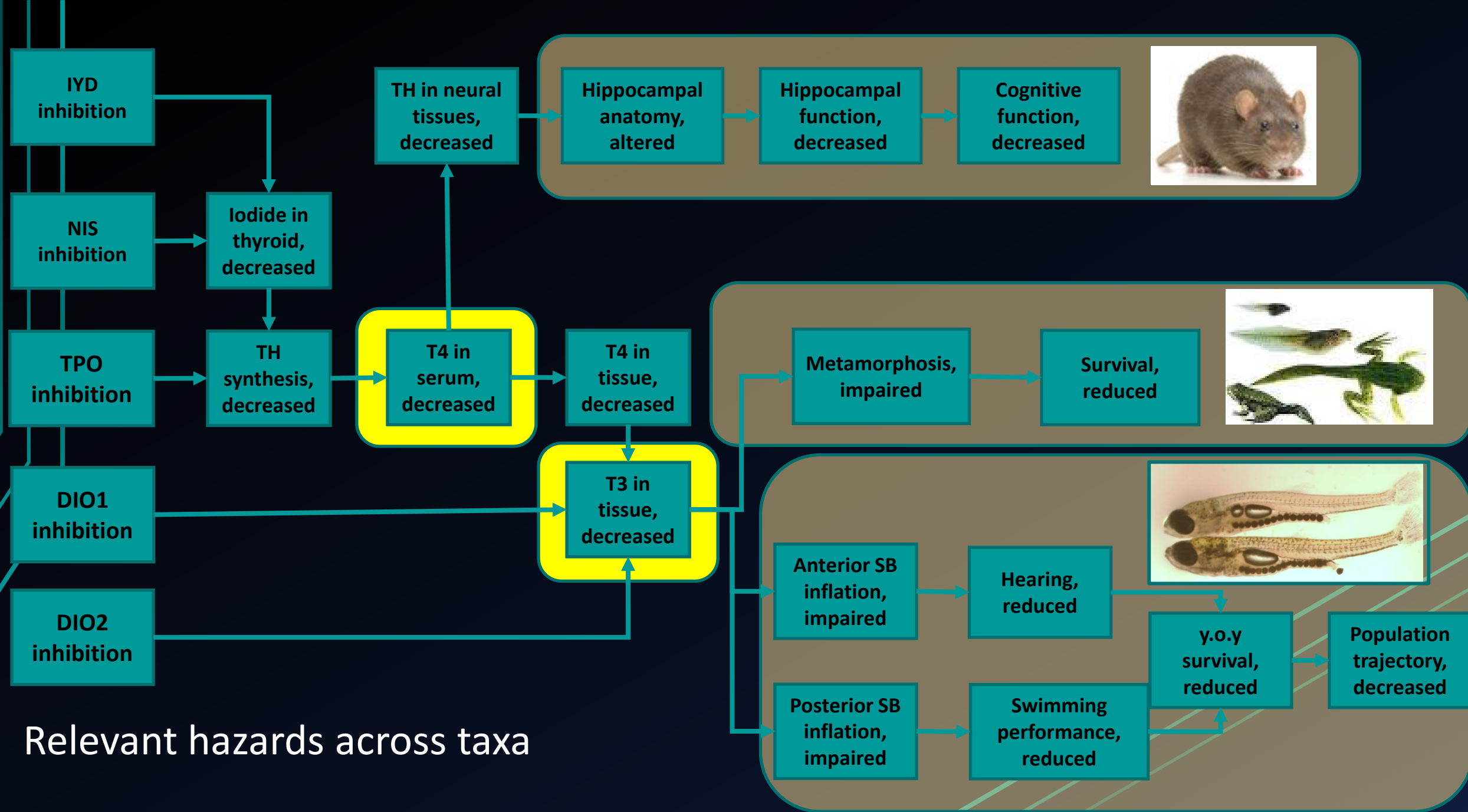
DIO2
inhibition

HT assay developed
>200 chemicals screened



Friedman et al., 2016. Tiered High-Throughput Screening Approach to Identify Thyropoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol Sci* 151 (1): 160-180. doi: 10.1093/toxsci/kfw034

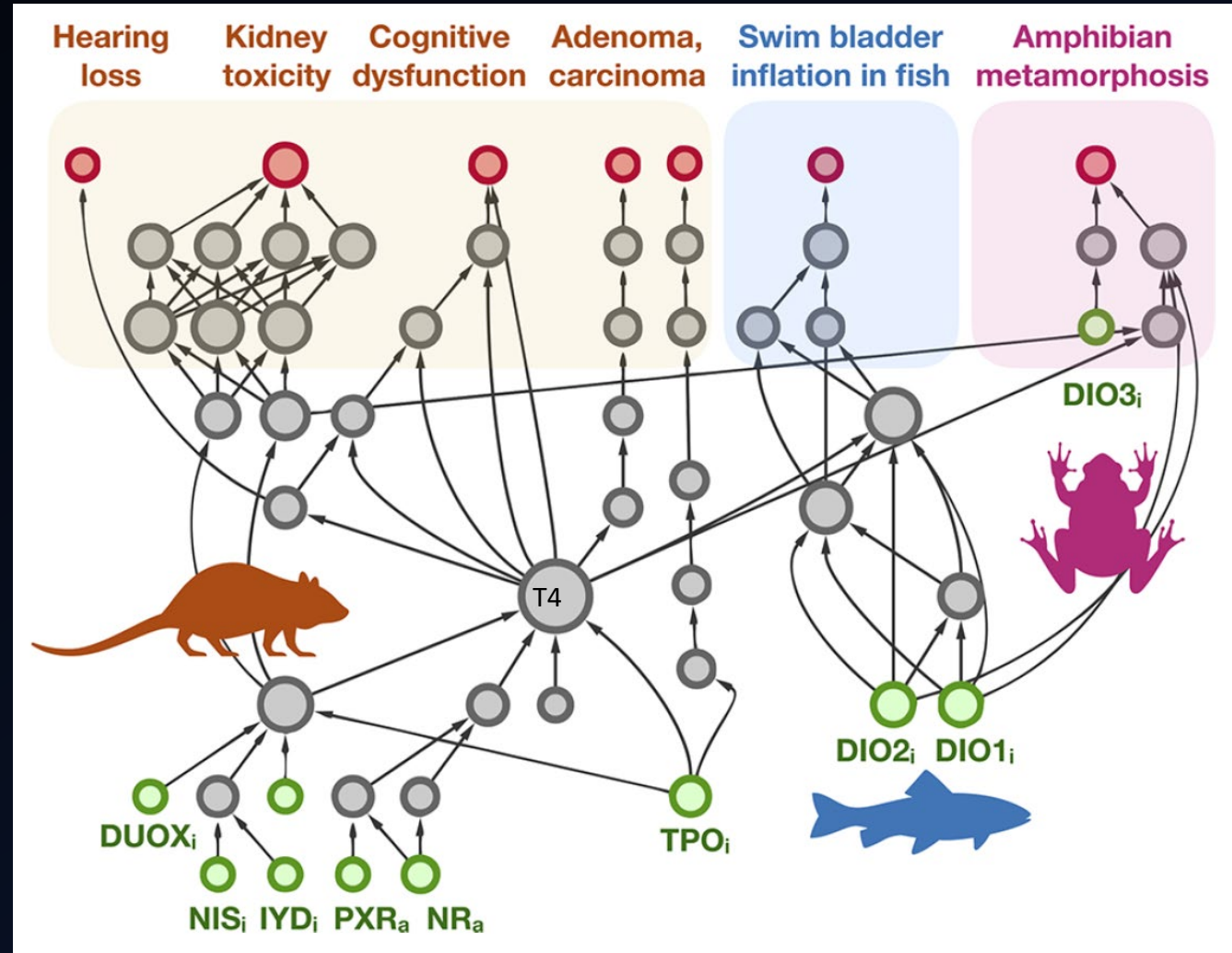




....greater complexity is captured via networks of AOPs that share common KEs and/or KERs.

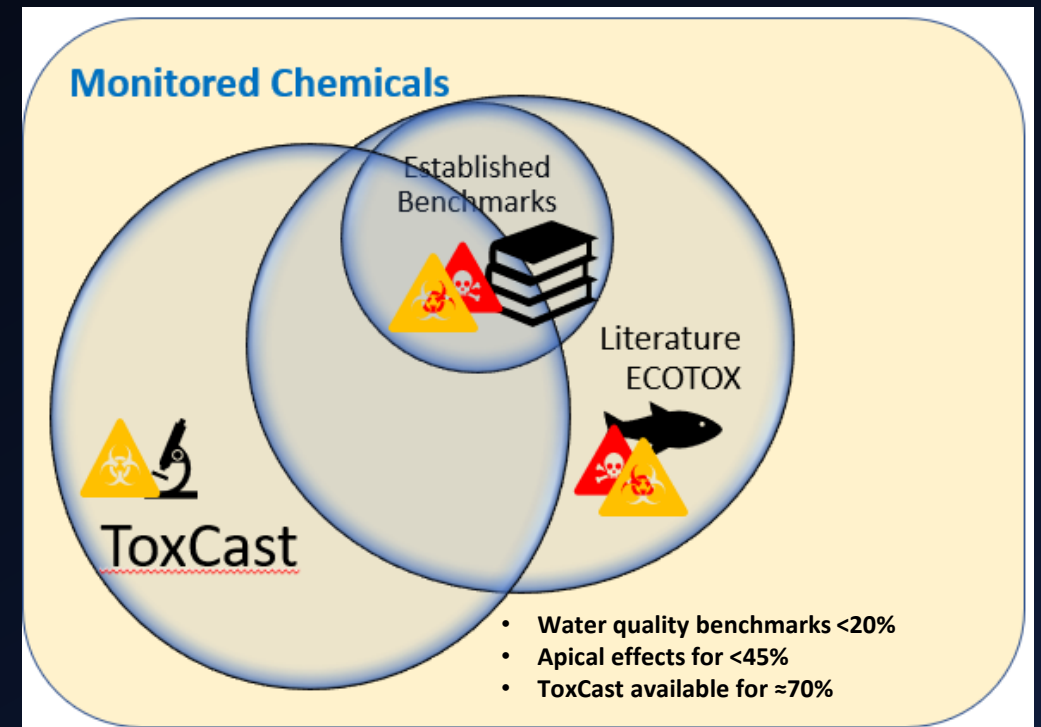
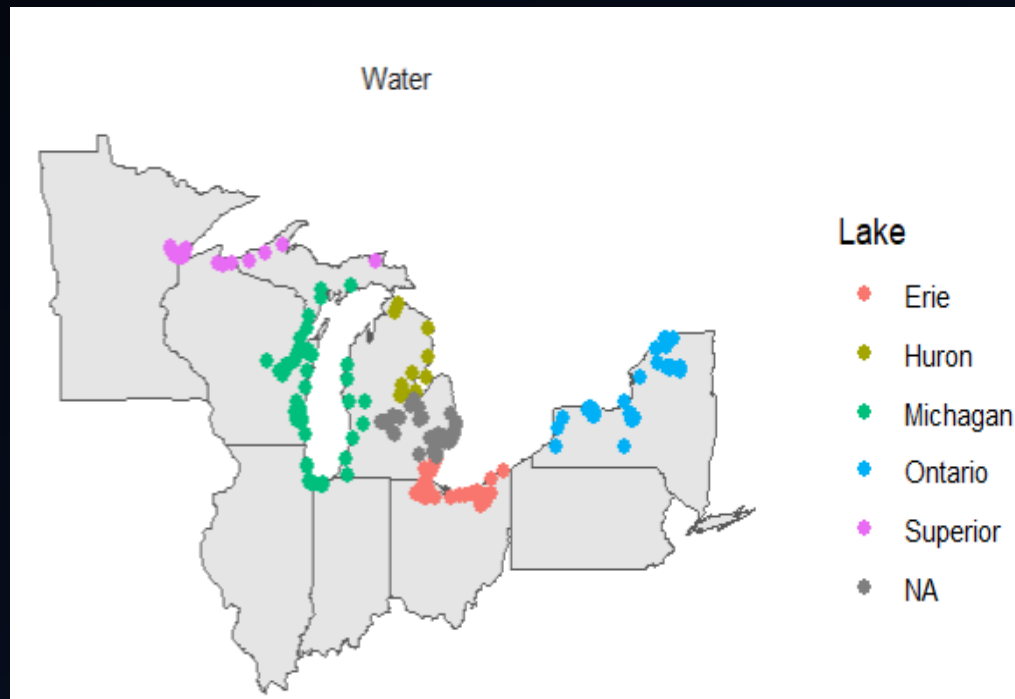
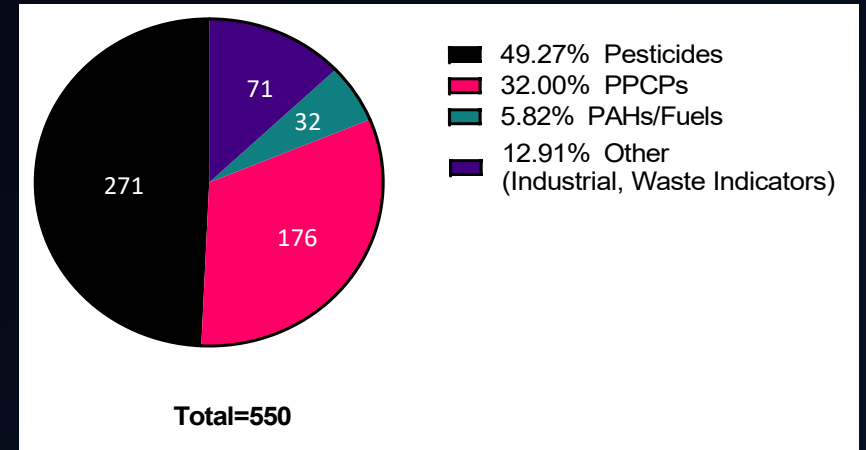
29 independent AOPs form a complex thyroid disruption AOP network formed through shared KEs.

T4 as a highly integrative node in the AOP network



Multiple stressors

- 2010-2018
- 830 unique compounds monitored; 550 detected
- Chemical Classes: antimicrobial disinfectants, antioxidants, detergent metabolites, dyes/pigments, fire retardants, flavors and fragrances, fuels, hormones, multi-use, PAHs, pesticides, pharmaceuticals, and personal care products (PPCPs), plastics additives, solvents, and sterols.
- Available toxicity benchmarks – highly variable; some intensively studied; some not at all

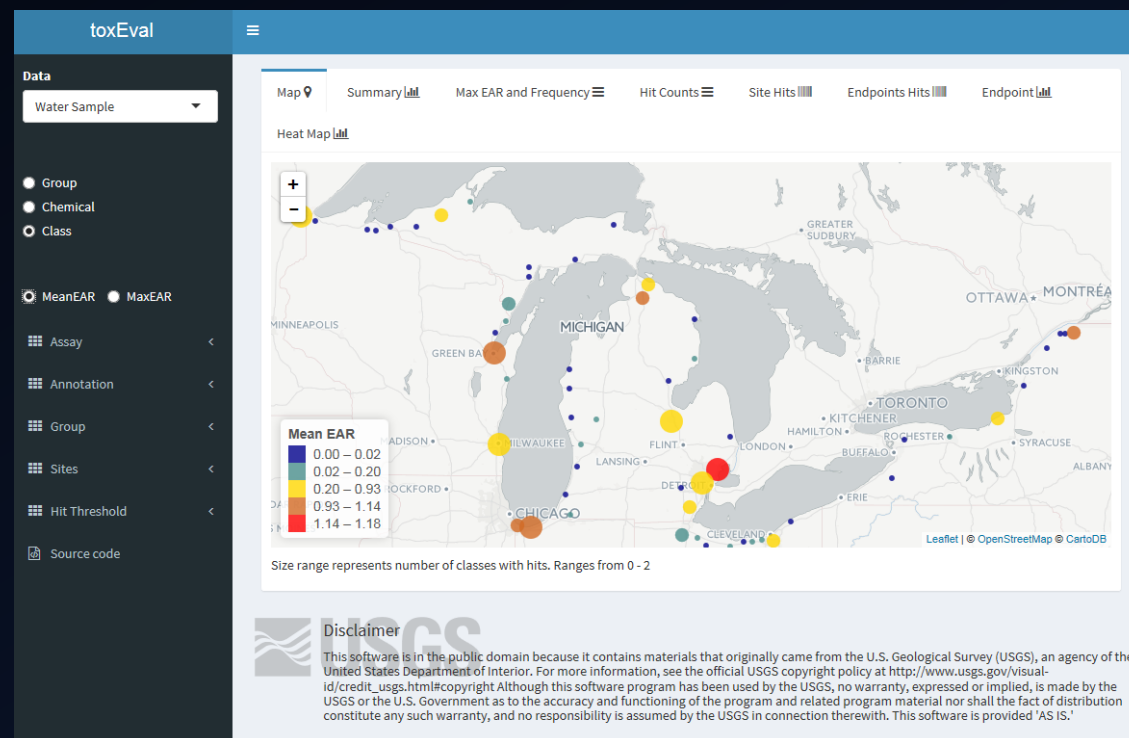


Exposure:Activity Ratios (EARs)

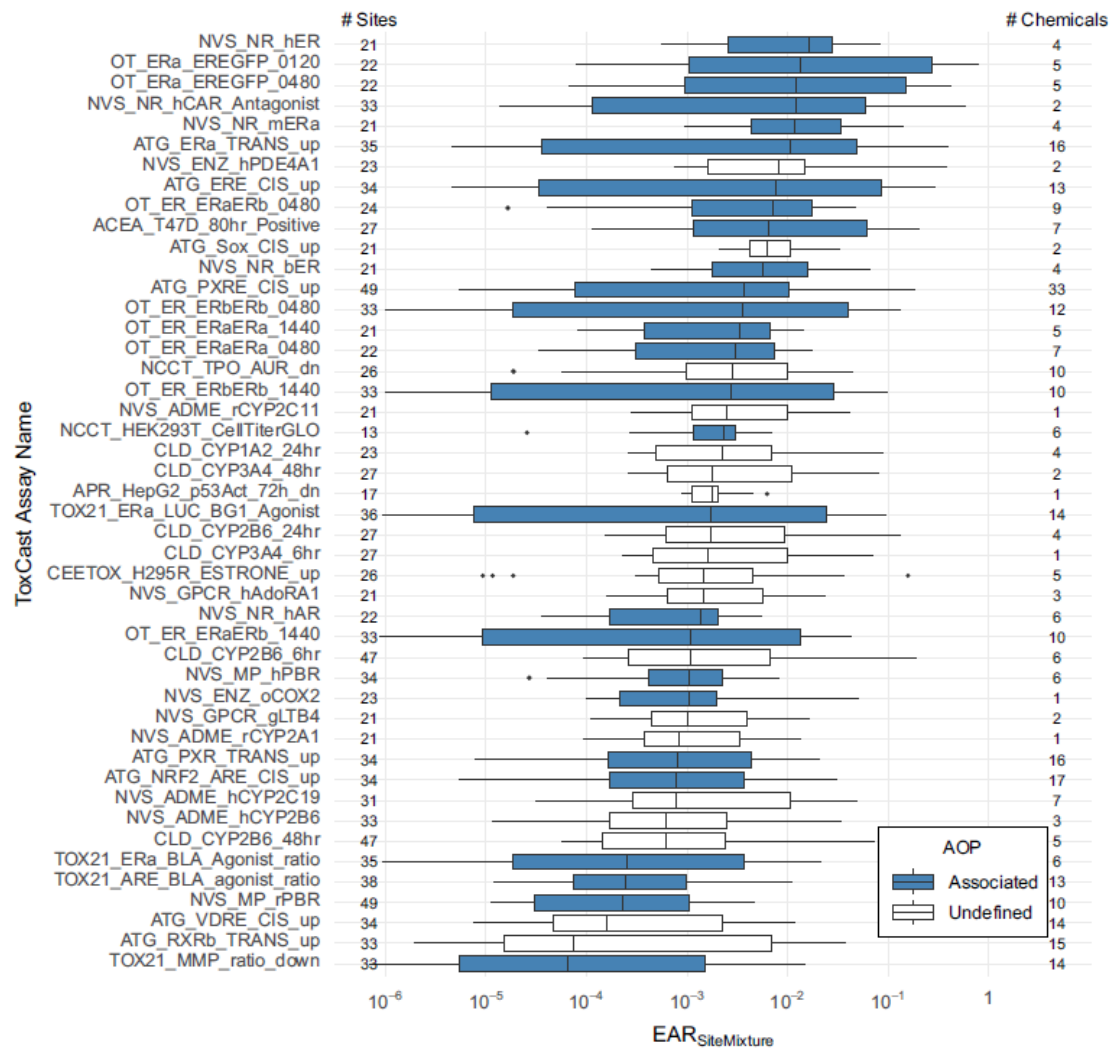
$$\text{EAR} = \frac{\text{Measured Concentration } (\mu\text{M})}{\text{Activity Concentration at Cut-off (ACC; } \mu\text{M})}$$



De Cicco, L.A., Corsi, S.R., Villeneuve D.L, Blackwell, B.R, and
Ankley, G.T., 2023, toxEval: Evaluation of measured concentration
data using the ToxCast high-throughput screening database or a ##
user-defined set of concentration benchmarks. R package version ##
1.3.0., <https://code.usgs.gov/water/toxEval>, doi:10.5066/P906UQ5I

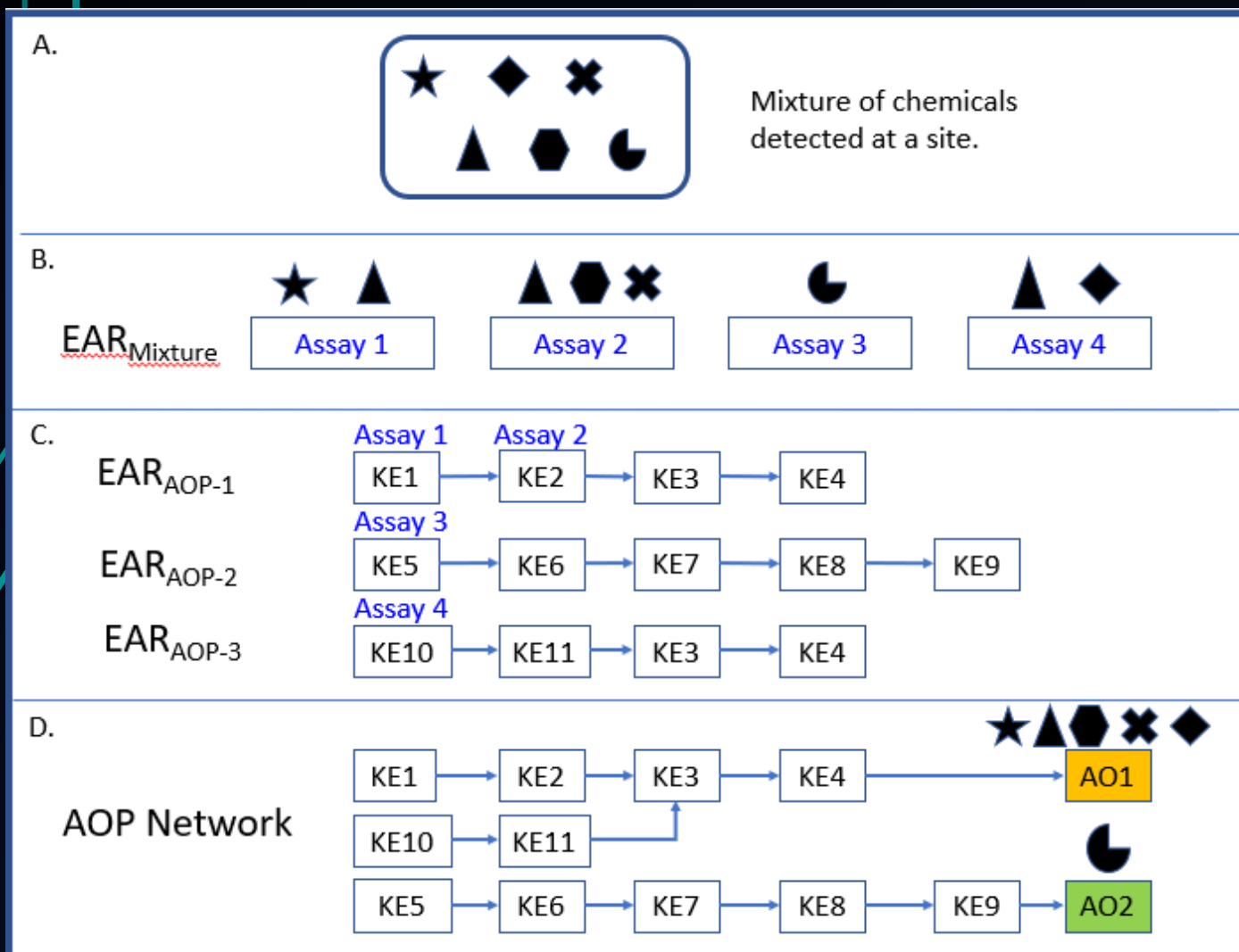


Expected bioactivity of multiple stressors



Corsi SR, De Cicco LA, Villeneuve DL, Blackwell BR, Fay KA, Ankley GT, Baldwin AK. Prioritizing chemicals of ecological concern in Great Lakes tributaries using high-throughput screening data and adverse outcome pathways. *Sci Total Environ.* 2019 Oct 10;686:995-1009. doi: 10.1016/j.scitotenv.2019.05.457.

Associating hazards with expected bioactivity



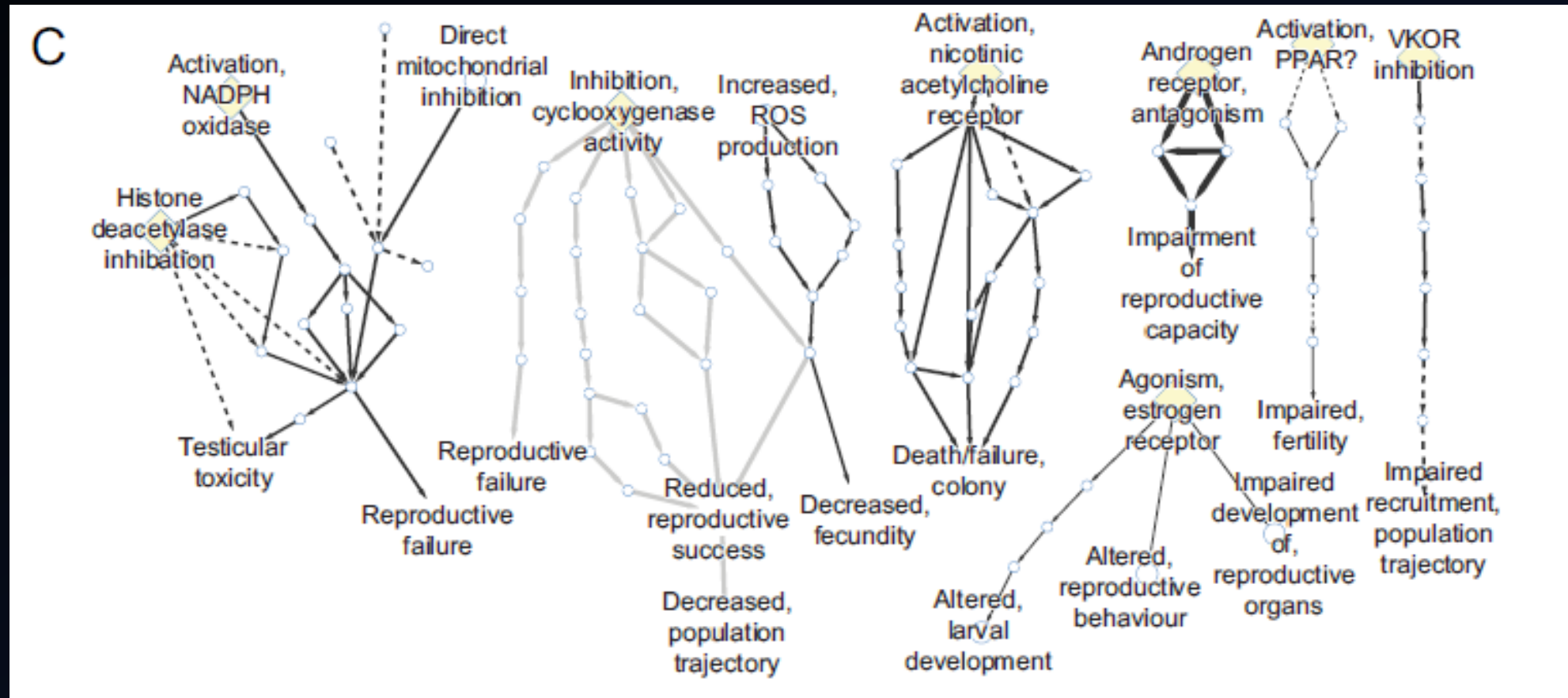
Considers cumulative effects of **detected** chemicals

Assume additivity within each ToxCast assay/endpoint

Assay endpoints map to key events
Redundant KEs not double-counted

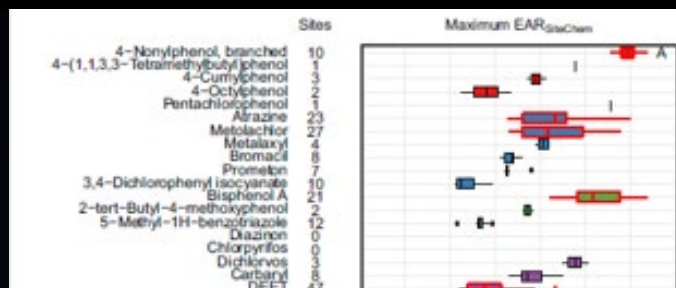
Considers cumulative impacts of multiple pathway perturbations on potential adverse outcomes.

Relevant AOP networks



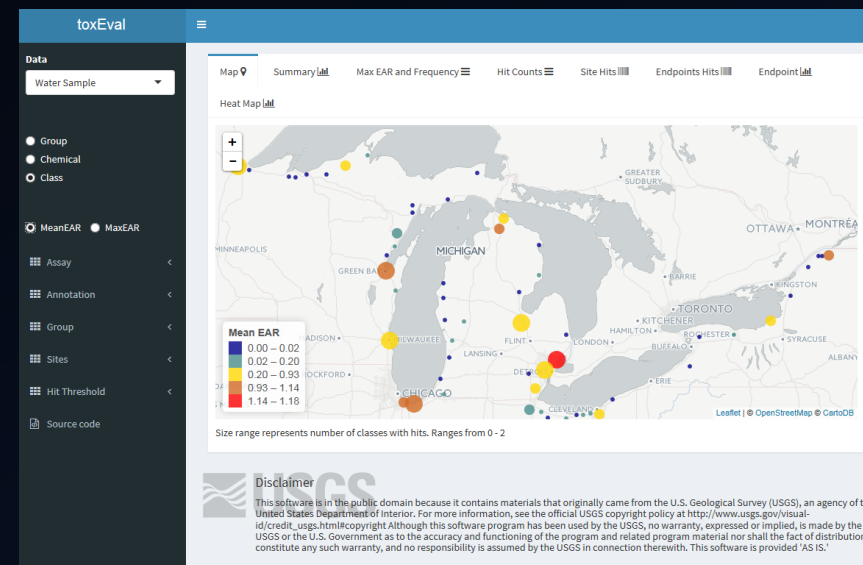
Pathway-Based Biological Effect Monitoring –

$$EAR = \frac{\text{Measured Concentration } (\mu M)}{\text{Activity Concentration at Cut-off (ACC; } \mu M)}$$



Use of ToxCast HTP screening data for risk-based prioritization

Blackwell et al. 2017, ES&T, 51(15): 8713-8724



<http://usgs-r.github.io/toxEval/index.html>



extract



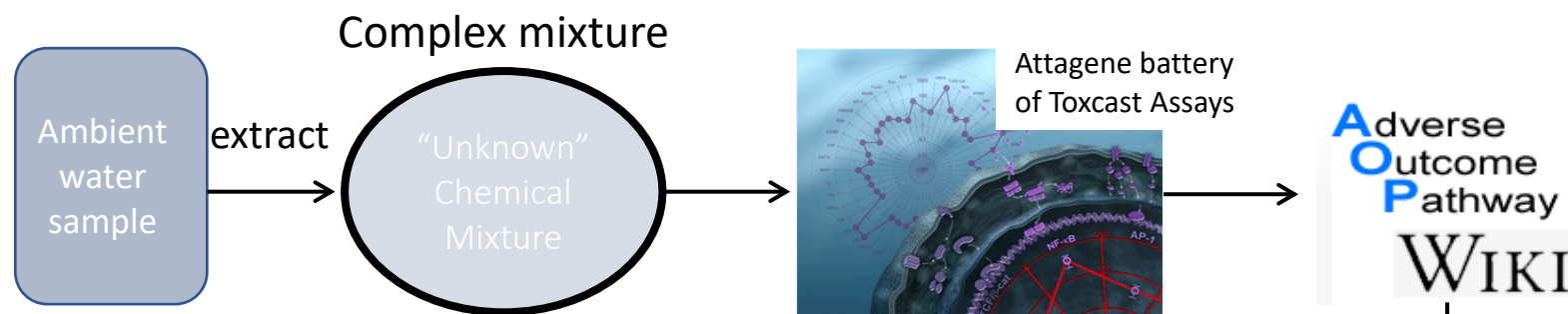
Multi-factorial assays for bioactivity surveillance (mixtures)

Blackwell et al. ES&T, 2019, 53(2): 973-983

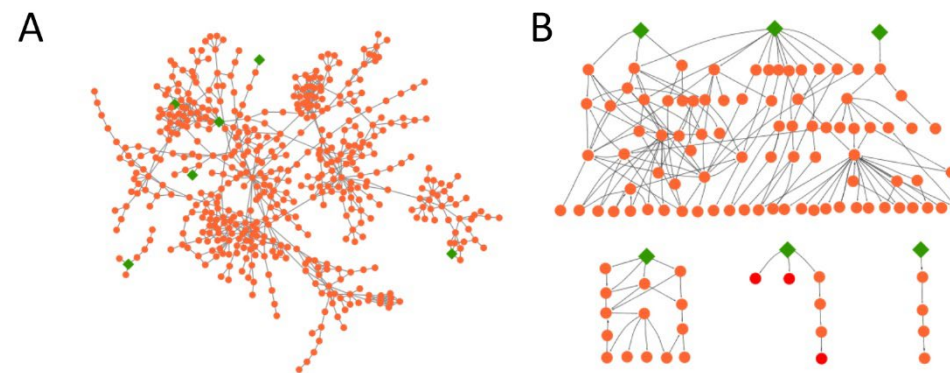


AOP Network Filtering

Example

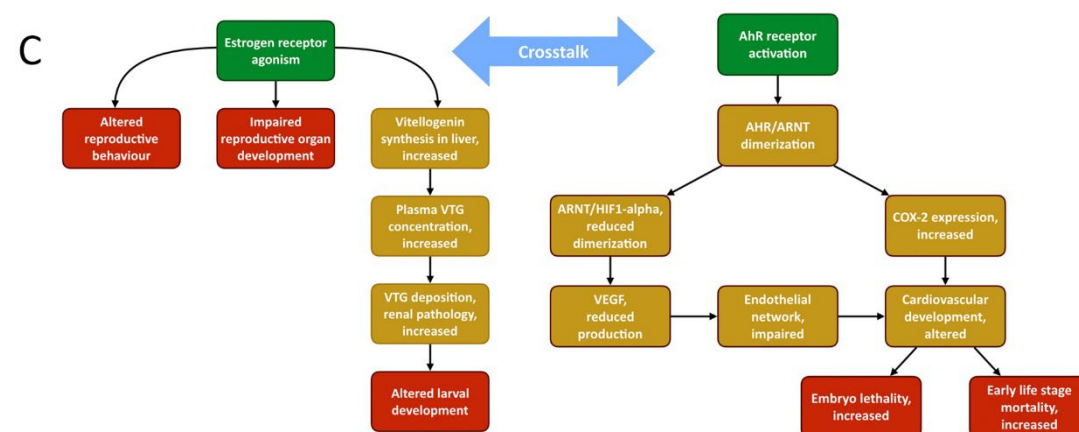


A. 6 MIEs identified as hits by Attagene assay are KEs in global AOP network.



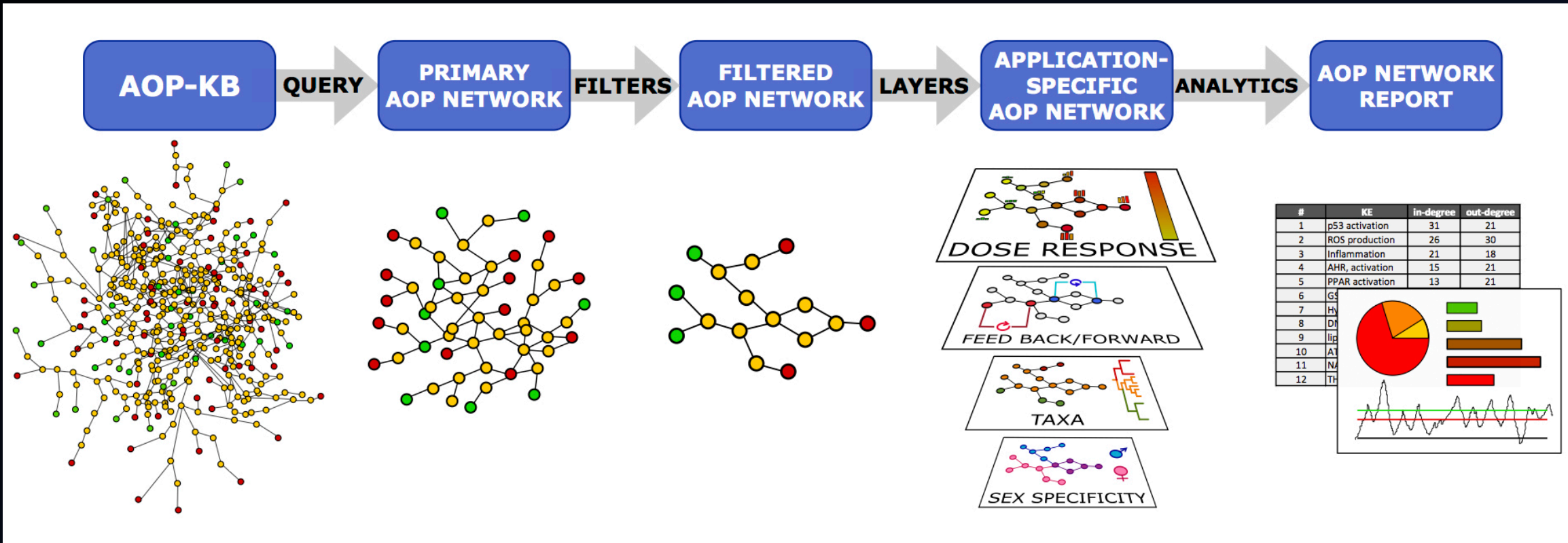
B. Network filtered to subset of AOPs directly linked to the 6 MIEs

C. Further filtered by taxonomic applicability.



Embryo lethality
Reproductive hazard

AOP Network Derivation – Filters and Layers



Example Filters

- Taxa
- Life-stage
- Confidence

Example Layers

- Positive/negative feedback
- Taxa
- Time-to-effect

Take Home

- An AOP network is an assembly of two or more AOPs that share one or more key events.
- AOP networks lay out the landscape of AOs that can result from a perturbation, or the landscape of perturbations that may cause AO.
- Tools based on graph theory and network science can be used to identify important features in AOP networks.
- In concept, filters and layers based on structured terms captured in AOP-Wiki can be used to tailor AOP to problem formulation or research question – improved ontologies needed.
- Application of AOP networks is in its infancy – active area of R&D.

Special Thanks

- AOP Networks Workgroup: April 2017, SETAC Pellston Workshop



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Xiaowei Zhang
Michelle Angrish
Nathan Pollesch