Improving systematic review and usability of NexGen/high throughput data in studies of chemical toxicity using AOPs

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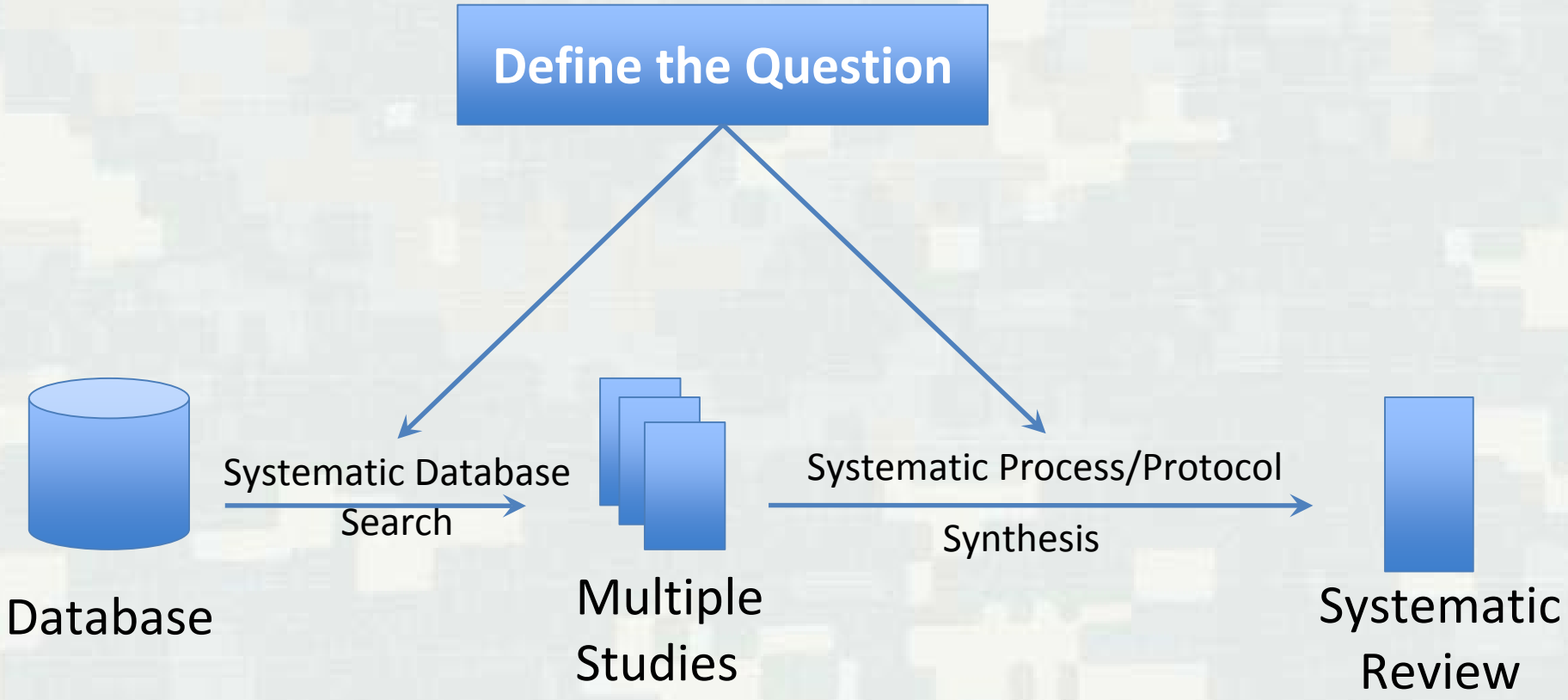
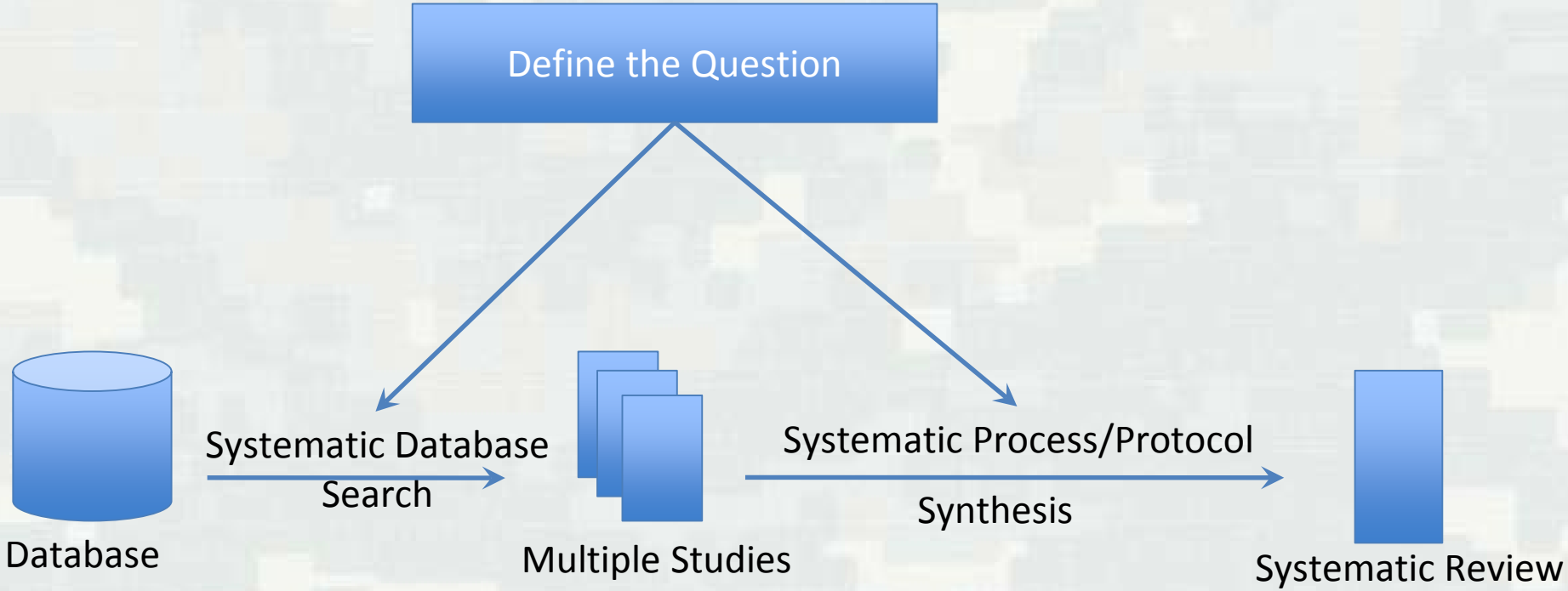
Overview

- **What we consider systematic review**
- **Study Quality**
  - Microarray study quality: SOAR
  - HTS assays: update on developments
- **Data Integration, Analysis and Graphical Synthesis**
  - Data Integration
    - Orthogonal assays (HTS)
    - Bayesian data integration (all data types)
    - Evidence Maps (graphical synthesis)
  - Adverse Outcome Pathway-based Data Integration
    - AOPXplorer

Define: Systematic Review



Define: Systematic Review



Predefined Protocol
Predefined Search Terms, Databases, Filtering Criteria

Predefined Protocol
Pre-defined study quality criteria, data integration methods, statistical analysis methods, and exit criteria

Study Quality for NexGen Mechanistic Studies

SOAR for Microarray Study Quality

- Specific to toxicology microarray studies
- Structured for in vitro and in vivo studies
- Asks questions about study design
- Most questions come from
  - ToxR Tool
  - MIAME standard (microarray data quality standard)
  - Fostel, et al 2007
- Scoring trained and evaluated on datasets of known quality
- McConnell, et al 2014 PLOS ONE:; DOI: 10.1371/journal.pone.0110379
- Developed by EPA/ORD/NCEA in collaboration with US Army Engineer Research and Development Center

HTS Study Quality Considerations

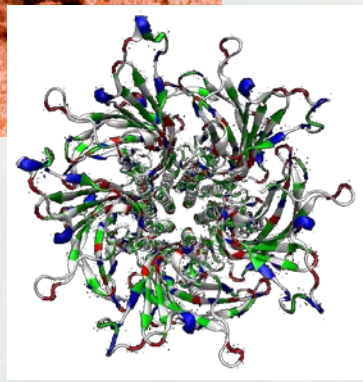
(Work in progress)

- If cell assay
  - Is the cell of the correct type/lineage?
    - BG1-Luc-4E2 may not actually be of ovarian lineage (http://web.expasy.org/cellosaurus/CVCL_6571)
  - If different lineage, will this impact interpretation?
  - Metabolically competent?
- If protein assay
  - Evidence that protein operates same as in cell?
  - Evidence that protein has same post-translational modifications as expected if in the cell?
- Transcriptional assays
  - Is a realistic or artificial promoter used? This will impact confidence in the result
- Dose range appropriate for question being studied?
- Are species relevant for question being studied?

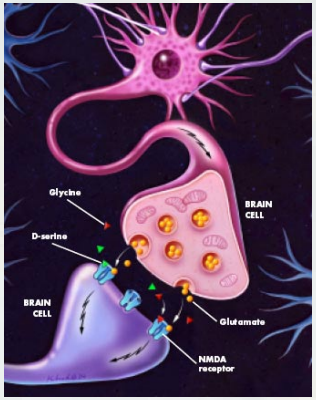
DATA INTEGRATION


Challenge
TOX21/ToxCast/
Toxicogenomics
Test Data
Modeling
AOPs
Decisions
Data-to-
Decisions Chasm

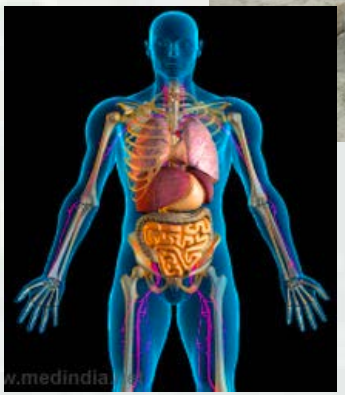
Exposure



Molecular Initiating Event



Cellular effects



Organ, Individual effects

Group, Populationeffe cts

Absorption, Distribution, Metabolism,Elimination

Toxicity Pathway

Mode of Action

Adverse Outcome Pathway

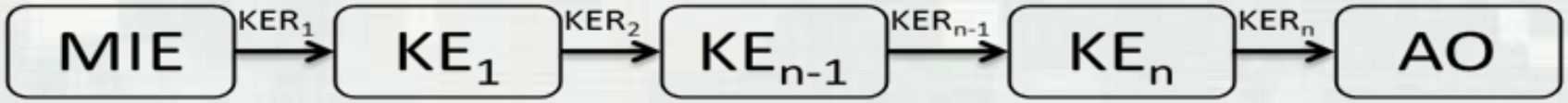
K. Crofton

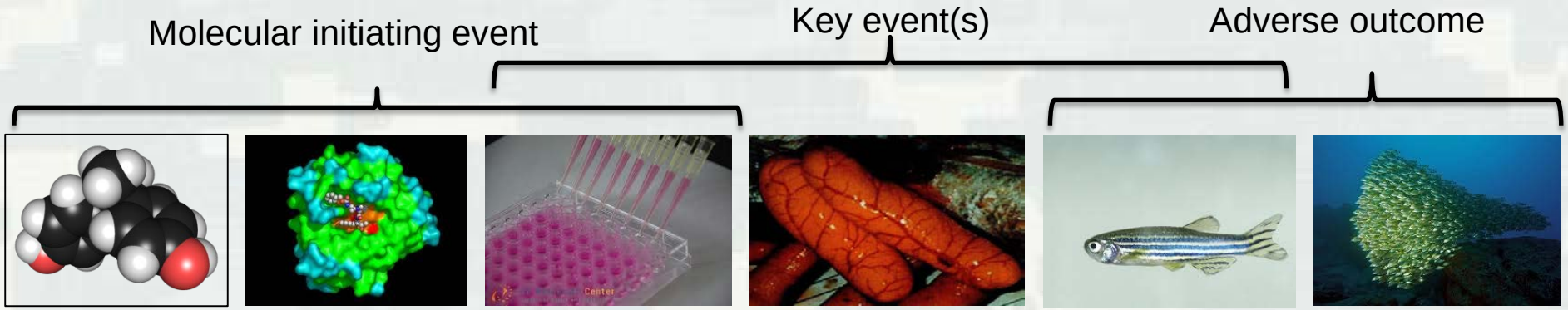
Rules of AOP Development

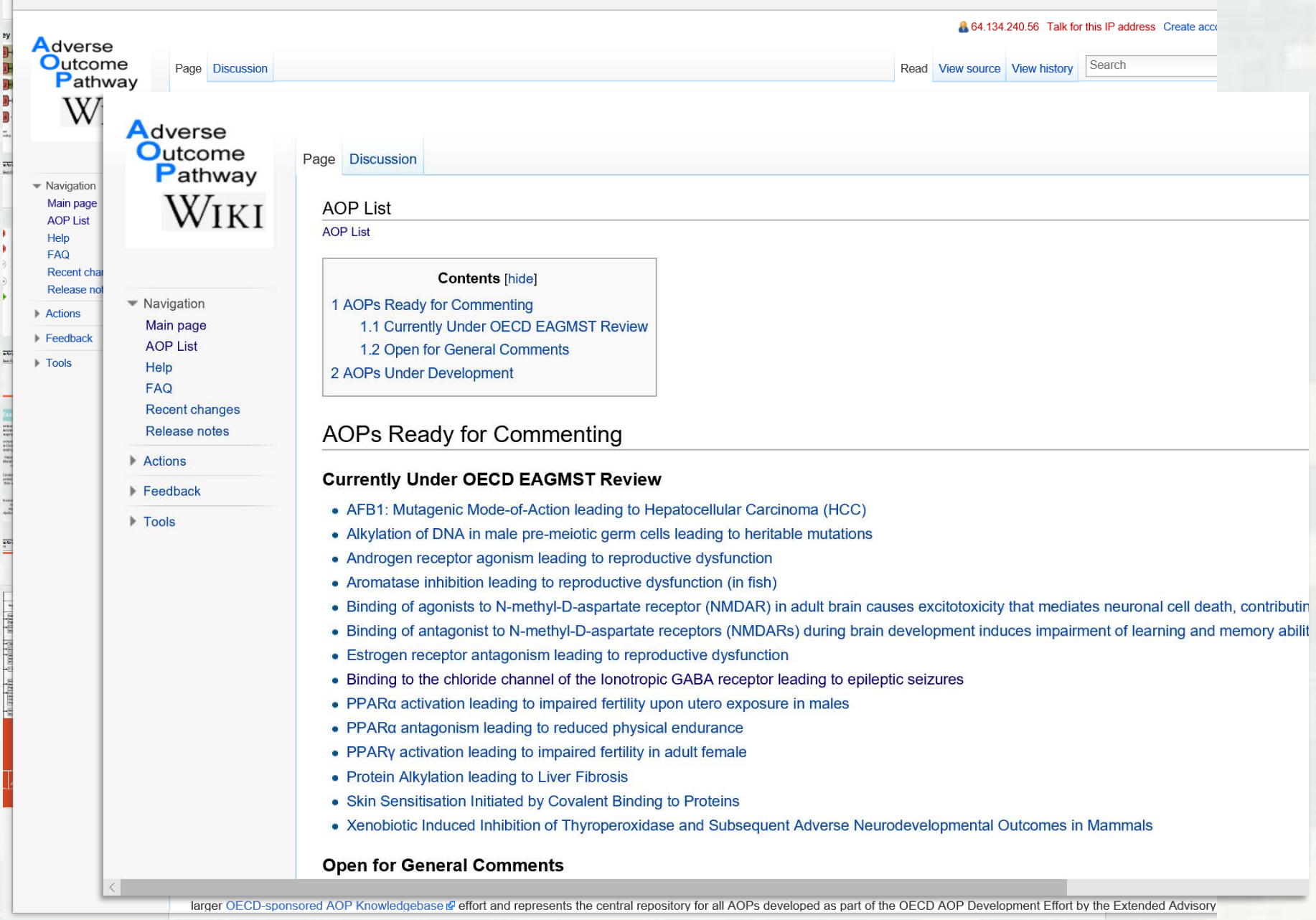
AOPs are not chemical-specific

AOPs are modular

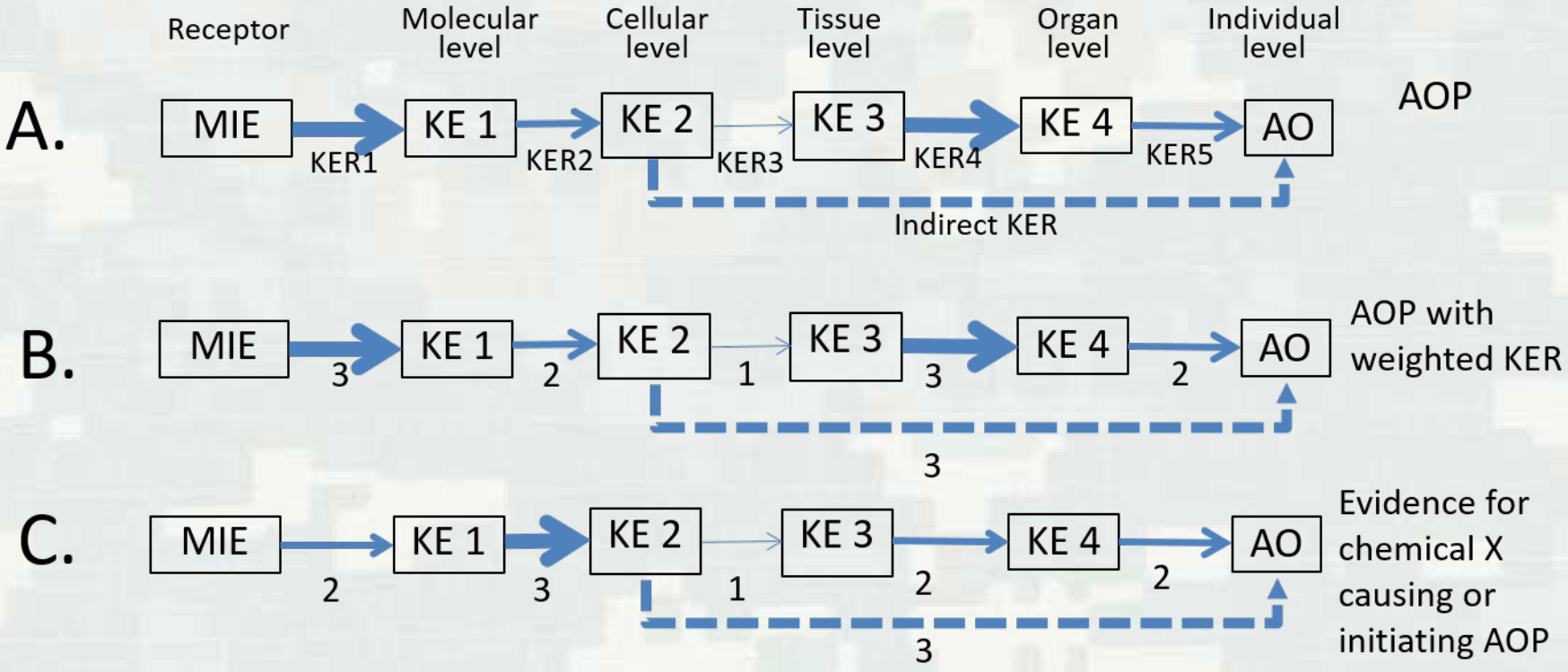
- Key Events – functional unit of observation – nodes
- Key Event Relationships – dose/response-response – edges

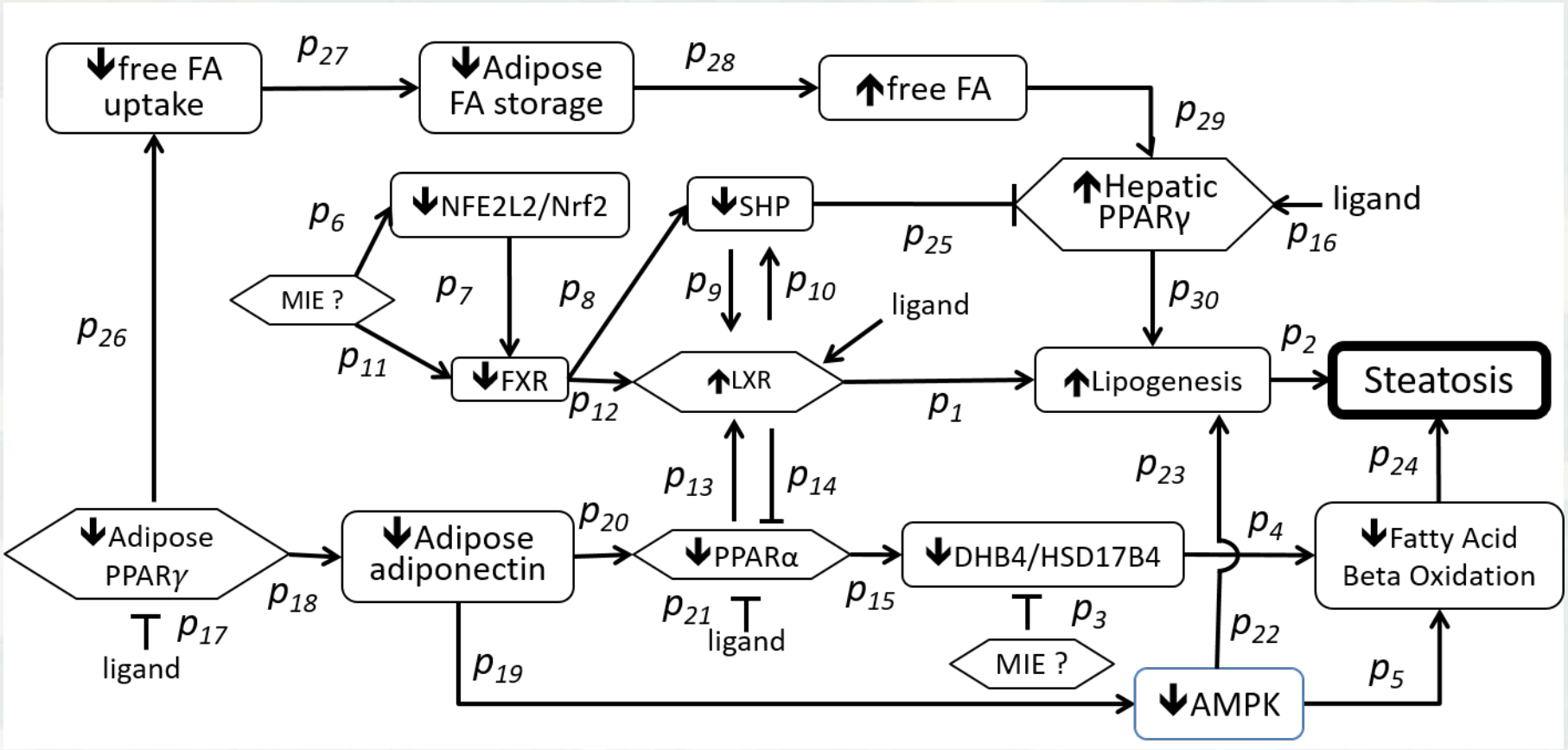


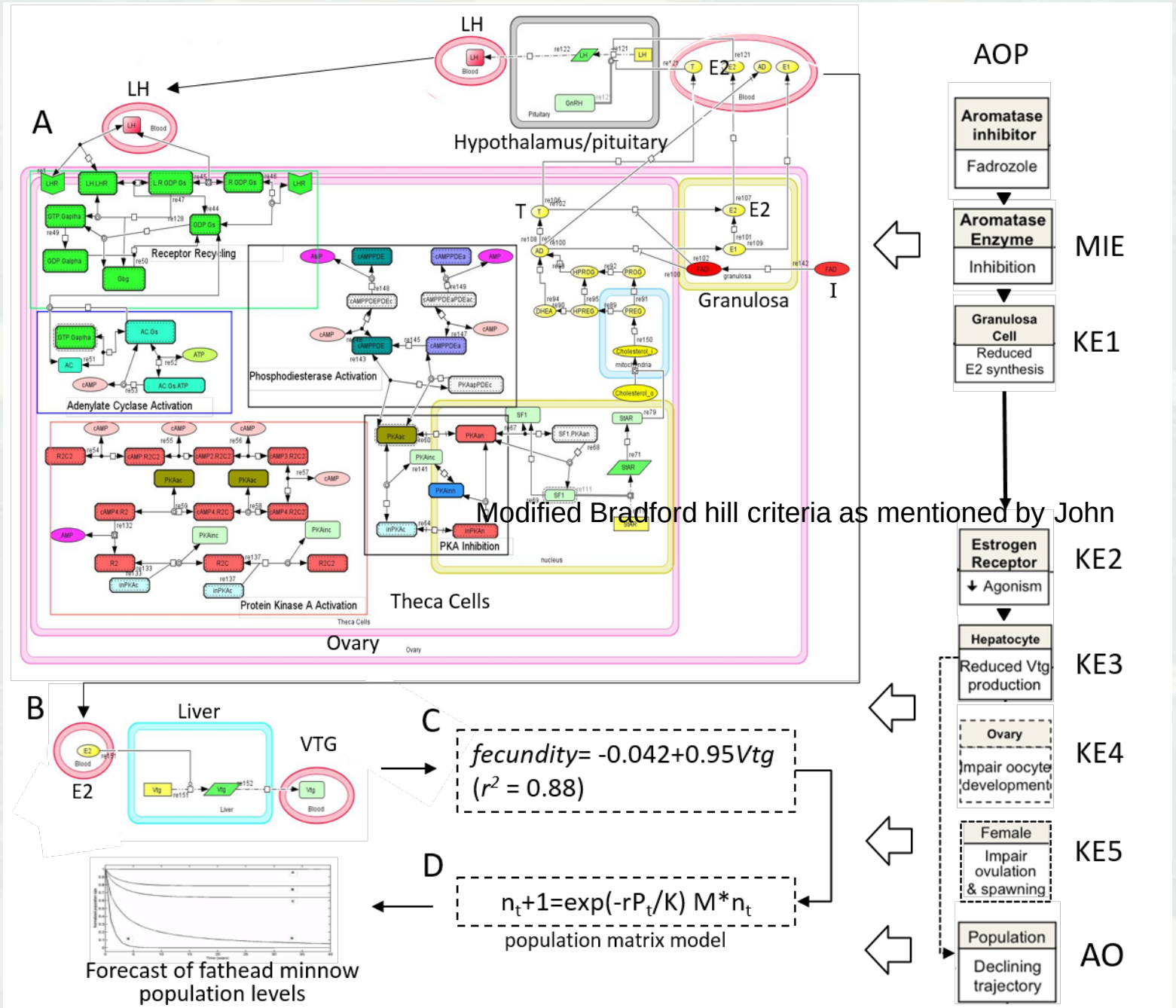



Adverse Outcome Pathway WIKI
Page Discussion
Navigation
Main page
AOP List
Help
FAQ
Recent changes
Release notes
Actions
Feedback
Tools
AOP List
AOP List
Contents [hide]
1 AOPs Ready for Commenting
1.1 Currently Under OECD EAGMST Review
1.2 Open for General Comments
2 AOPs Under Development
AOPs Ready for Commenting
Currently Under OECD EAGMST Review
AFB1: Mutagenic Mode-of-Action leading to Hepatocellular Carcinoma (HCC)
Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations
Androgen receptor agonism leading to reproductive dysfunction
Aromatase inhibition leading to reproductive dysfunction (in fish)
Binding of agonists to N-methyl-D-aspartate receptor (NMDAR) in adult brain causes excitotoxicity that mediates neuronal cell death, contributin
Binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilit
Estrogen receptor antagonism leading to reproductive dysfunction
Binding to the chloride channel of the Ionotropic GABA receptor leading to epileptic seizures
PPARα activation leading to impaired fertility upon utero exposure in males
PPARα antagonism leading to reduced physical endurance
PPARγ activation leading to impaired fertility in adult female
Protein Alkylation leading to Liver Fibrosis
Skin Sensitisation Initiated by Covalent Binding to Proteins
Xenobiotic Induced Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals
Open for General Comments
larger OECD-sponsored AOP Knowledgebase effort and represents the central repository for all AOPs developed as part of the OECD AOP Development Effort by the Extended Advisory

AOP as a framework to integration and weight of evidence



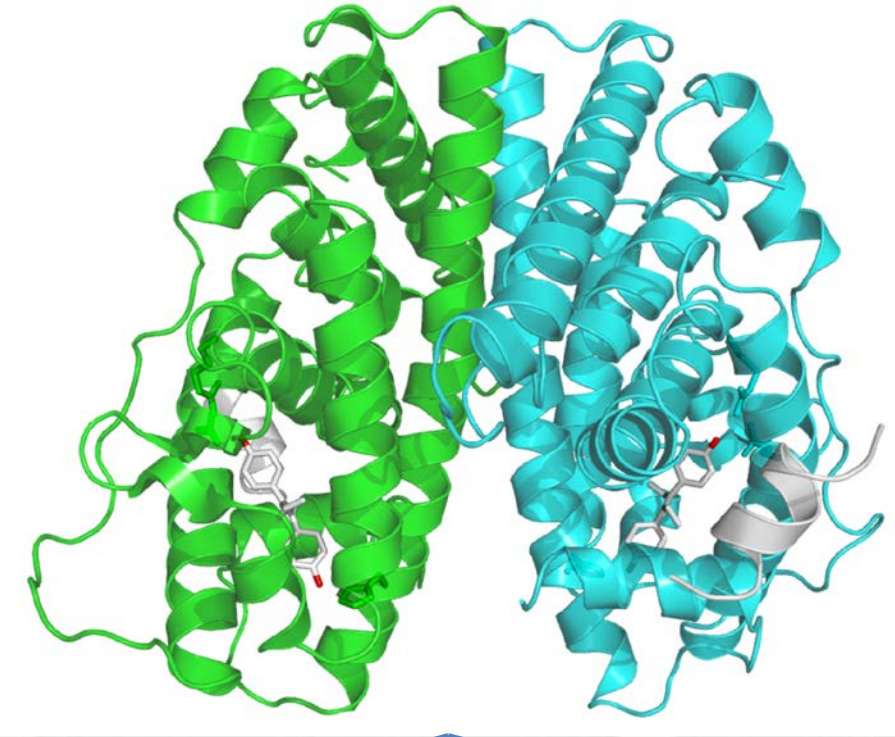

↓free FA uptake
p_{27}
↓Adipose FA storage
p_{28}
↑free FA
p_{29}
↑Hepatic PPARγ
ligand
p_{16}
↓SHP
p_{25}
↓NFE2L2/Nrf2
p_6
MIE ?
p_7
p_8
p_9
p_{10}
ligand
p_{30}
p_{26}
p_{11}
↓FXR
p_{12}
↑LXR
p_1
↑Lipogenesis
p_2
Steatosis
p_{13}
p_{14}
p_{23}
p_{24}
↓Adipose PPARγ
p_{18}
ligand
p_{17}
↓Adipose adiponectin
p_{20}
↓PPARα
p_{21}
ligand
p_{15}
↓DHB4/HSD17B4
p_3
MIE ?
p_4
↓Fatty Acid Beta Oxidation
p_{22}
p_{19}
↓AMPK
p_5


A
LH
Hypothalamus/pituitary
Receptor Recycling
Adenylate Cyclase Activation
Phosphodiesterase Activation
PKA Inhibition
Protein Kinase A Activation
Theca Cells
Ovary
T
E2
I
Granulosa
B
Liver
VTG
E2
C
fecundity= -0.042+0.95Vtg
(r^2 = 0.88)
D
n_t+1=exp(-rP_t/K) M*n_t
population matrix model
Forecast of fathead minnow population levels
AOP
Aromatase inhibitor
Fadrozole
Aromatase Enzyme
Inhibition
MIE
Granulosa Cell
Reduced E2 synthesis
KE1
Modified Bradford hill criteria as mentioned by John
Estrogen Receptor
↓ Agonism
KE2
Hepatocyte
Reduced Vtg production
KE3
Ovary
Impair oocyte development
KE4
Female
Impair ovulation & spawning
KE5
Population
Declining trajectory
AO

Application to Developing Screening Level Risk Assessments

- Identify all available data for a chemical or mixture
- Use AOPs to identify potential adverse outcomes (hazard ID)
- Use concentration-response or dose-response data to calculate a POD for an AOP
  - Use sufficient key event – key event sufficient to infer adversity based on network theory
- Reverse dosimetry on POD (if in vitro data) to estimate adult POD
- Determine a safe margin from the POD (divide by 100 if a 100x safe margin is desired)

Orthogonal Assays



Estrogen Receptor alpha

Transactivation Assays Luciferase or beta-lactamase

Cell Proliferation Assays MCF-7 proliferation

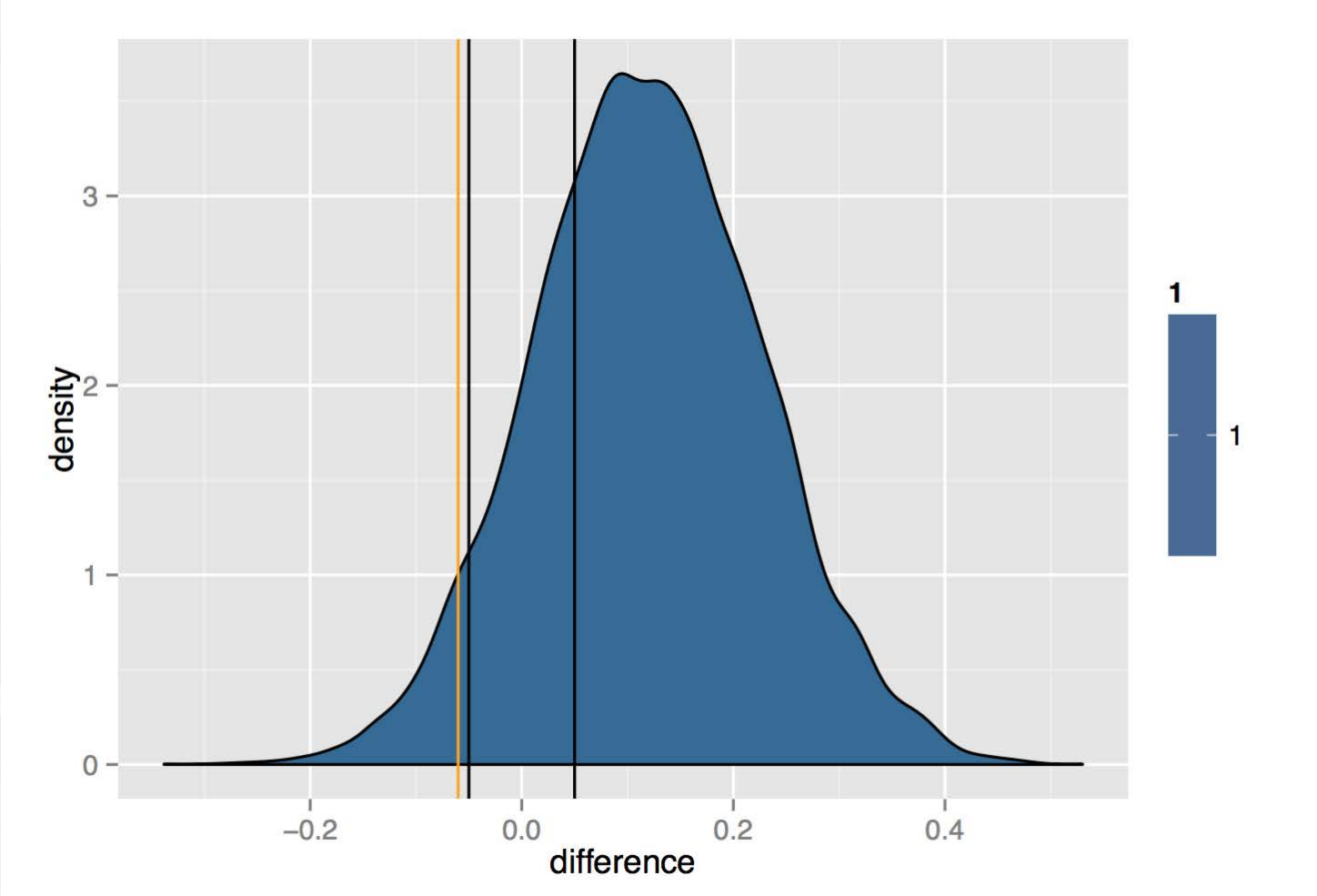
Cell-Free Assays Binding/Competition

Bayesian Data Integration

- **Question**: Is tumor rate in controls from study 1 different from tumor rate in controls from study 2?

- **Statistical testing using ROPEs**
  - ROPE: region of practical equivalence
  - Region where we say it's all equivalent to the null hypothesis
  - So, for a Bayesian "t-test" situation, the null hypothesis is centered at 0, and we'd put a ROPE up that flanks it by maybe 0.5 on either side

HDIs and ROPEs



95% Highest Density Interval (HDI): From Orange line (5% frequency) and above on the curve

ROPE: Area between the two black lines (+/- 5%)

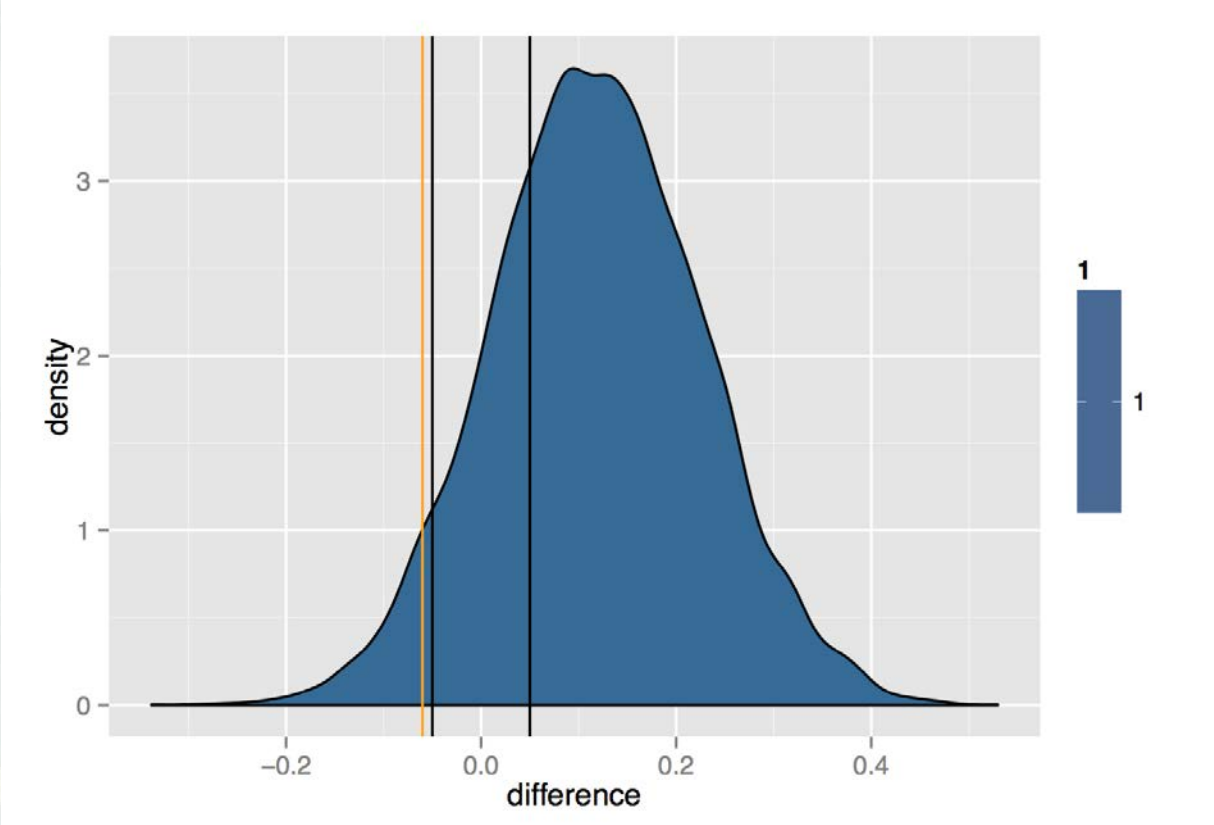
ROPE and HDI Decision Rules

- If the 95% HDI is completely within the ROPE, then accept null hypothesis.
- If the 95% HDI contains zero, then zero difference is a credible value, then accept null hypothesis.
- If the 95% HDI does not contain zero and the 95% HDI is within the ROPE, cannot accept or reject null hypothesis. More data is required.
- If the 95% HDI is completely outside the ROPE, reject null hypothesis.

Approach

- **Question**: Is tumor rate in controls from study 1 different from tumor rate in controls from study 2?
- *Markov Chain Monte Carlo (MCMC using Stan)*
  - Model 1: tumor rates in controls study 1
  - Model 2: tumor rates in controls study 2
- *Model set-up (for both studies)*
  - Flat uninformative prior for both (Beta(1,1))
  - Likelihood modeled as Bernoulli
  - Posterior: Beta distribution using information from the likelihood
- *Calculate the difference in tumor rates from MCMC*
  - Null hypothesis: difference in tumor rates is within a ROPE centered on 0 +/- 5% (this is a rather generous ROPE)

HDIs and ROPEs



95% Highest Density Interval (HDI): From Orange line (5% frequency) and above on the curve

ROPE: Area between the two black lines (+/- 5%)

Conclusion: The tumor rates are substantially the same from both studies, and likely represent values that are on either side of the mean due to random sampling

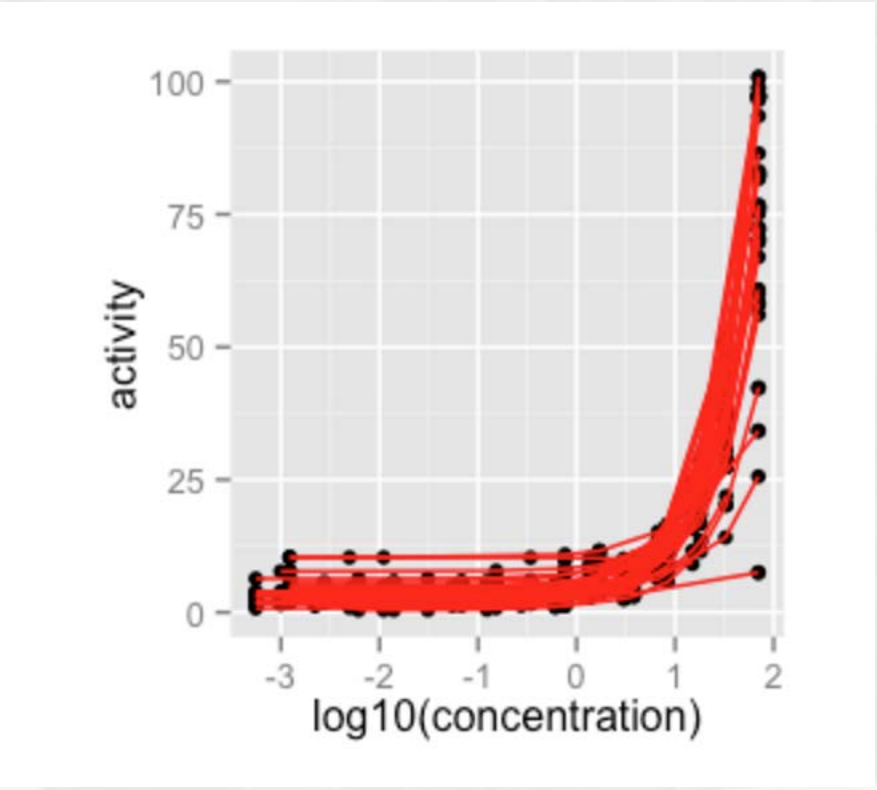
Larger Bayesian Analysis Context

- The tumor rates in controls are likely from the same overall distribution
- We have confidence that we can combine the data from both studies to create a more credible model for statistical analysis
- **New Question**: does chemical X change the tumor rates in exposed mice?
- **Answer**: coming soon, stay tuned!

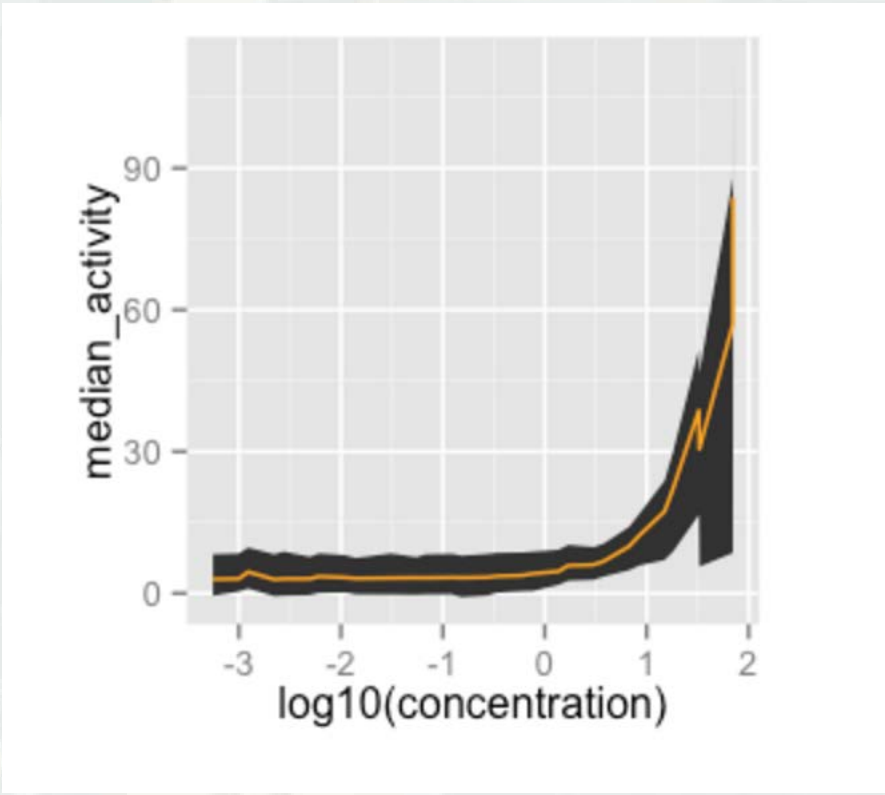
Is Oxybenzone an EDC?

- **Approach**
  - *Orthogonal HTS Data:*
    - PubChem AID 743075 (part of Tox21)
      - ER alpha agonist assay: ER-alpha-UAS-bla GripTite™
    - PubChem AID 743079 (part of Tox21)
      - ER alpha agonist assay: BG1-Luc-4E2
  - *Bootstrap metaregression (R aop package)*
    - https://github.com/DataSciBurgoon/aop/releases
    - Data from the 2 orthogonal assays were combined and bootstrap together (instead of bootstrapping each assay independently)
  - *Point of Departure determination (R aop package)*
    - https://github.com/DataSciBurgoon/aop/releases

Is Oxybenzone an EDC?

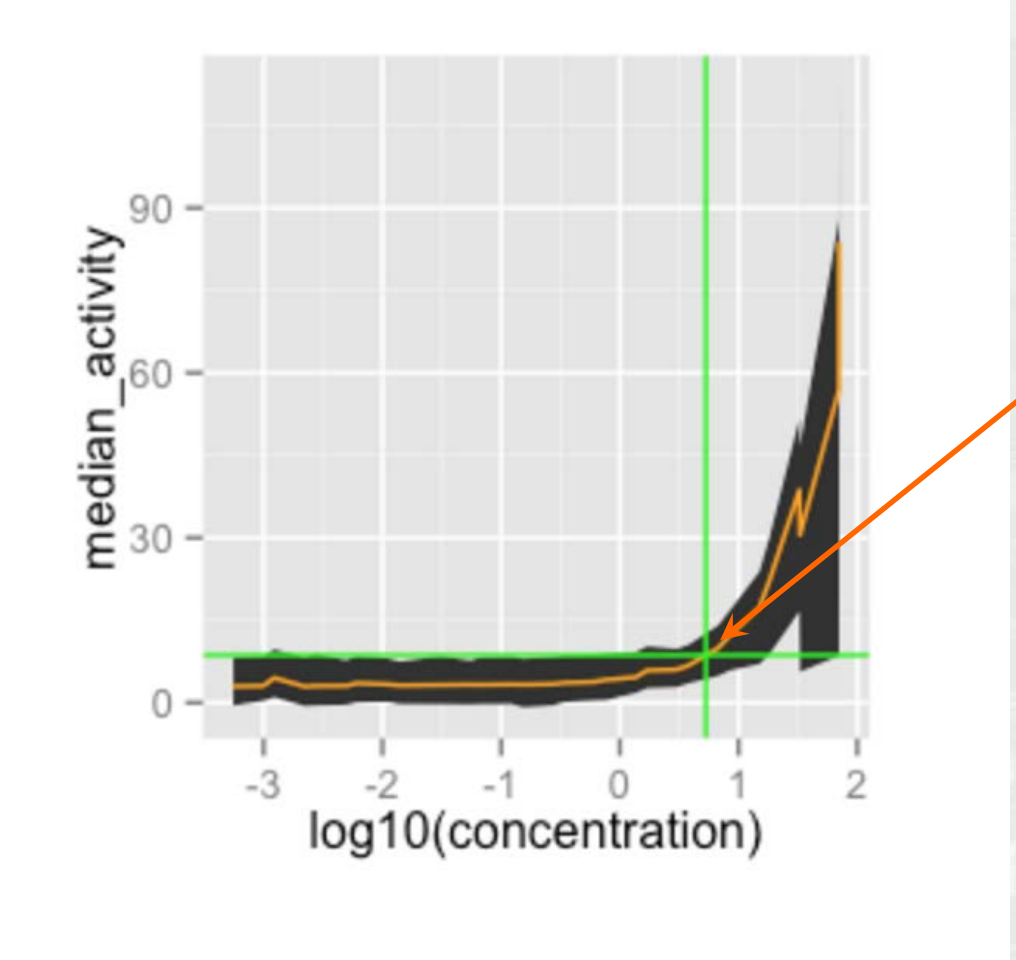


Spaghetti plot



95% Confidence Envelope + Median

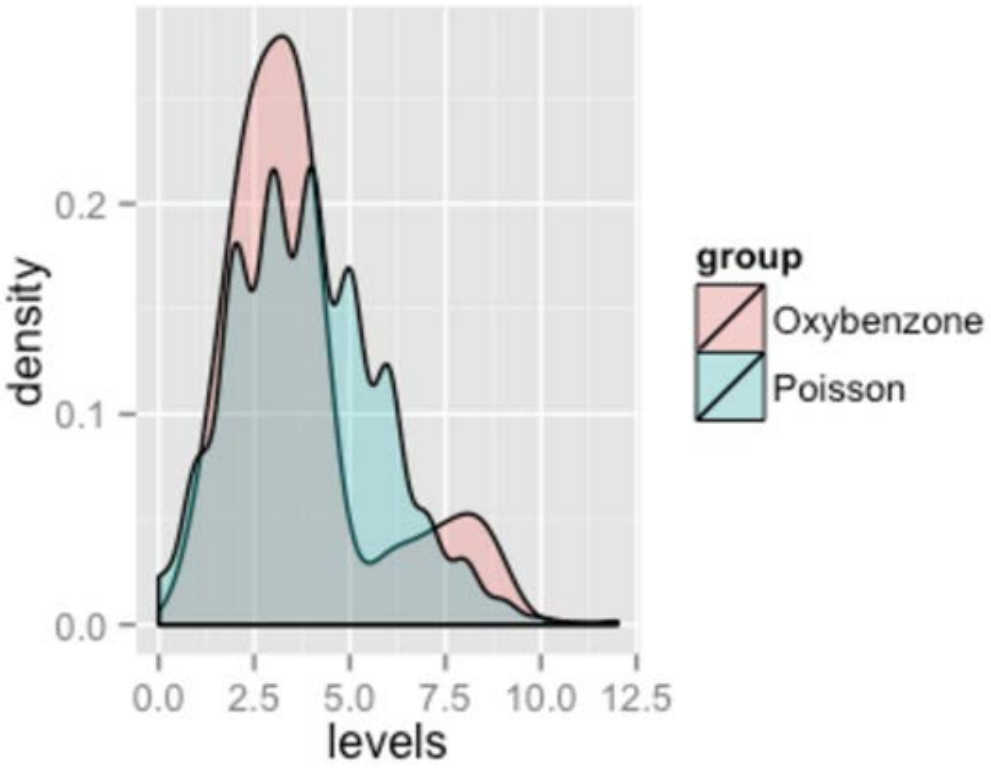
Is Oxybenzone an EDC?



Point of Departure: 5.3uM

Oxybenzone is likely an EDC.

Risk Screening Level for Oxybenzone Estrogenic Activity



Oxybenzone skin absorption data is modeled adequately by a Poisson (3.9) distribution

Data reported in Gonzalez, et al, 2006, British Journal of Dermatology

Risk Screening Level for Oxybenzone Estrogenic Activity

- Oxybenzone MW: 228.24
- Assume: 5L of blood in human
- Given:
  - POD: 5.3uM
- Human POD by skin absorption (protect 1:10,000 people): 432g
- Margin of Exposure (MOE): 100x
- Human Risk Screening Level: 4.32g

- *Real-world application of oxybenzone sunscreen*
  - 28g of sunscreen applied; 4% oxybenzone = 1.12g of oxybenzone applied
  - 3 applications gets us within the 100x MOE (Human Risk Screening Level of 4.32g)

Oxybenzone

Pro-Estrogenicity Arguments
- Evidence in 2 estrogen receptor agonist assays
- Assays are different tissues of origin
- One assay: full-length natively expressed ER
- One assay: ER ligand-binding fusion protein
- Both assays from human tissues

Attenuating Statements
- Not a complete system -- may lack paracrine factors
- Lack of pharmacokinetic model
- Do have skin absorption and urine elimination rates

Contra-Estrogenicity Arguments
- Both assays are cells in monoculture -- not organs or organoids
- Cells may not be able to metabolize oxybenzone

Conclusion: Oxybenzone is an EDC
Confidence: Level 7 (Scale 1-10)
Risk Screening: 4.32g

Confidence Level: We are creating a protocol that describes our levels of confidence on a 1-10 scale (10 being highest confidence)

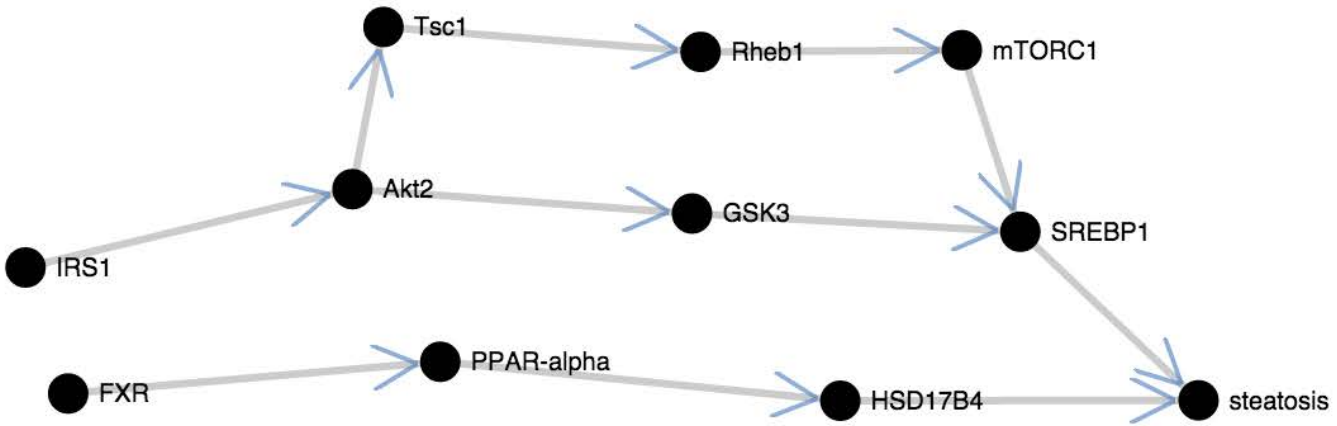
AOPXplorer

- Tool being developed at US Army ERDC to facilitate analysis of NexGen toxicology data
- Predict adverse outcomes using toxicogenomic and HTS data
- Overlays data onto adverse outcome pathways (AOPs)
- Using Machine Intelligence and causal network theory to make predictions of adverse outcomes using AOPs and your data
- Facilitate Screening Risk Assessment development and publication
- Ongoing development

Sneak Peak of AOPXplorer

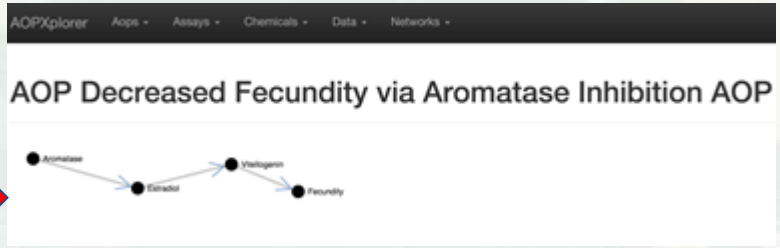
AOPXplorer Aops Assays Chemicals Networks Sign Up Sign In

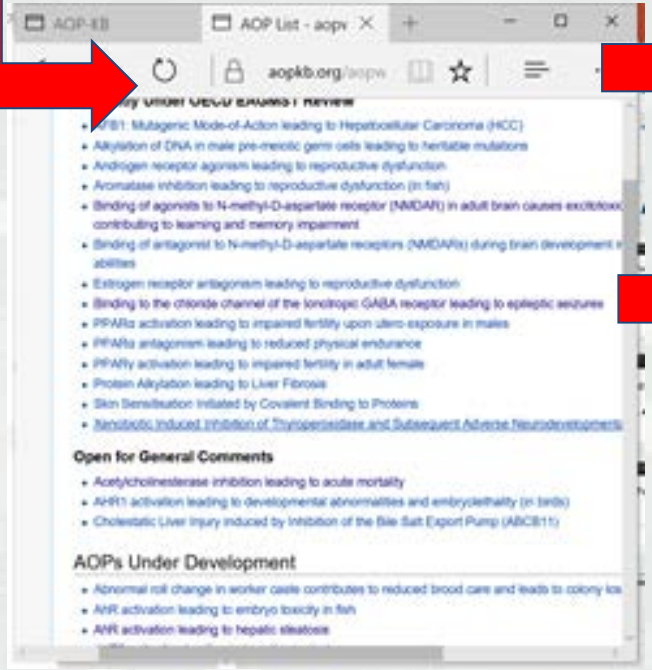
Steatosis AOP Network



Incorporation of AOPs from AOPwiki

Fish fecundity AOPs



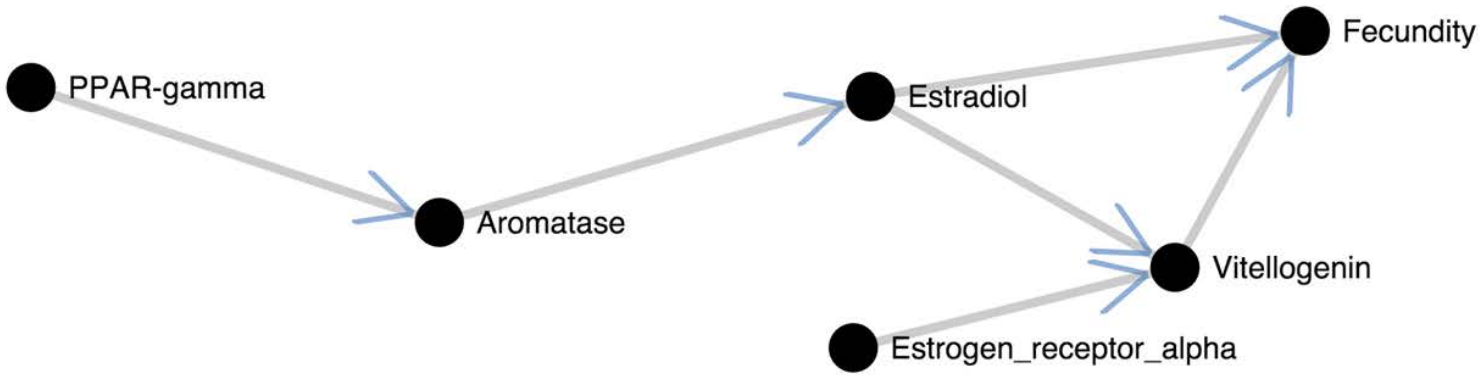


AOPwiki

Fish Fecundity AOP Network

AOPXplorer Aops Assays Chemicals Data Networks

Decreased Fecundity AOP Network



AOPXplorer

- So far, all of the analysis code, ontologies, etc are all up on github
  - https://github.com/DataSciBurgoon/
  - All of this is a work in progress, and improvements are constantly being made

- AOPXplorer web interface is still under development
- Coordinating with AOP-KB/wiki

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Erin Yost

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Ila Cote

Cisco

Kyle Painter*

* previously ORISE Fellows at US EPA