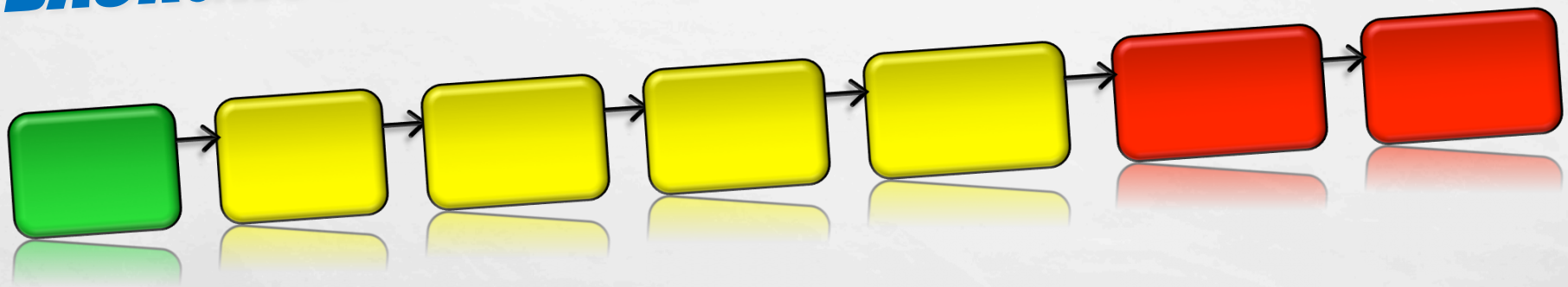
ADVERSE OUTCOME PATHWAYS

BACKGROUND AND PRINCIPLES



Daniel L. Villeneuve, Ph.D.

US EPA, Mid-Continent Ecology Division*, 6201 Congdon Blvd, Duluth, MN, 55804

OUTLINE

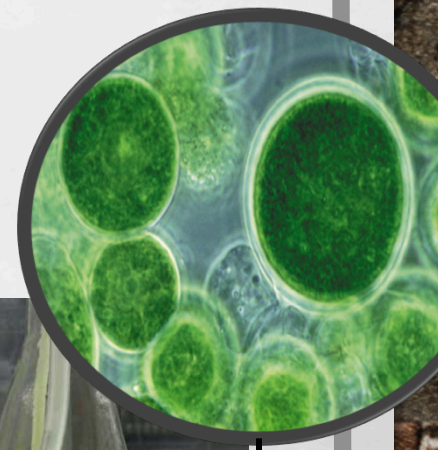
- BACKGROUND
- ADVERSE OUTCOME PATHWAYS – DEFINED
- EXAMPLES OF APPLICATIONS
- PRINCIPLES OF AOP DEVELOPMENT

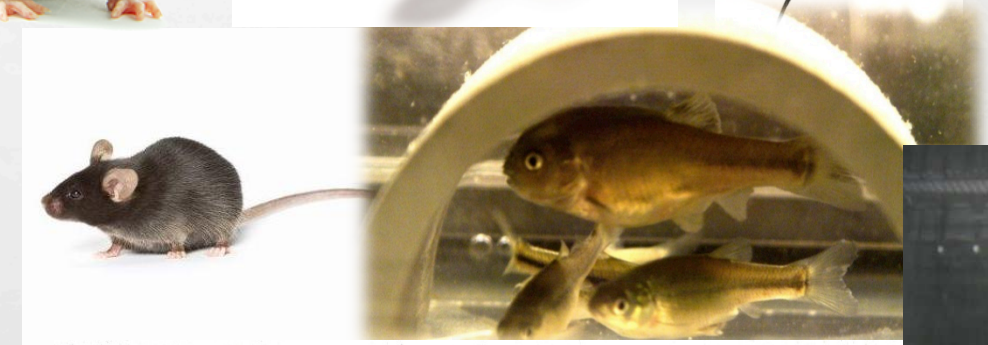
Wide range of diagnostic tests are employed in medicine

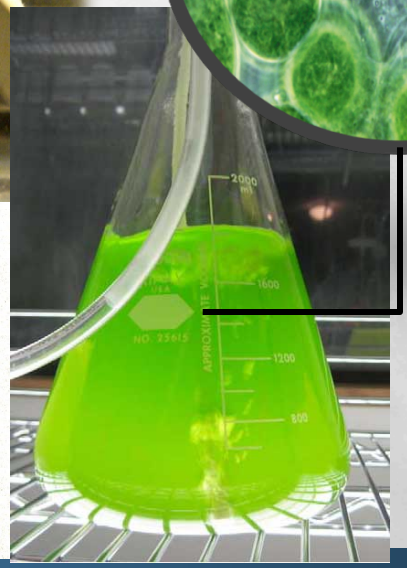
Doctors explain to patients, what the results of those tests mean relative to health.

TOXICOLOGY

- HAS GENERALLY FAVORED DIRECT OBSERVATION OF APICAL ADVERSE EFFECTS
  - EXPENSIVE
  - TIME-CONSUMING
  - ETHICAL/SOCIETAL CONCERNS



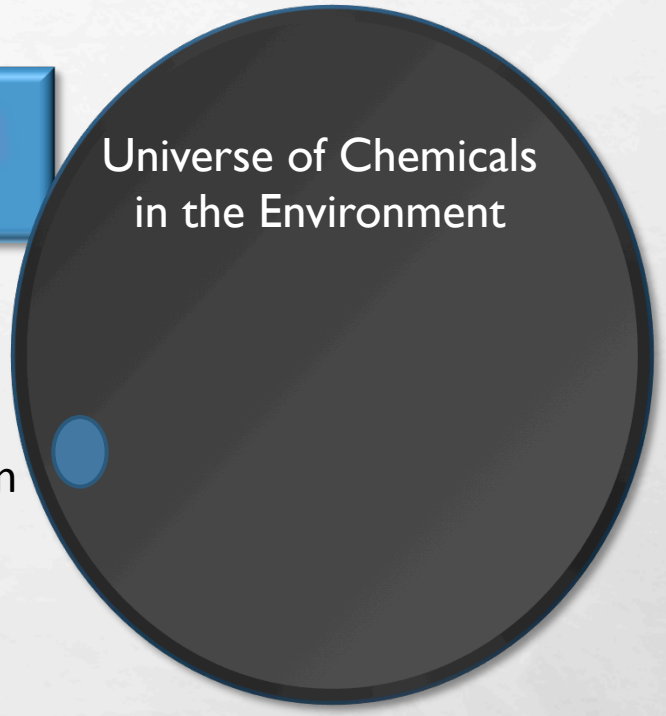




Introduction

The Great Chemical Unknown

[Scientific American October 28, 2010]



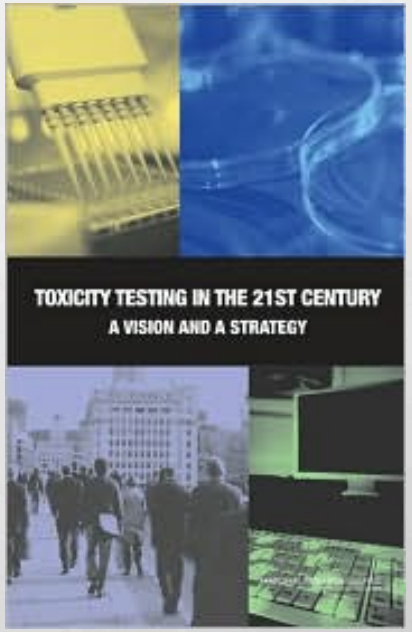
- Very limited toxicity characterization for most chemicals in commerce.

The Vision

"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

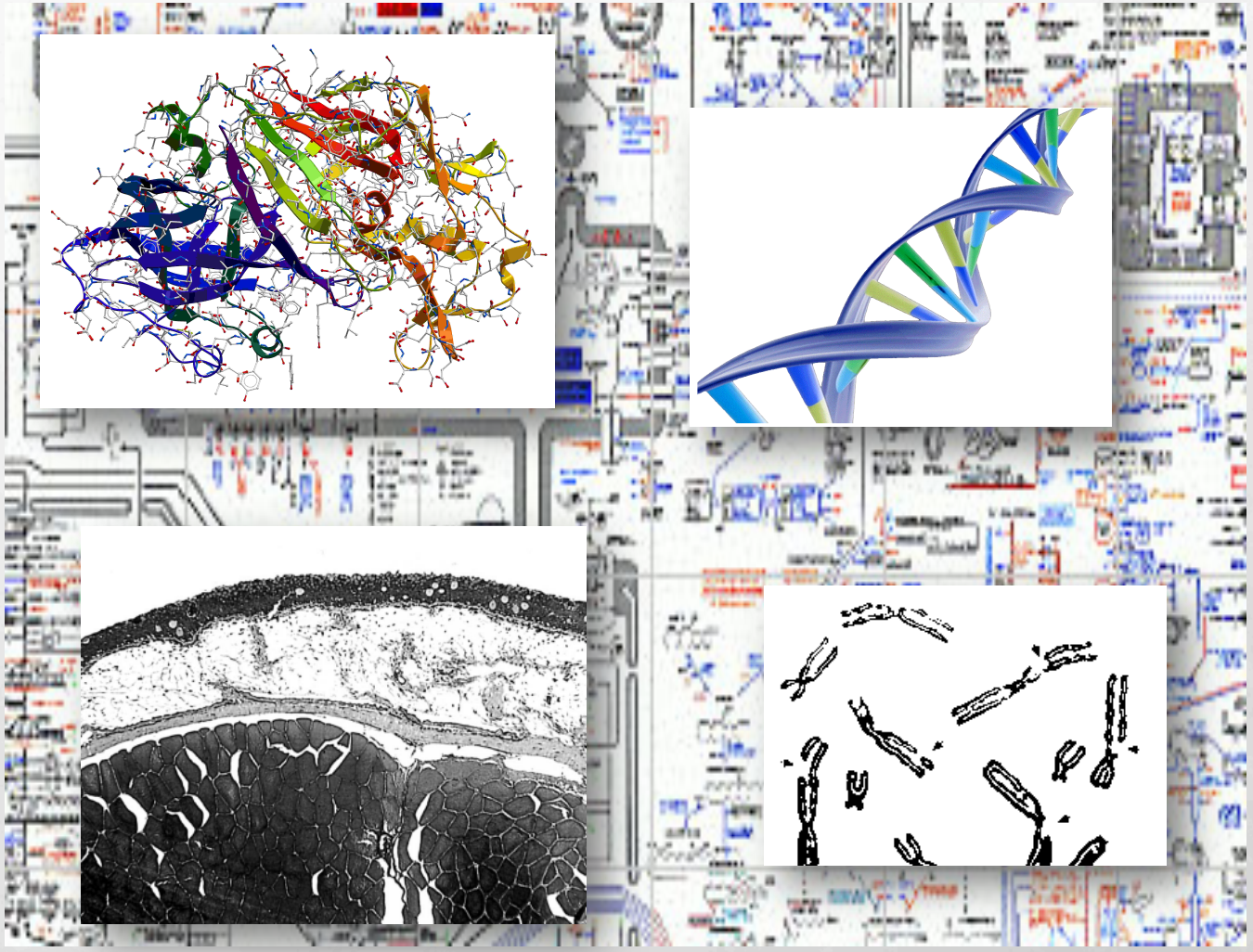
"The vision emphasizes the development of suites of predictive, high-throughput assays ….."

"The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves."



Examples

- Enzyme activities
- Gene expression
- Hormone concentrations
- Metabolite abundance
- Histological changes
- DNA damage
- Epigenetic modifications
- Lipid or protein abundance



ToxCast

> 600 assays, >2000 chemicals, ToxCast HTS Assays

EPA United States Environmental Protection Agency

~500 Total Endpoints

Biochemical Assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

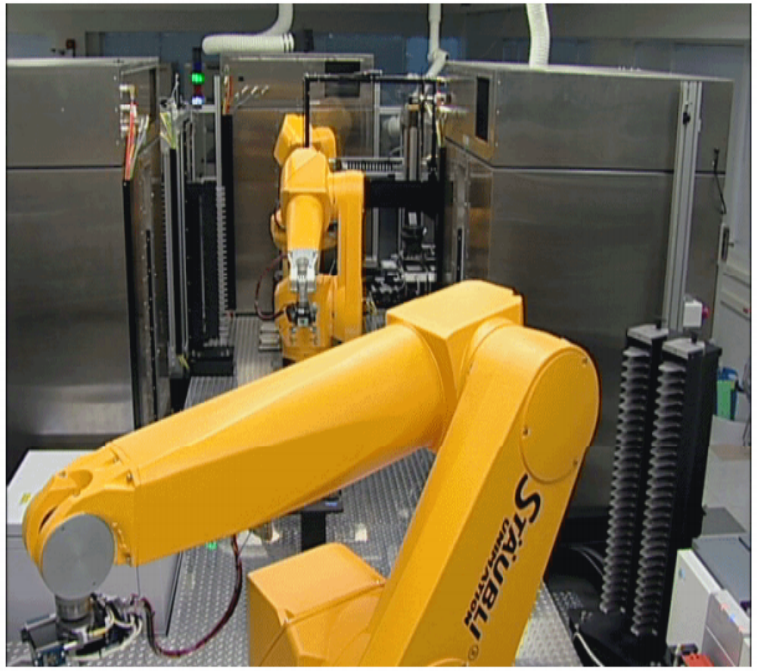
Cellular Assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
  - Rat hepatocytes
  - Mouse embryonic stem cells (Sid Hunter)
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Primarily Human / Rat
Exception: Zebrafish development (Stephanie Padilla)

- 1536 well HTS
- 10,000 chemicals
- 25 assays per year

Tox21

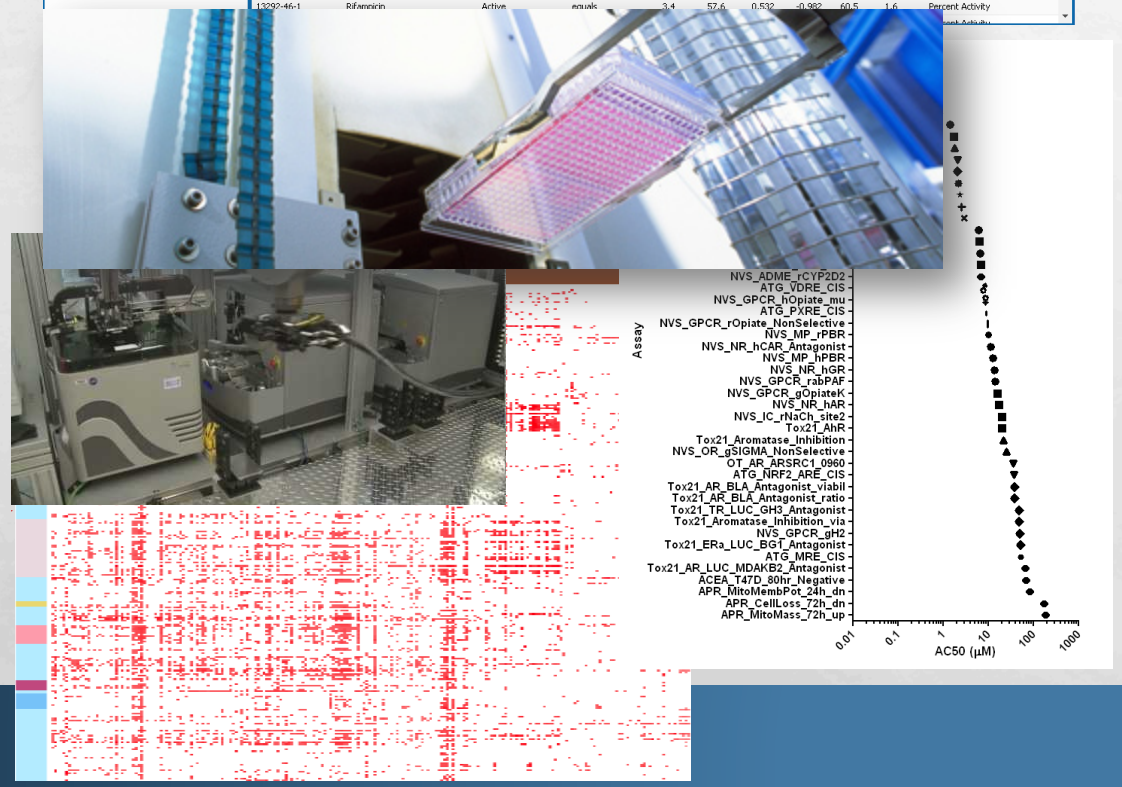


EPA United States Environmental Protection Agency

NIEHS National Institute of Environmental Health Sciences

NCGC NIH Chemical Genomics Center

FDA



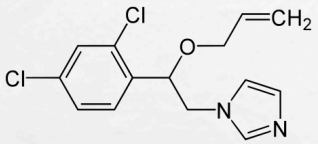
Vision

21st Century Toxicity Testing is here….

We can rapidly and cost effectively generate pathway-based data

- Activity of 1000s of chemicals in 100s of pathways.

Conceivable that majority of chemicals in commerce could be “tested” within the decade.



1-{2-(2,4-Dichlorophenyl)-2-[(prop-2-en-1-yl)oxy]ethyl}-1*H*-imidazole

Chemistry Dashboard

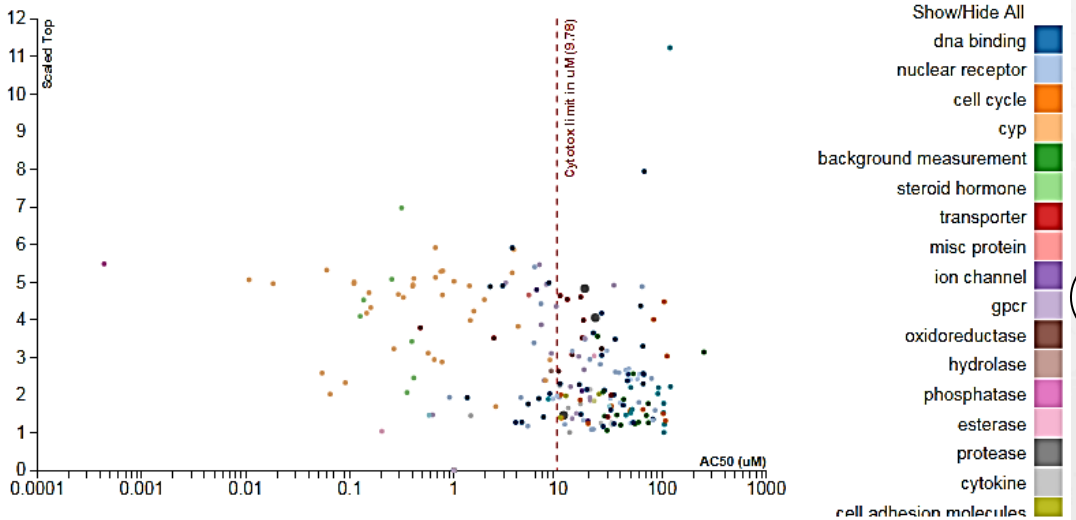
Submit Comment | Share | Copy

ToxCast

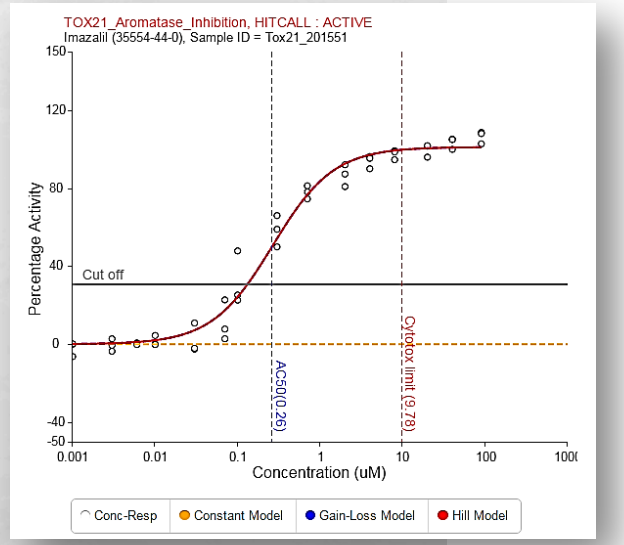
PubChem

ScrubChem (...

Chemical Activity Summary



Show/Hide All
dna binding
nuclear receptor
cell cycle
cyp
background measurement
steroid hormone
transporter
misc protein
ion channel
gpcr
oxidoreductase
hydrolase
phosphatase
esterase
protease
cytokine
cell adhesion molecules



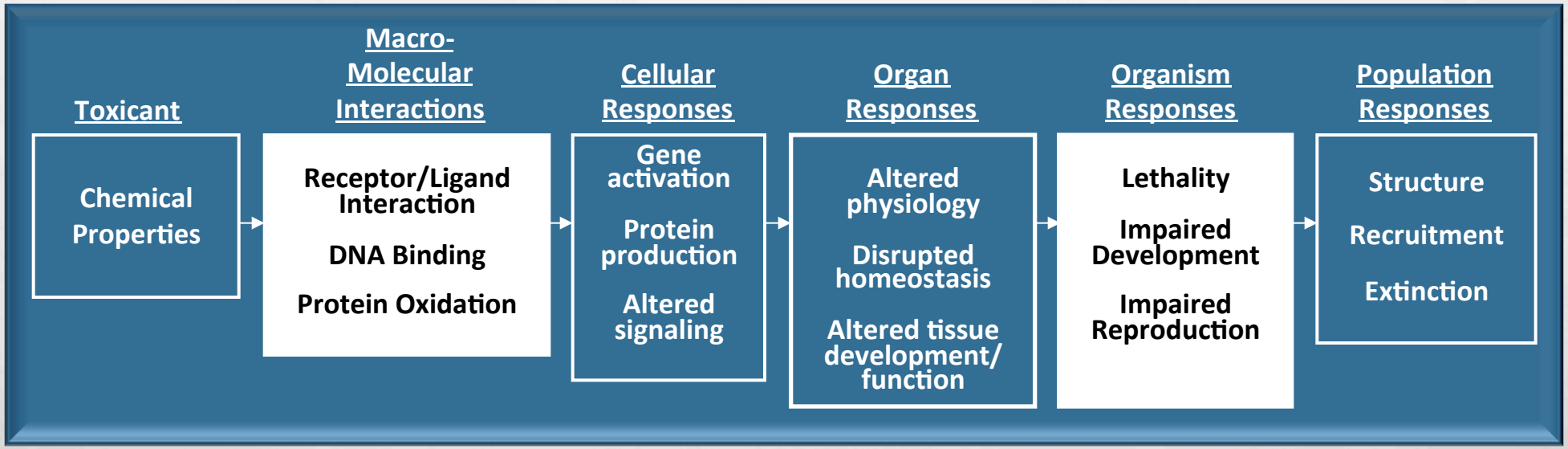
Download as: TSV | Excel Show: Inactive | Background

Assay Name	Hit Call	Top	Scaled ...	AC50	log AC50	Intended Target Fa...
agonist						
TOX21_Aromatase_Inhibition	ACTIVE	101	3.23	0.266	-0.575	cyp

Adverse Outcome Pathway

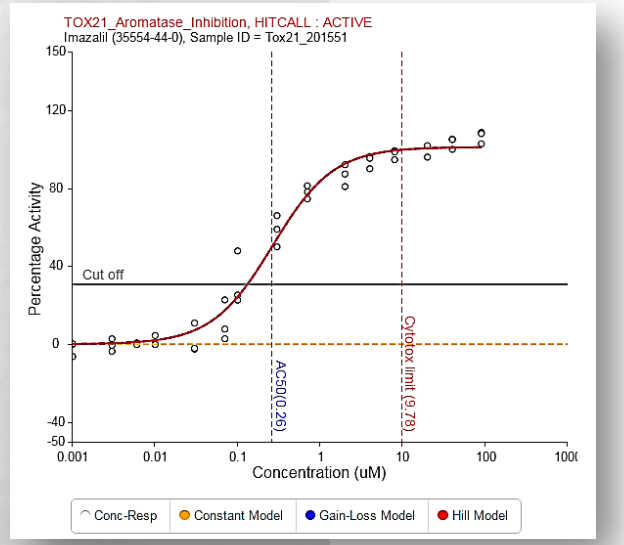
An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.

(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)



- Helps us organize what we know
- Utilize mechanistic data to support risk-based decision-making

Potential evidence for more specifically-acting toxicity

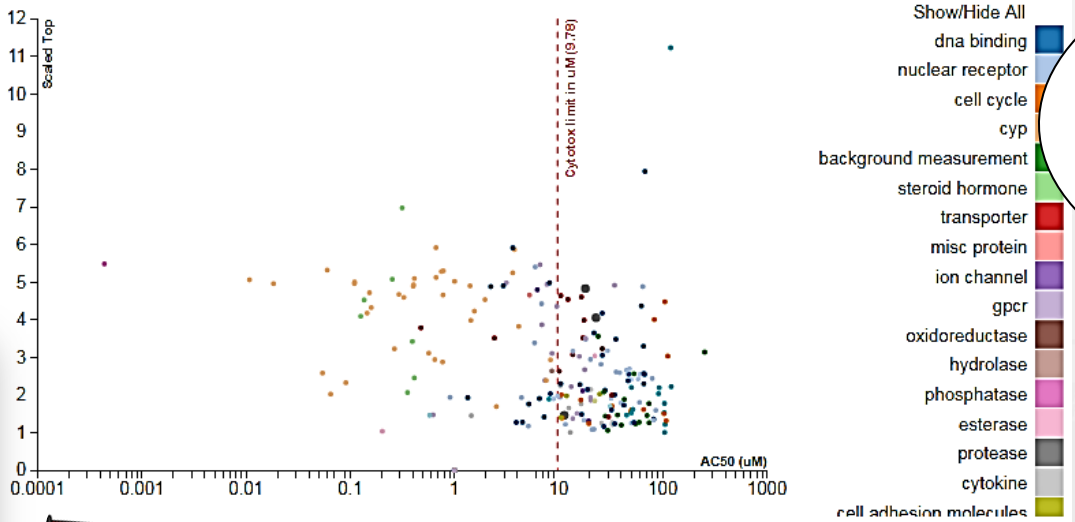


Chemistry Dashboard

Submit Comment | Share | Copy

ToxCast

Chemical Activity Summary



Evidence for Aromatase inhibition

There's an AOP for that….reproductive hazard

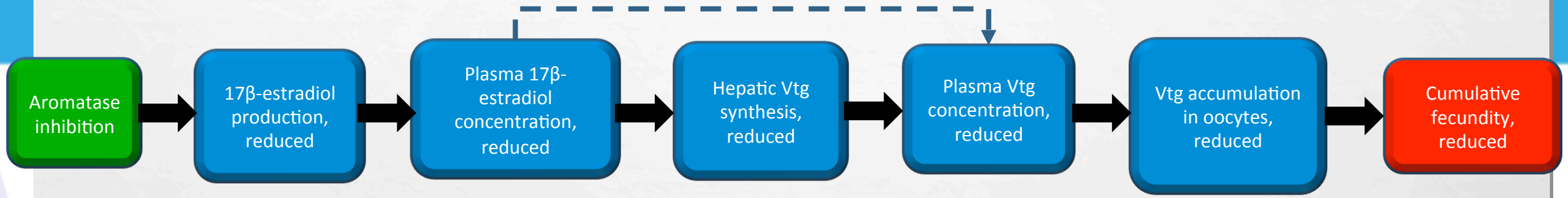
Download as: TSV | Excel Show: Inactive | Background

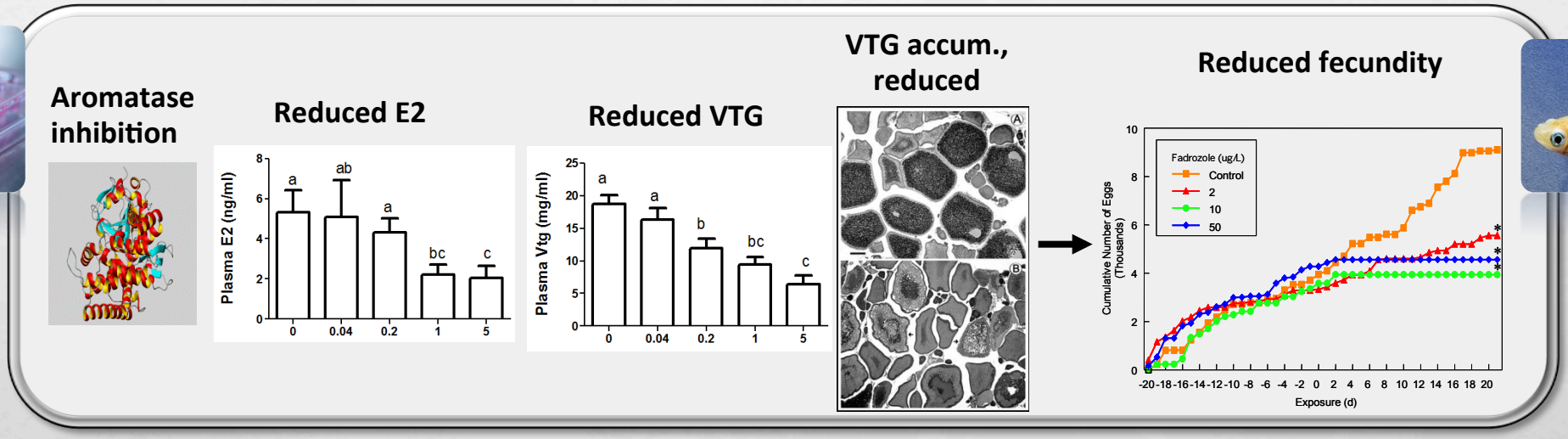
Assay Name	Hit Call	Top	Scaled …	AC50	log AC50	Intended Target Fa…
agonist						
TOX21_Aromatase_Inhibition	ACTIVE	101	3.23	0.266	-0.575	cyp

Potential hazard to vertebrate reproduction

AOP 25: Aromatase inhibition leading to reproductive dysfunction

Example of an AOP: https://aopwiki.org/aops/25

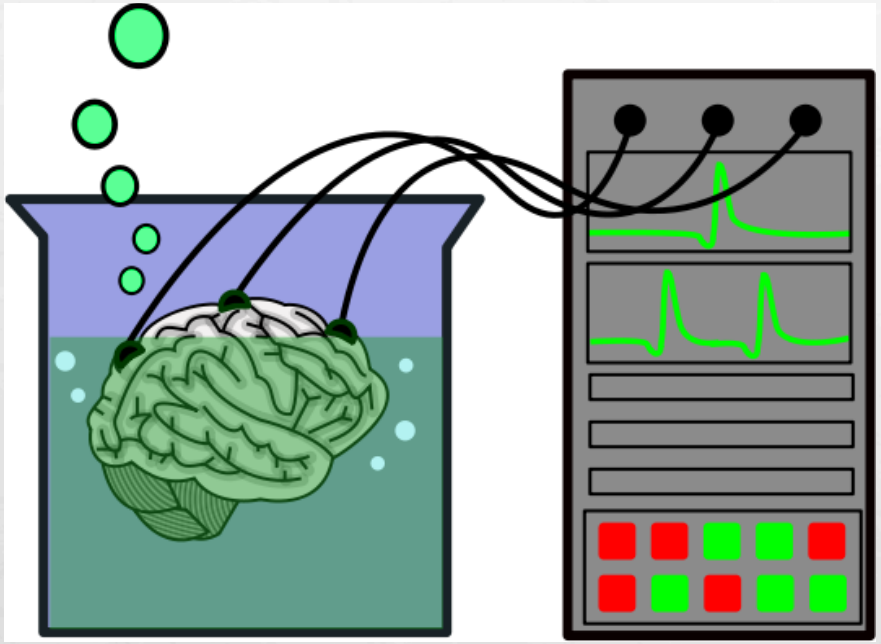




Not only is it biologically plausible – its supported by empirical evidence

Consistent profile of effects have been observed with other cyp19 inhibitors and in other species :
Prochloraz, fathead minnow: *Toxicol. Sci. 2005. 86: 300-308*
Propiconazole, fathead minnow: *Toxicol. Sci. 2013. 132: 284-297.*
Letrozole, Japanese medaka: *Compar. Biochem. Physiol. Pt. C, 2007, 145: 533-541*

Adverse Outcome Pathway



- AOPs organize knowledge
- Present it systematically
- Manner that is accessible and usable

AOP as diagnostic manual: explains what it means for health and why.

What AOPs are not:

- **AOPs are not risk assessments**
  - Do not explicitly address exposure
- **AOPs are not synonymous with HTT or pathway-based assays**
  - Aid interpretation of HTT and pathway-based assay data in the context of apical hazard
- **AOPs are not Computational Models**
  - Computational models that align with AOPs and can be used to simulate KERs along the AOP and predict state of KEs under various conditions/scenarios termed qAOPs.
- **AOPs are not a panacea**
  - Don't solve challenges of in vitro / in vivo extrapolation
  - Don't account for all known biology or all possible modulating variables

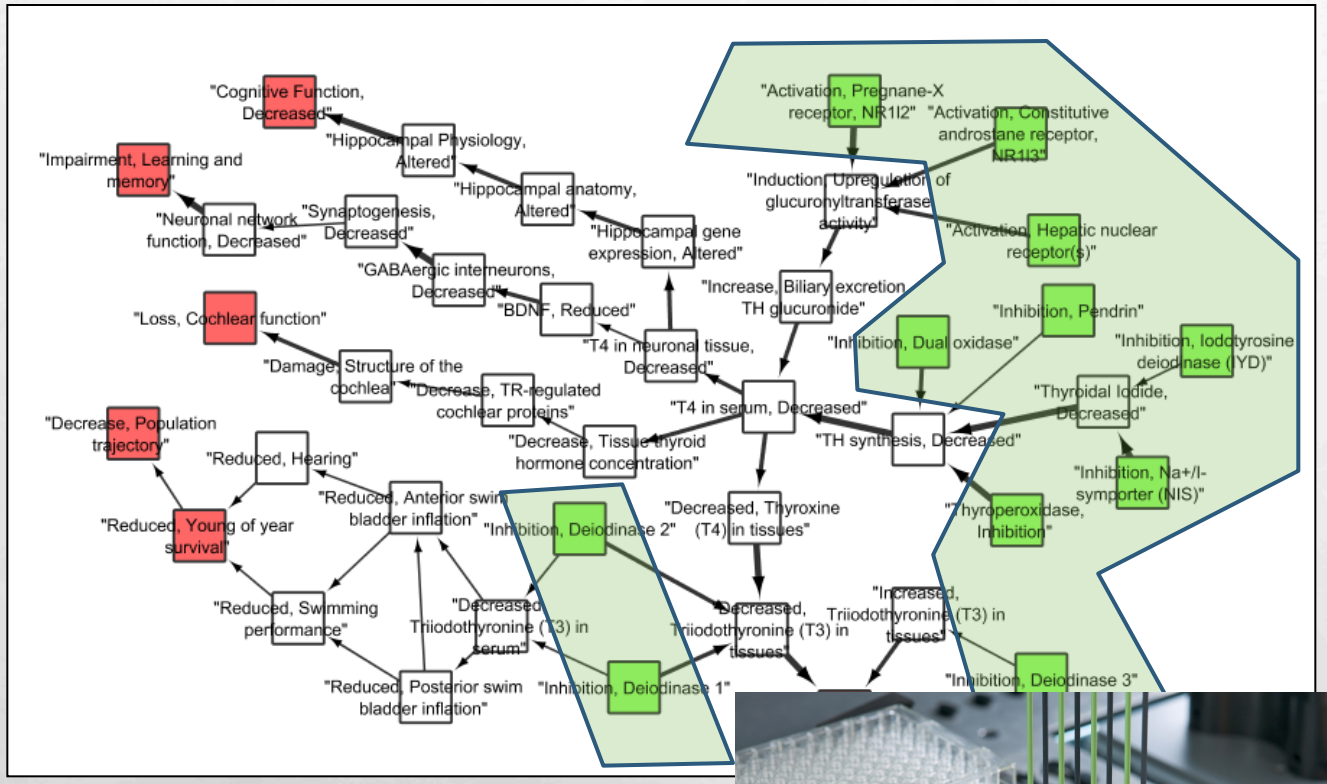
What AOPs can do for us:

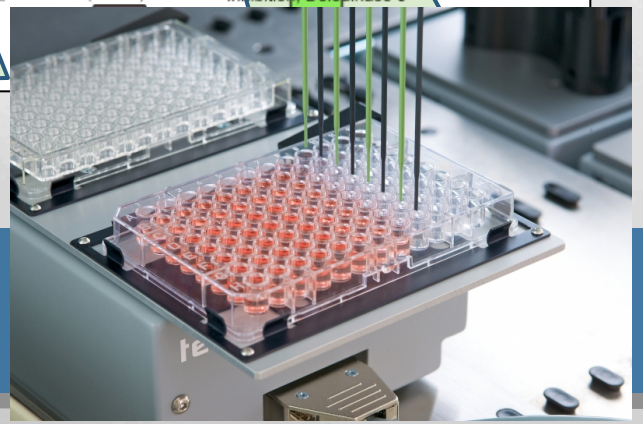
- Enhance use of mechanistic data in regulatory decision-making
- Support hypothesis-driven testing - target in vivo testing on endpoints of concern
- Inform appropriate cross-species extrapolation & focus testing on species, life-stages, taxa of concern
- Aid a strategic, knowledge-driven approach to evaluating complex mixtures
- Identify critical knowledge & evidence gaps that impede application

EXAMPLE APPLICATIONS

- DEVELOPMENT OF ALTERNATIVE TESTING APPROACHES
- SUPPORTING TIERED TESTING STRATEGIES / IATA
- FRAMEWORK FOR ORGANIZING AND EVALUATING EVIDENCE
- QUANTITATIVE BMD ESTIMATION
- BIOACTIVITY-BASED ENVIRONMENTAL MONITORING

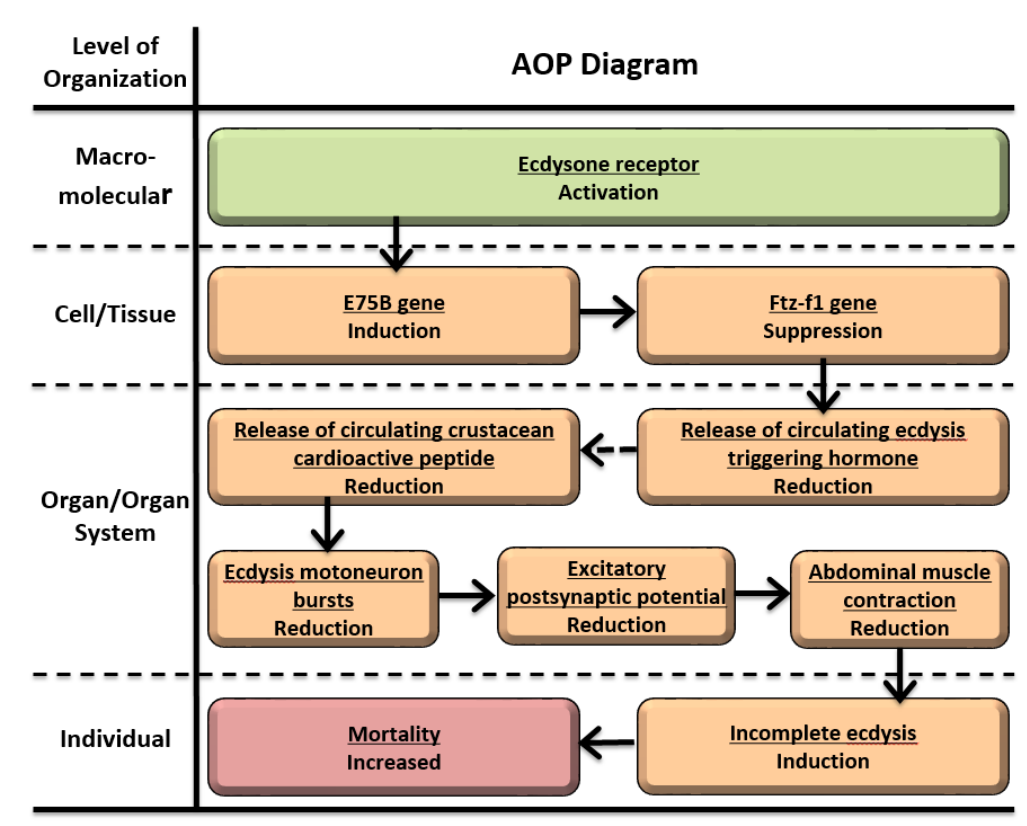
ALTERNATIVE TESTING APPROACHES

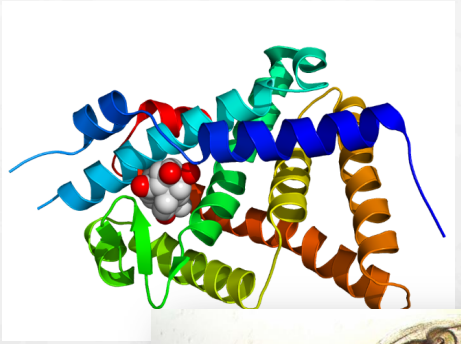




IYD inhibition	HT assay under development
NIS inhibition	HT assay under development
TPO inhibition	HT assay developed >1000 chemicals screened
DIO1 inhibition	HT assay developed >100 chemicals screened
DIO2 inhibition	HT assay developed >100 chemicals screened

ALTERNATIVE TESTING APPROACHES





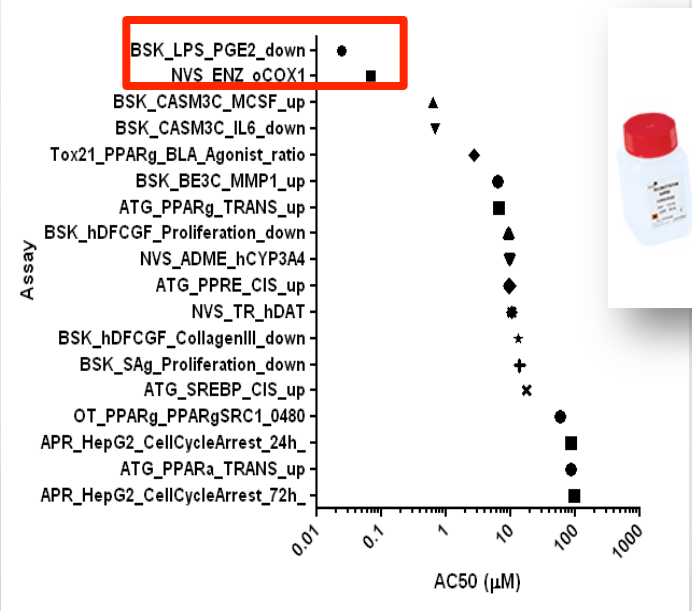
Identify assays that could be incorporated and establish why they are relevant

https://aopwiki.org/aops/4

Song Y, Villeneuve DL, Toyota K, Iguchi T, Tollefsen KE. Ecdysone Receptor Agonism Leading to Lethal Molting Disruption in Arthropods: Review and Adverse Outcome Pathway Development. Environ Sci Technol. 2017 Apr 18;51(8): 4142-4157. doi: 10.1021/acs.est.7b00480.

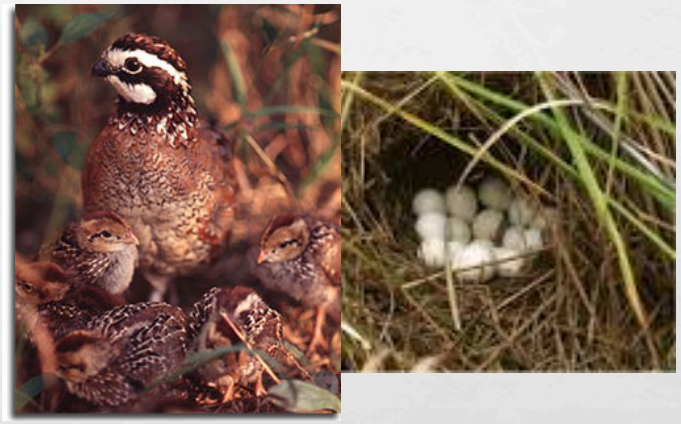
TIERED TESTING / IATA

Cyclooxygenase inhibition → Prostaglandin E2 concentrations in eggshell gland mucosa, reduced → Ca^{2+} and HCO_3 transport to shell-gland lumen impaired → Eggshell thickness reduced → Crushed eggs, breeding failure



High Throughput Screening

In vivo – 96 h

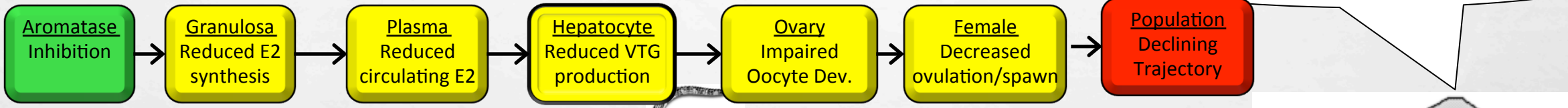


Avian reproduction study
(OPPTS 850.2300; OECD 206)

$>250,000
>30 weeks to perform

TIERED TESTING / IATA

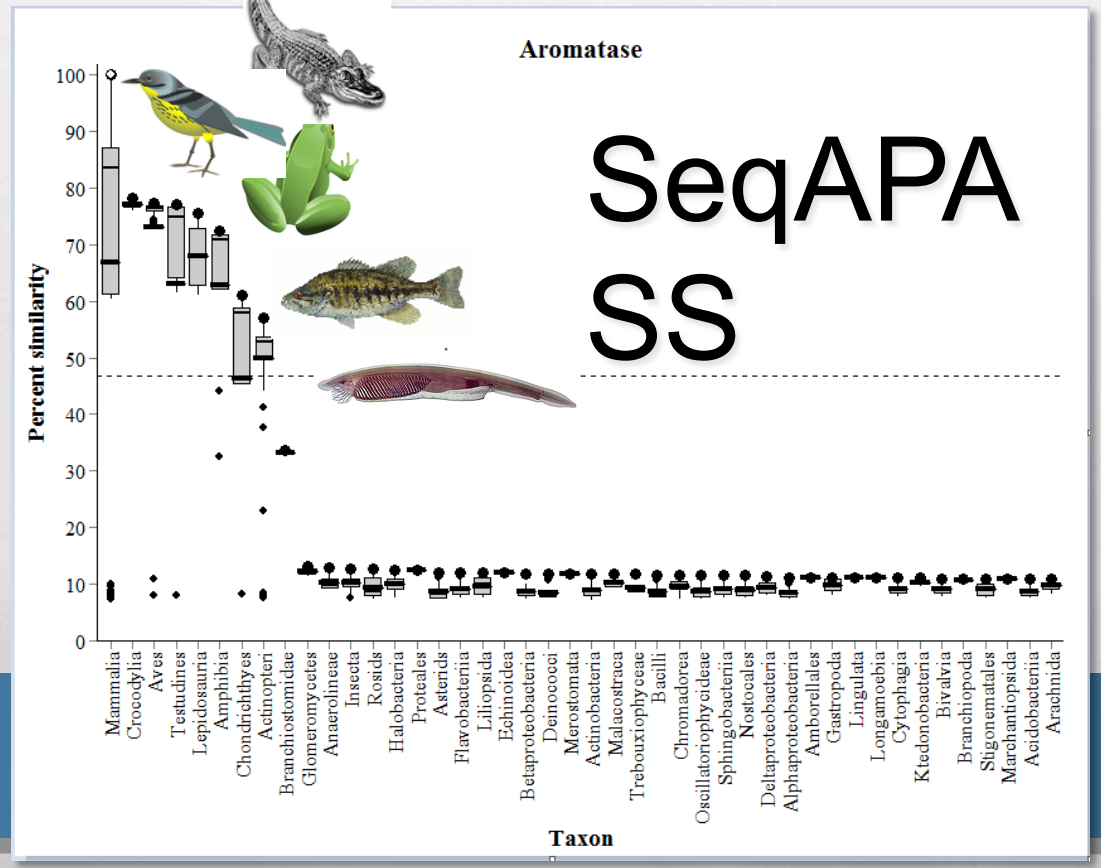
Invertebrate tests are unlikely to drive criteria setting



Aromatase first appears in common ancestor to amphioxus and vertebrates (Baker 2011).

MIE – likely applicable to most vertebrates

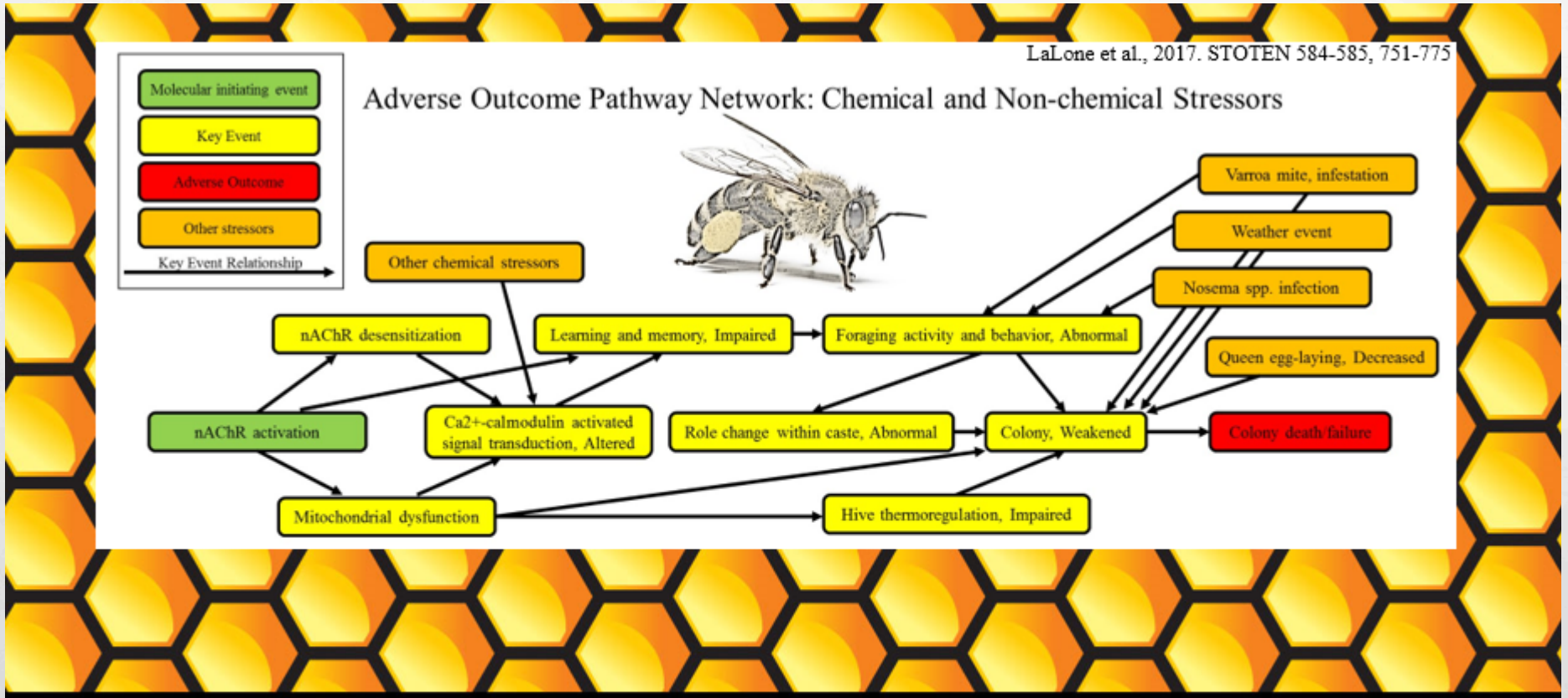
Vitellogenesis-related key events likely applicable to oviparous vertebrates



SeqAPASS

Baker ME. 2011. Molec. Cell. Endocrinol. 334:14-20

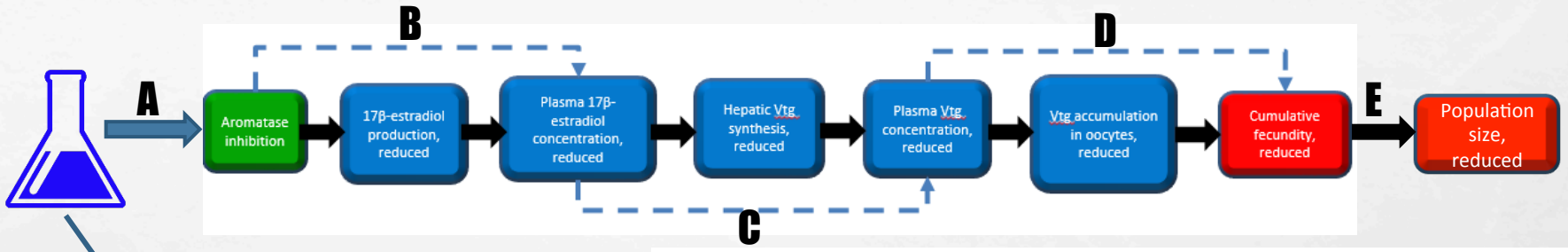
FRAMEWORK FOR ORGANIZING AND EVALUATING EVIDENCE



Using an adverse outcome pathway network to describe the weight of evidence linking nicotinic acetylcholine receptor activation to honey bee colony failure

AOP:88
AOP:89
AOP:77
AOP:87
AOP:79
AOP:178
AOP:81
AOP:179
AOP:181
AOP:185

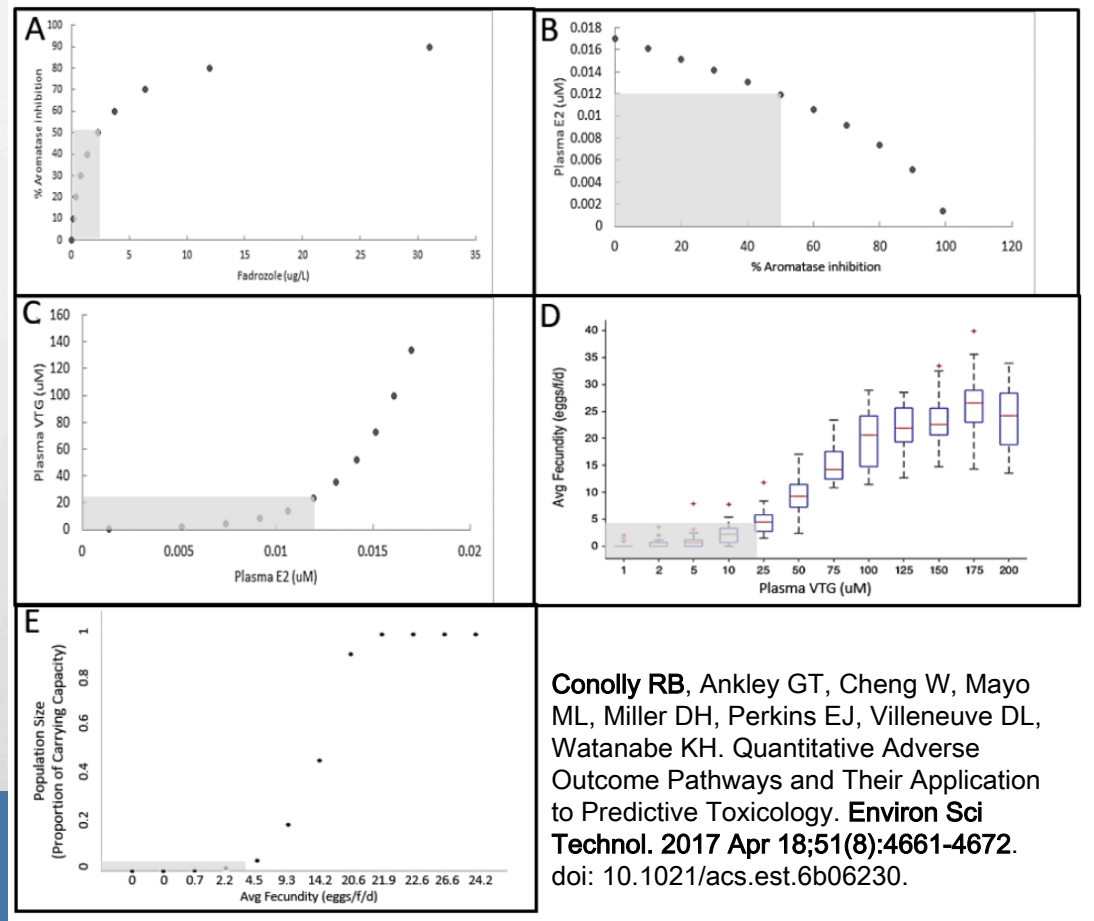
QUANTITATIVE BMD ESTIMATION



Equipotent concentration of reference chemical

Model-derived response-response relationships for major KERs along the AOP.

Steady state, after compensation assumed.



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.

APPLICATIONS

Examples are not comprehensive

Some of the most prominent applications to date

An International AOP Development Program

- 2011: US EPA/ORD AOP discovery and development program
- 2012: OECD AOP development programme initiated
- 2013: First guidance document – developing and assessing AOPs
- 2014: Case studies in AOP development and revised guidance

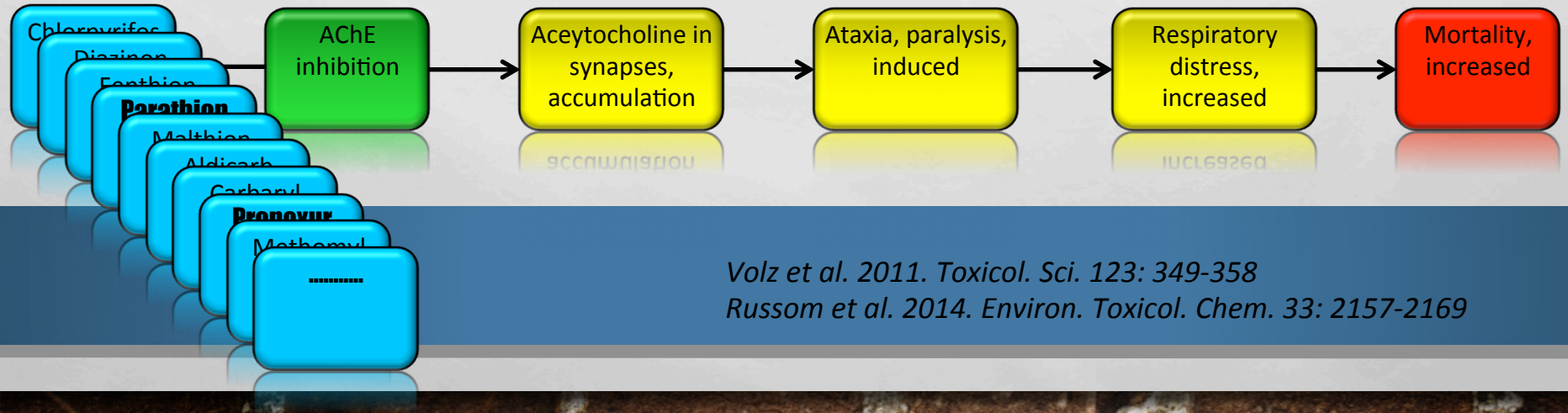
Principles of AOP Development

1. AOPs are not chemical-specific

- Not trying to describe what a single chemical does

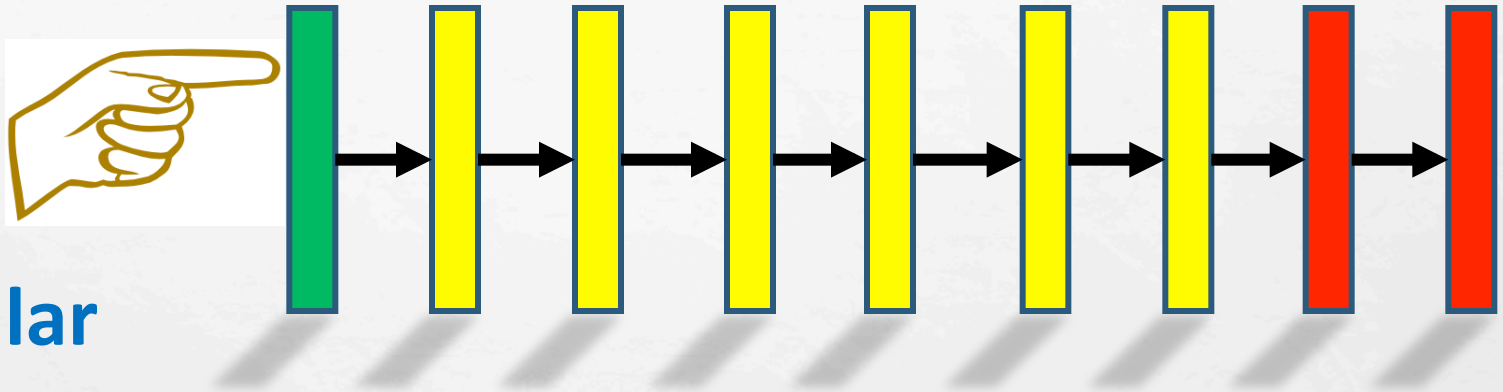
- Trying to describe what <u>ANY</u> chemical that perturbs the MIE with sufficient potency and duration is likely to do- Biological motifs of failure

- *Describing* AOP does not require chemical-specific information.
- *Applying* those motifs in a predictive context requires understanding chemical-specific properties (e.g., potency, ADME) that dictate the magnitude and duration of perturbation at the MIE.



Volz et al. 2011. Toxicol. Sci. 123: 349-358
Russom et al. 2014. Environ. Toxicol. Chem. 33: 2157-2169

Principles of AOP Development



2. AOPs are Modular

Key Events (KEs)

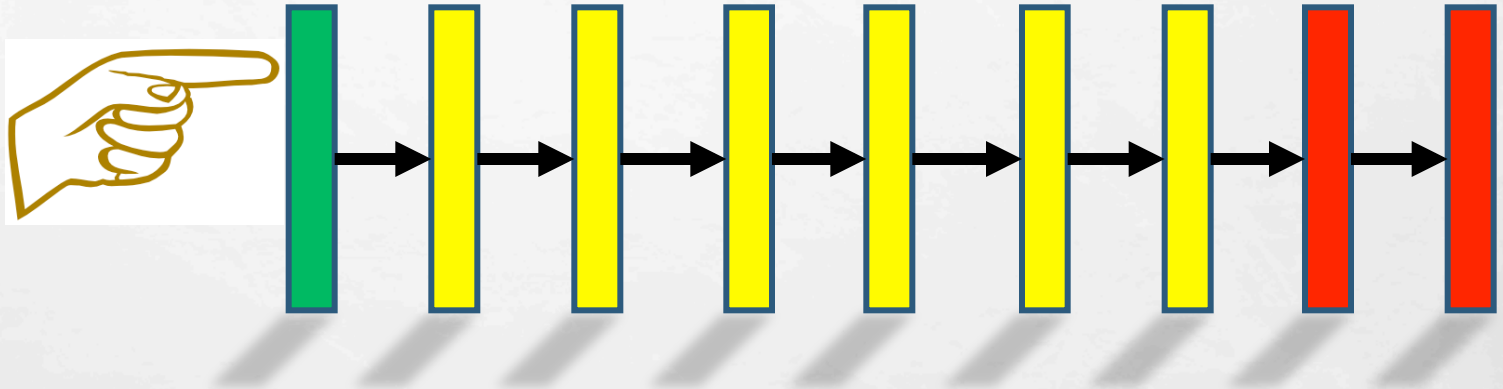
- *Functional unit of observation/verification*

- Observable Δ biological state (measurable)
- Essential (but not necessarily sufficient)

- Description
- Methods for observing/measuring
- Taxonomic applicability

Principles of AOP Development

2. AOPs are Modular

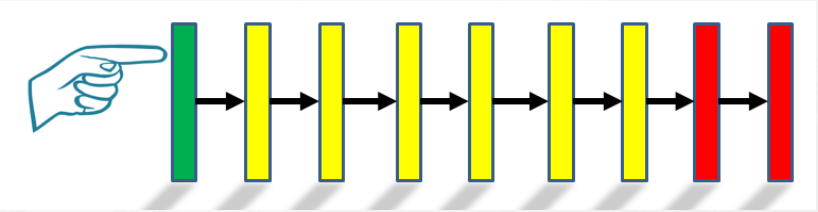


"Special case" KEs

- Molecular initiating event (MIE) – A specialized type of KE that represents the initial point of chemical interaction, on the molecular level, within an organism, that results in a perturbation that starts the AOP.

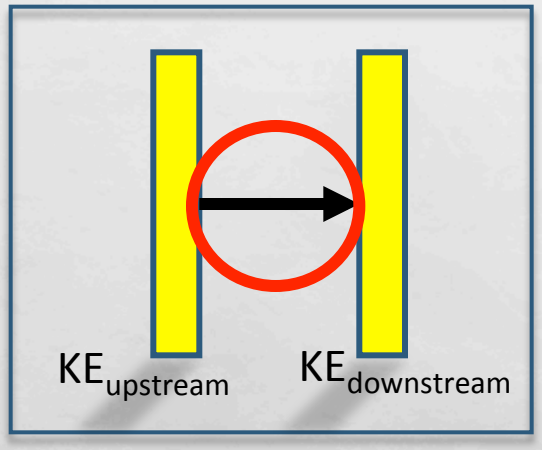
- Adverse Outcome (AO) – A specialized type of KE that is generally accepted as being of regulatory significance on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test.

Principles of AOP Development



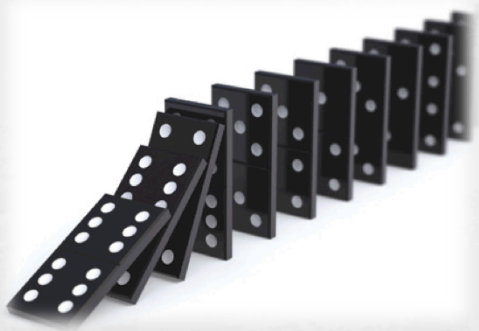
2. AOPs are Modular

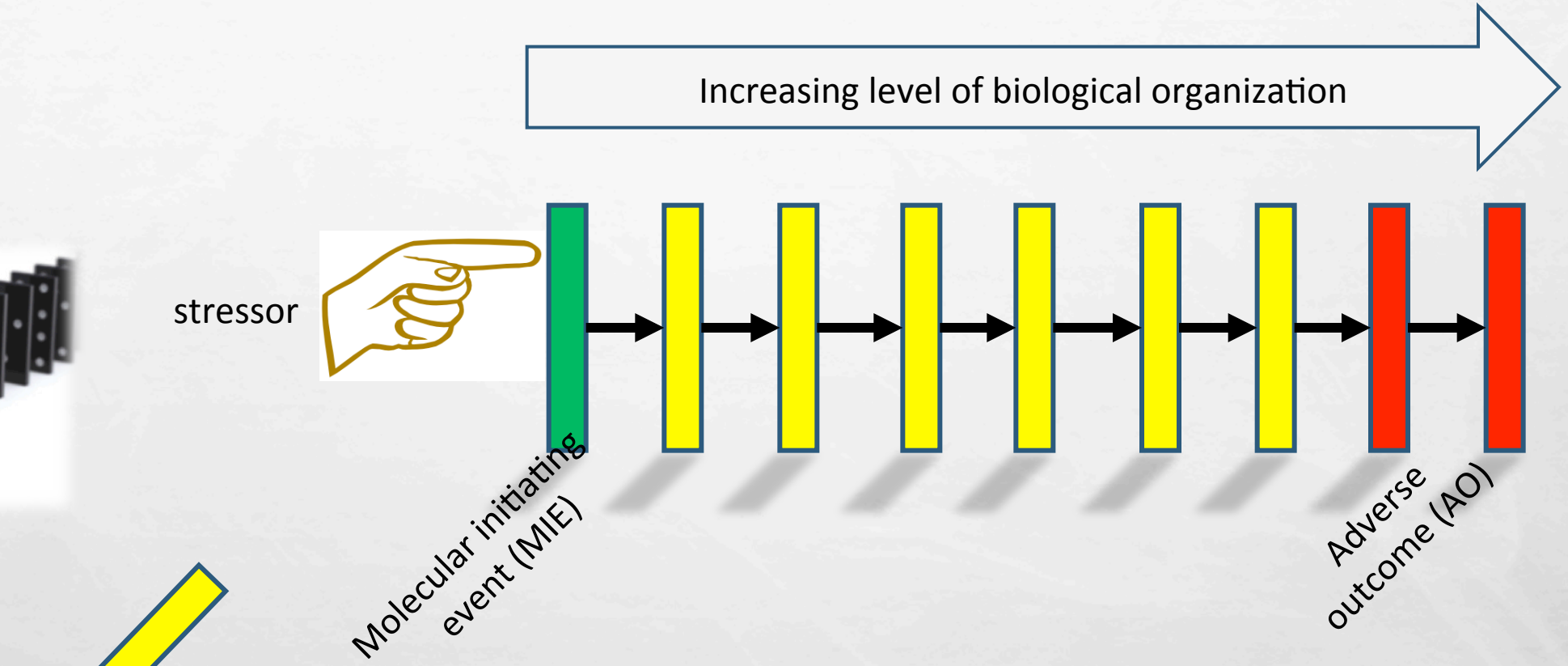
Key Event Relationships (KERs):
Functional unit of inference/extrapolation



- Define a directed relationship
- Describes the conditions and likelihood Ke_{up} will trigger KE_{down}.
- State of KE_{up} provides some ability to predict or infer state of KE_{down}

- Supported by plausibility and evidence
- Quantitative understanding

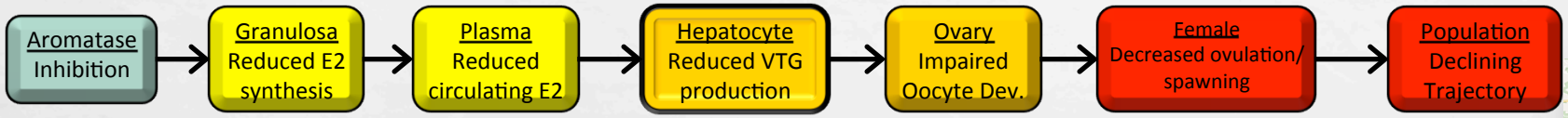




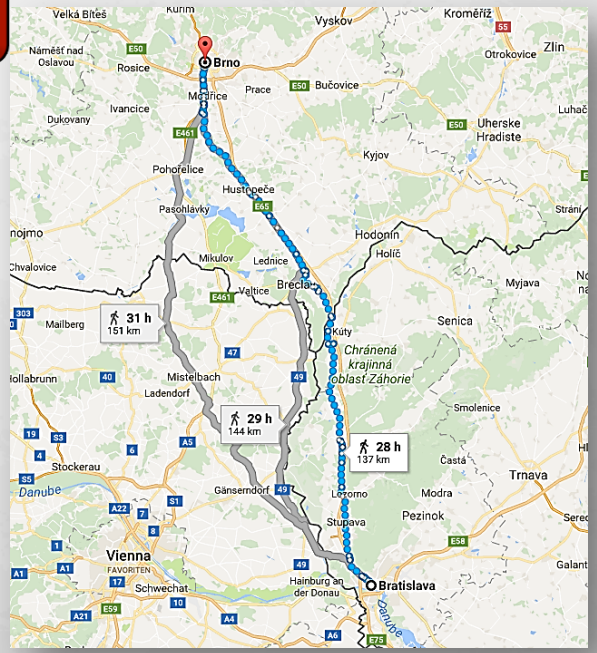
- Essential but not necessarily sufficient
- Defines the conditions under which up-stream domino will cause the next in the sequence to fall

Principles of AOP Development

3. Individual AOPs are a pragmatic functional unit of development and evaluation



- By convention AOP consists of a single sequence of key events connecting an MIE to AO (no branches)
- AOP is a pragmatic simplification of complex biology

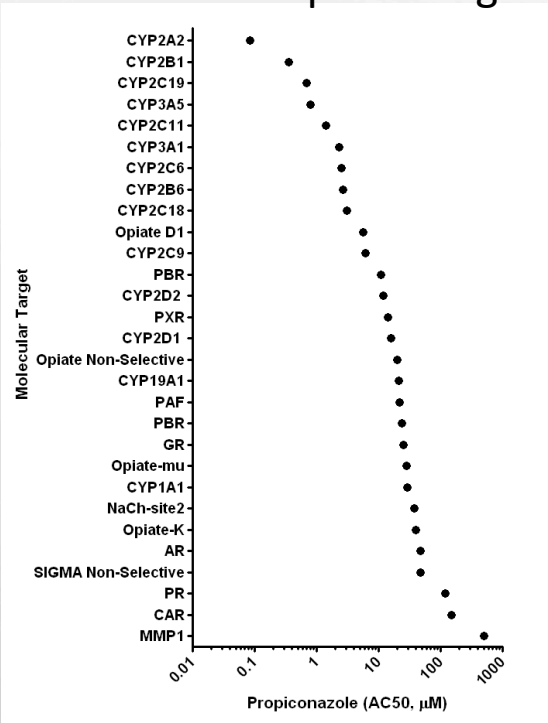


One set of directions from point A to point B, not the map of all possible routes

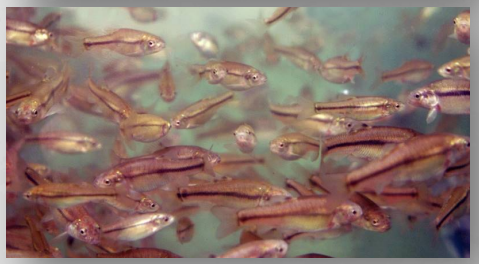
Principles of AOP Development

4. For most real-world applications, AOP networks are the functional unit of prediction

Chemicals with multiple biological activities



Exposure to multiple chemicals

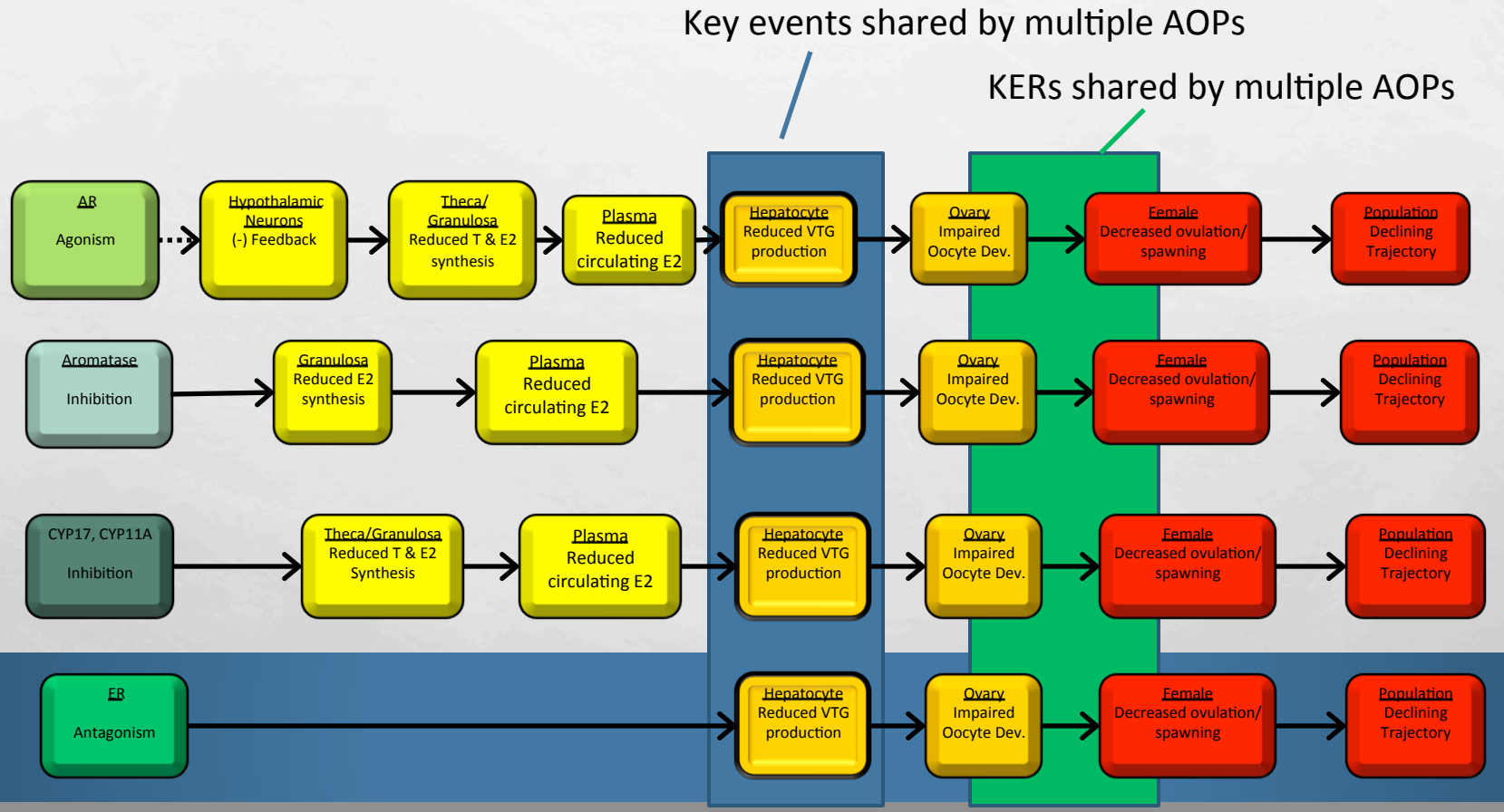


Diuron
Imidacloprid
Linuron
Terbuthylazine
2,4-D
MCPP
Propachlor ESA
Butalbital
Diclofenac
Furosemide
Gemfibrozil
Ibuprofen
Naproxen
Phenobarbital
Phenytoin
Sulfamethoxazole
Triclosan
Acebutolol
Albuterol
Amitriptyline

AOPs are not triggered in isolation. They interact.

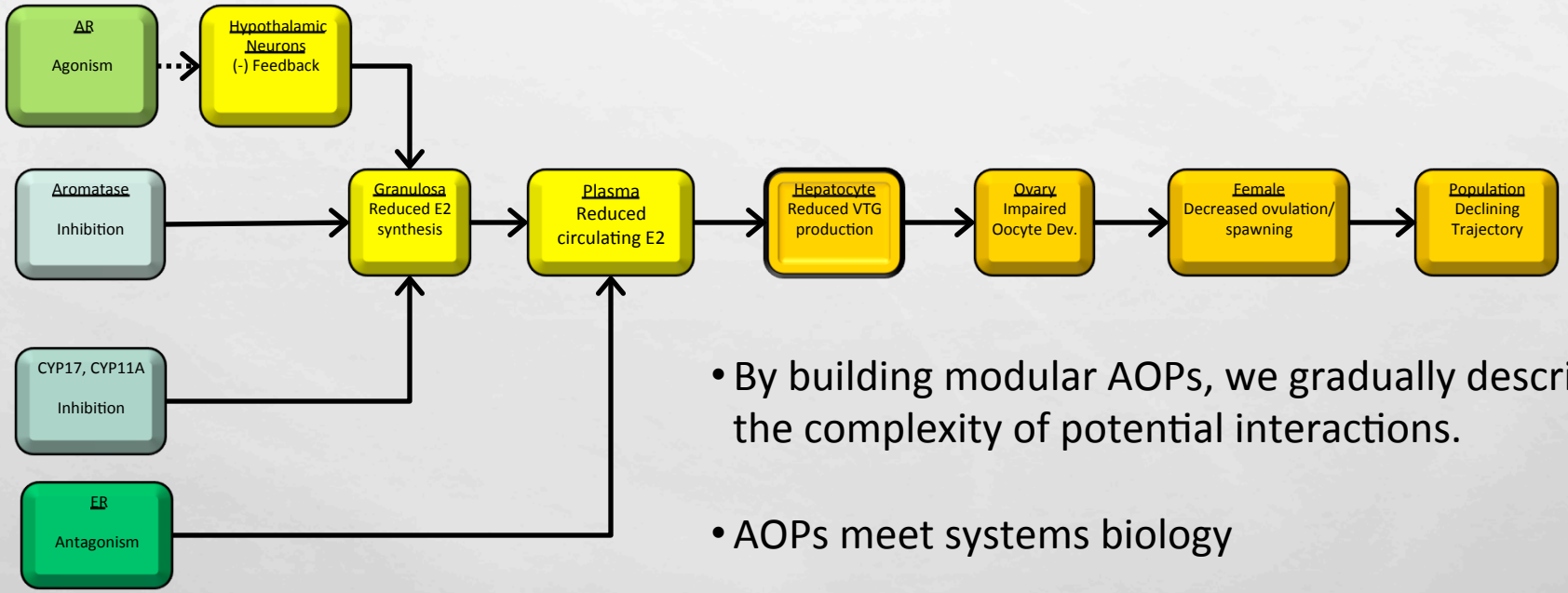
Principles of AOP Development

4. For most real-world applications, AOP networks are the functional unit of prediction

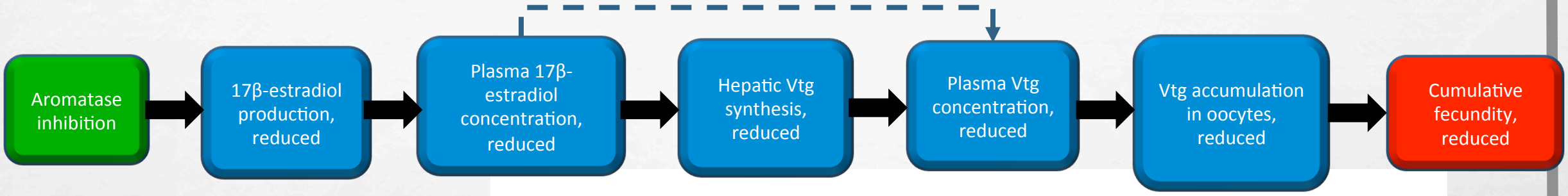


Principles of AOP Development

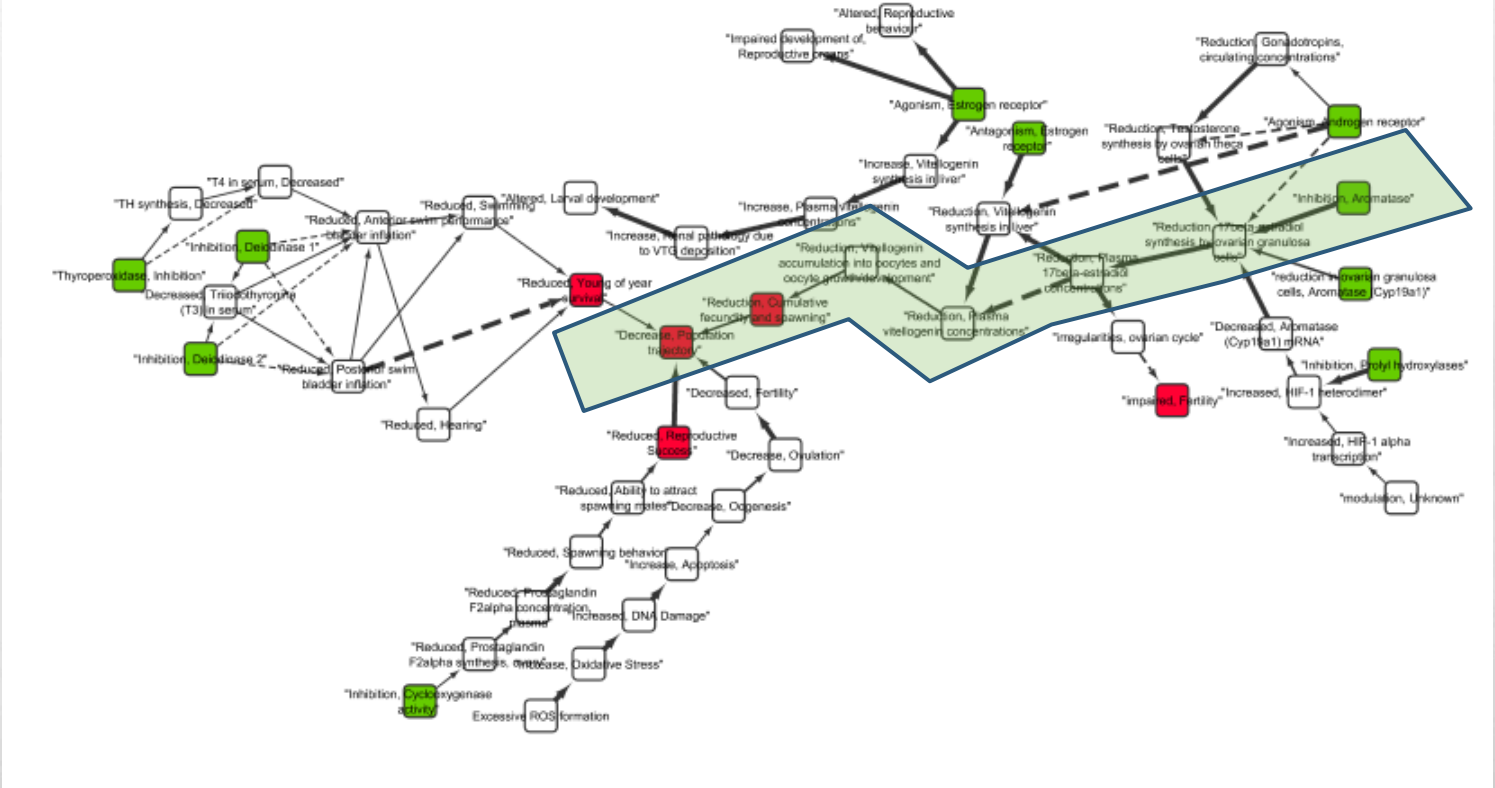
4. For most real-world applications, AOP networks are the functional unit of prediction



- By building modular AOPs, we gradually describe the complexity of potential interactions.
- AOPs meet systems biology



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



AOP 25: Aromatase inhibition leading to reproductive dysfunction (in fish)

Principles of AOP Development

5. AOPs are living documents

- AOPs are a way of organizing existing knowledge

- As methods for observing biology evolve:
  - New possibilities for KEs
  - Ability to measure KEs with greater precision/accuracy
- As new experiments are published:
  - Weight of evidence supporting (or rejecting) KERs grows
  - New AOPs and new branches in AOP networks discovered
- There is no objective "complete AOP"
  - There is only useful or not useful for a given application

Principles of AOP Development & The AOP-KB

AOPs are modular

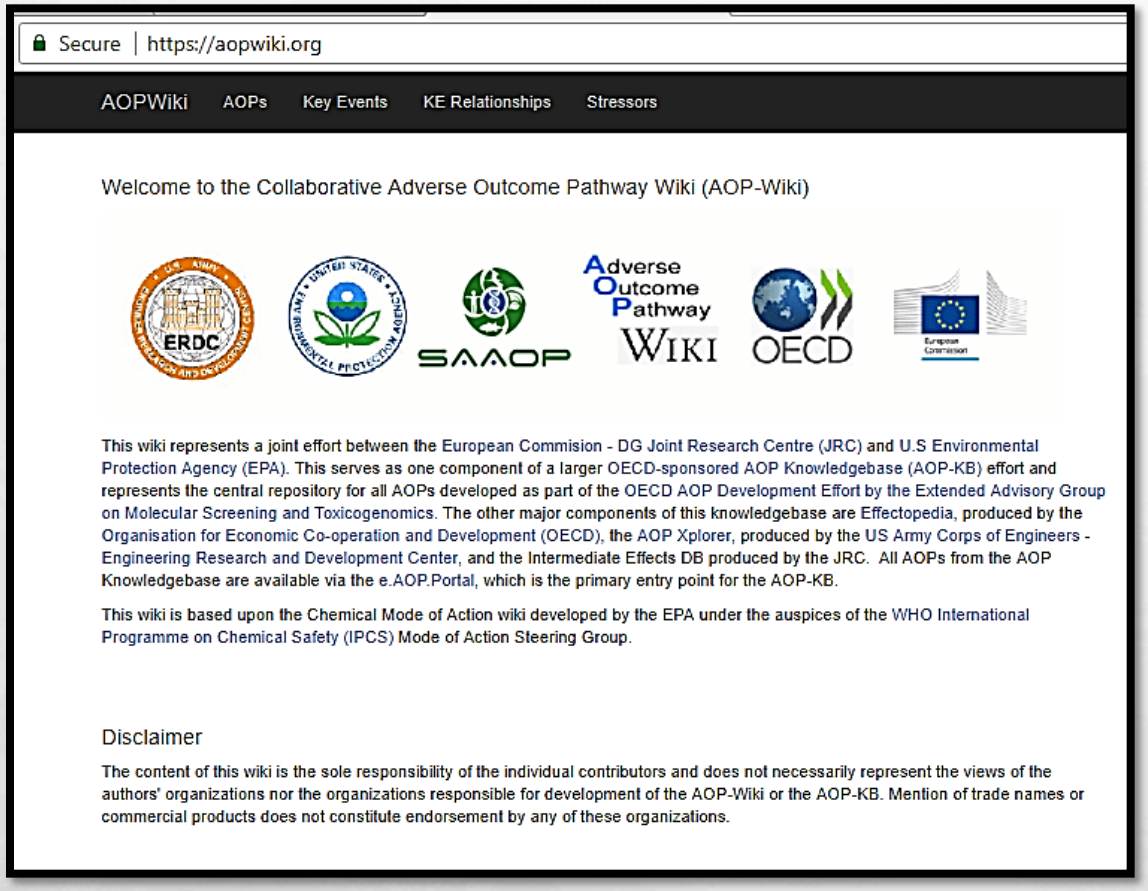
- KEs and KERs are shared by multiple AOPs
- No need to re-write the same descriptions over and over
- Reusability (best practices)

AOPs are living documents

- KE and KER descriptions can be expected to evolve over time
- As descriptions are updated and expanded – all AOP descriptions they link to update automatically

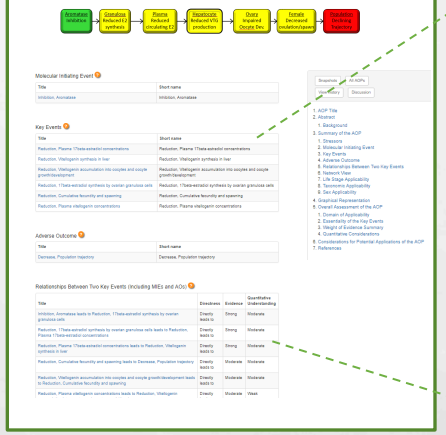
•AOP networks for prediction

- Entry of structured information in KB allows for de-facto assembly of AOP networks.



Aopwiki.org

AOP Page



KE Pages

- Description
- Measurement/ detection
- Domain of applicability

KER Pages

- Title
- Description
- Biological plausibility
- Empirical support
- Inconsistencies and uncertainties
- Quantitative understanding

Acknowledgements

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