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SCI

Introducing AOPs - Basics

Presented by Ludek Blaha, MU

… using and acknowledging materials from many others:

Dan Villeneuve – US EPA

Markus Hecker – University of Saskatchewan, Canada

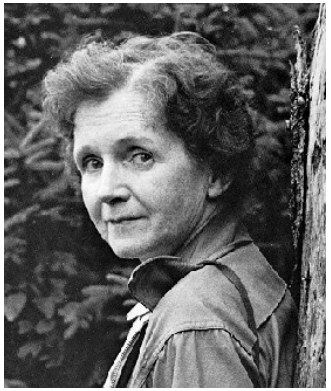
Mirjam Luijten – RIVM, Netherlands

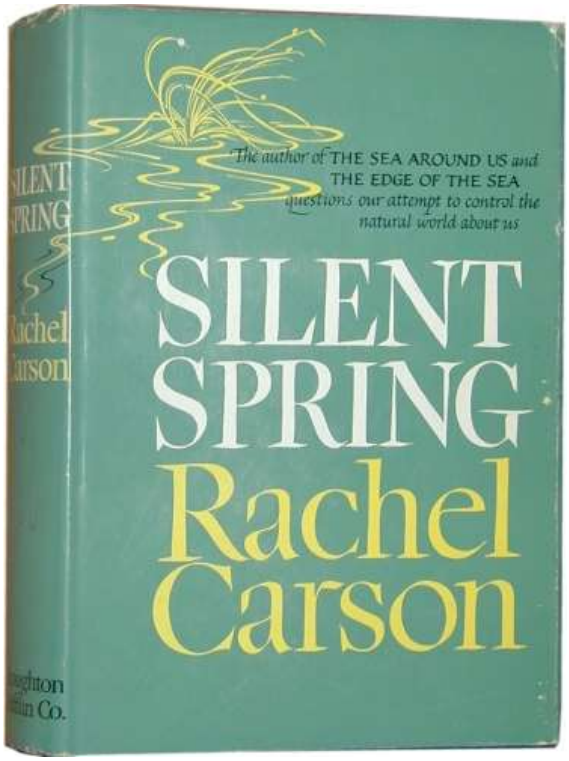
and many other AOP-developers and trainers

15th RECETOX Summer School
3rd HMB4EU Training School
Brno 18th June 2019, MU-RECETOX, Brno, Czech Republic

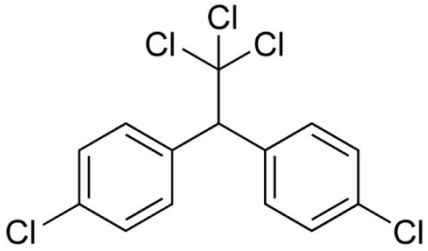
HBM4EU
science and policy
for a healthy future

1962



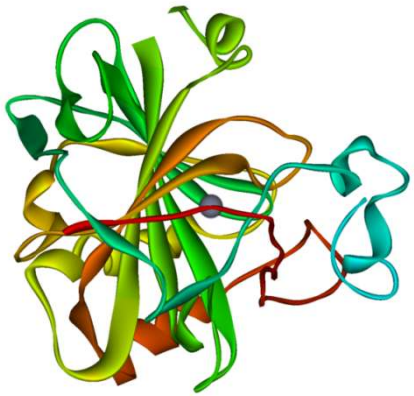


© Patuxent Wildlife Refuge, MA, USA

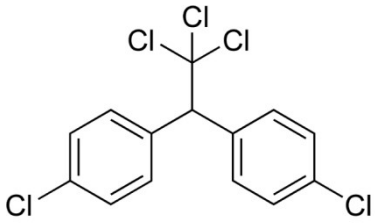


http://www2.ucsc.edu/scpbrg/

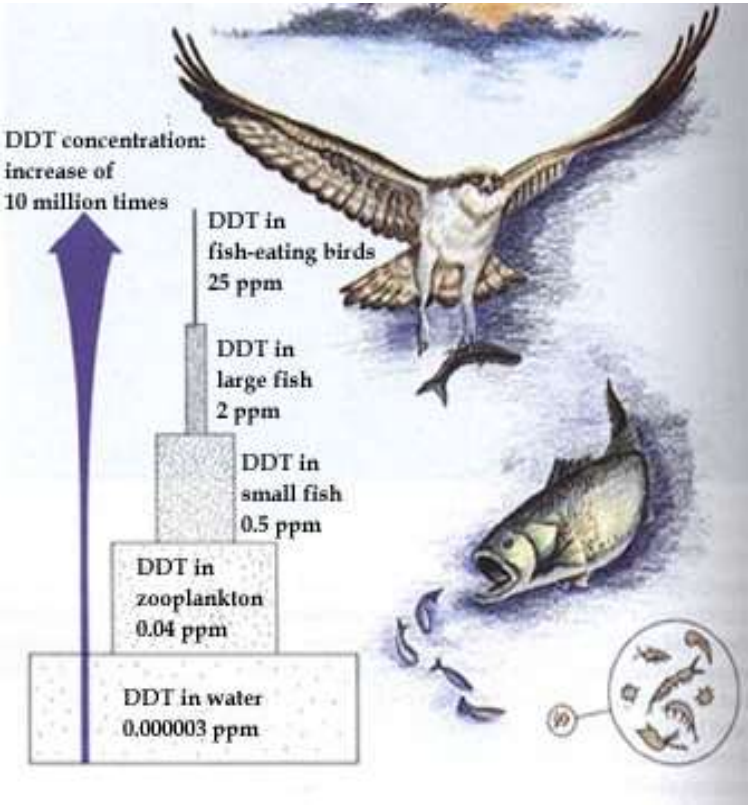
Bitman et al. *Science* 1970, 168(3931): 594



Biochemistry
bird carbonate dehydratase
+ several other mechanisms

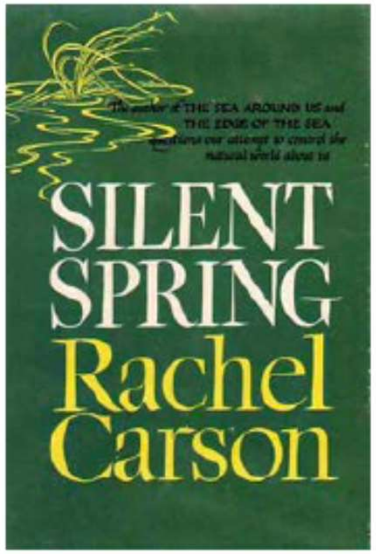


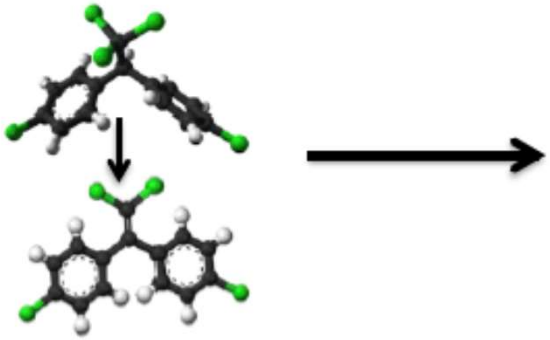
In situ: bioaccumulation
-> bird population decline



In vivo: shell thinning

Introduction





p,p'-DDE

Contribution of DDT to population declines in sensitive bird populations

Perhaps the most well known incident in wildlife ecotoxicology

Helped spark the environmental movement, and in part the mission of EPA

Introduction

A central challenge for regulatory toxicology

How do we identify the other chemicals that may cause similar adverse effects

Before we see impacts on human health or wildlife populations

Traditional Approach

Avian reproduction study
(OPPTS 850.2300; OECD 206)

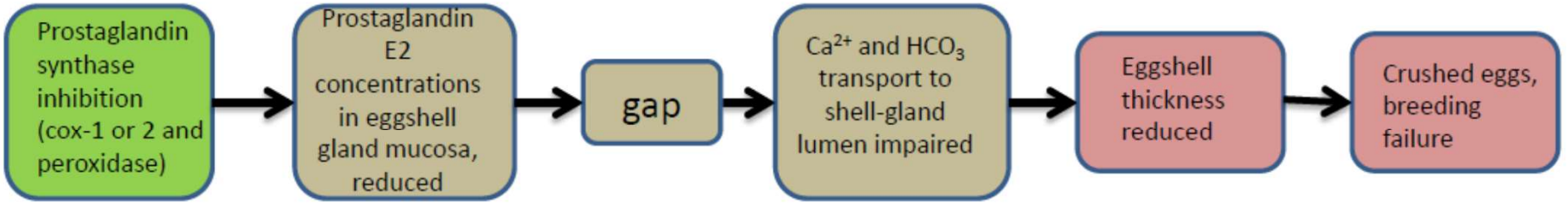
$>250,000
>30 weeks to perform

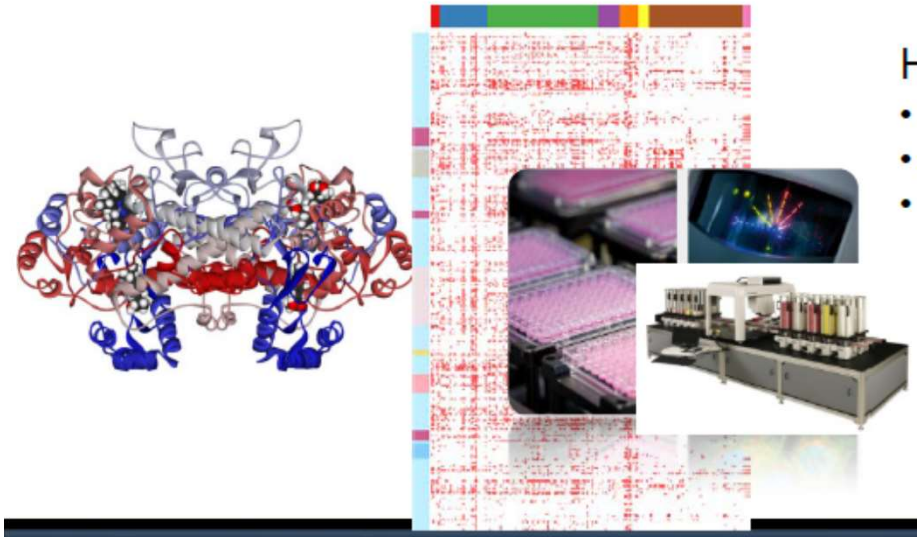
Introduction

If we understand HOW chemicals cause adverse outcomes

And…biological activities that lead to/are associated with progression toward those AOs

Creates opportunities to use new types of data for hazard id and/or risk-based decision-making

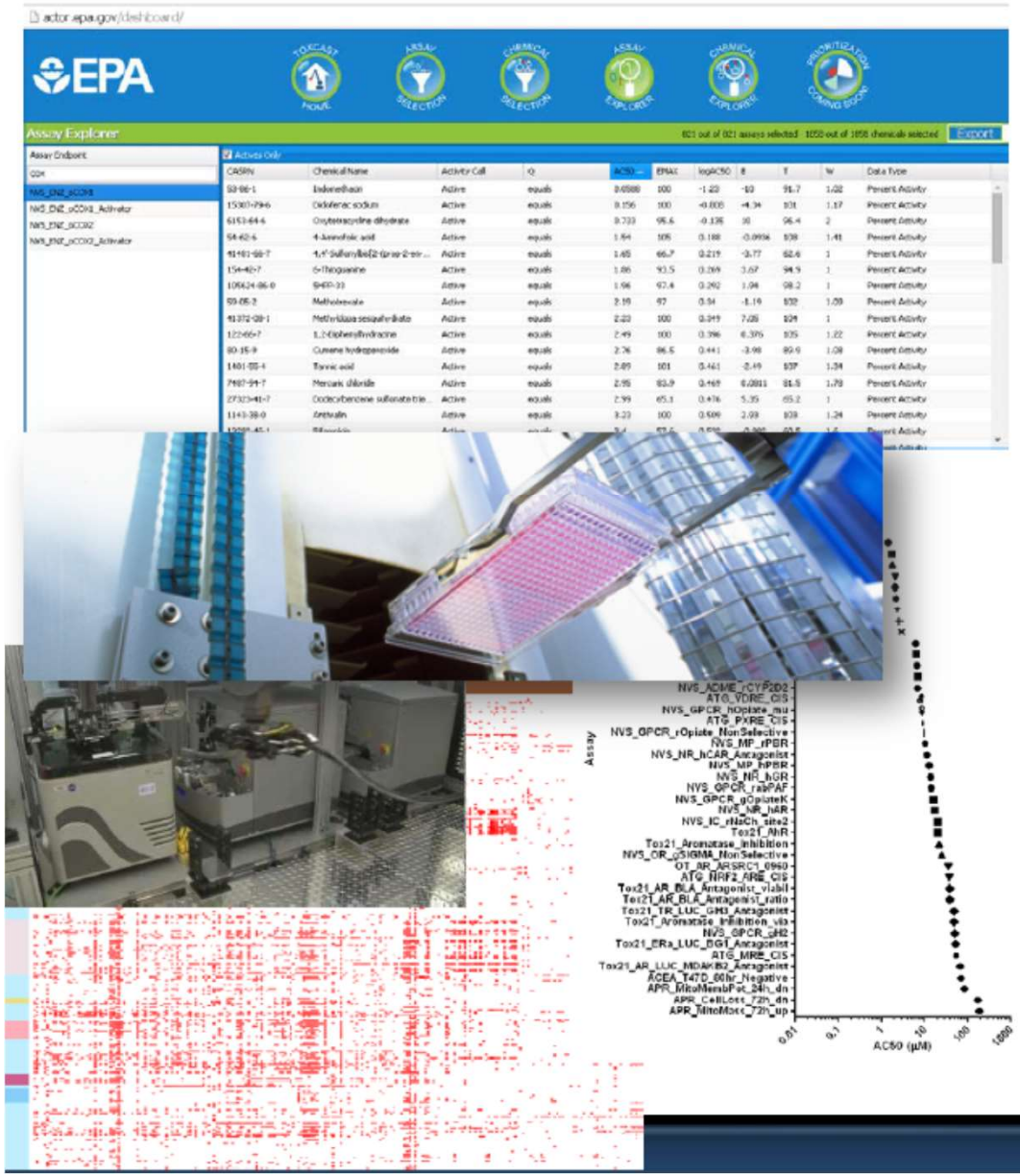




High throughput toxicology

- > 600 in vitro assays
- days
- ≈ $ 20,000

Toxicity Testing in the 21st Century



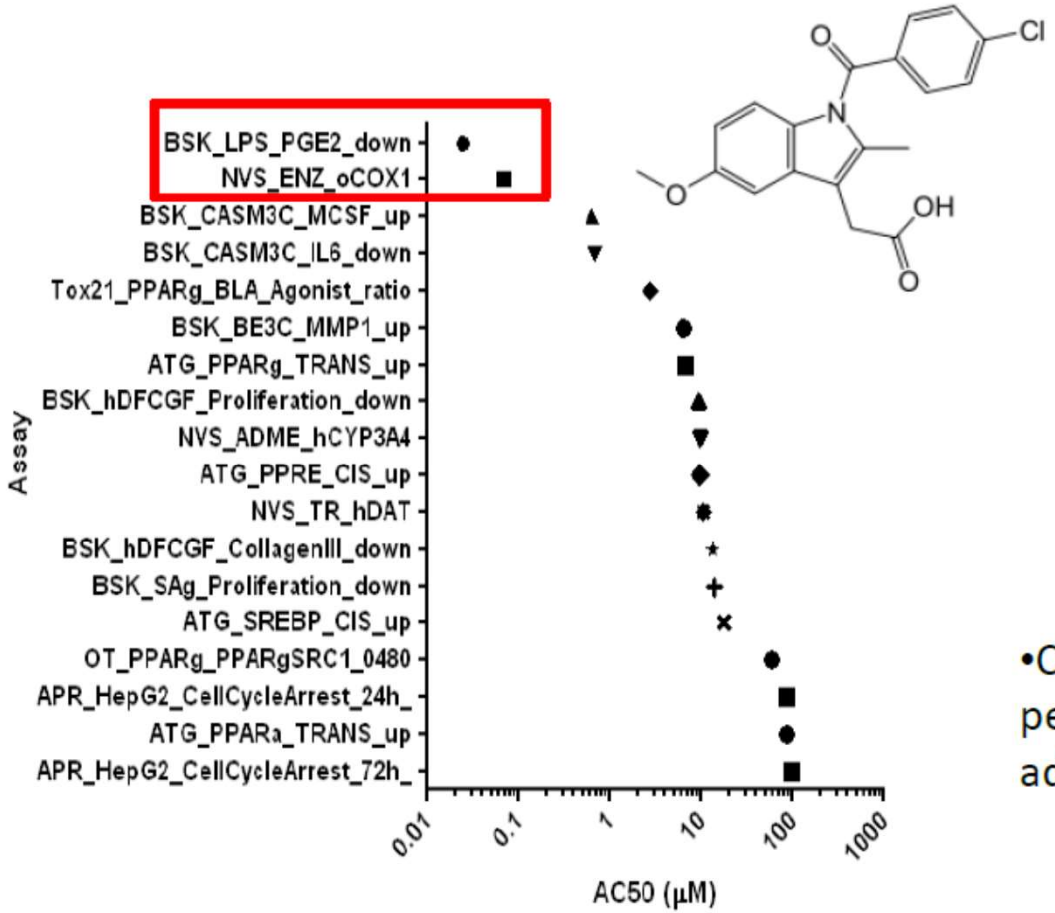
21st Century Toxicity Testing is here….

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.

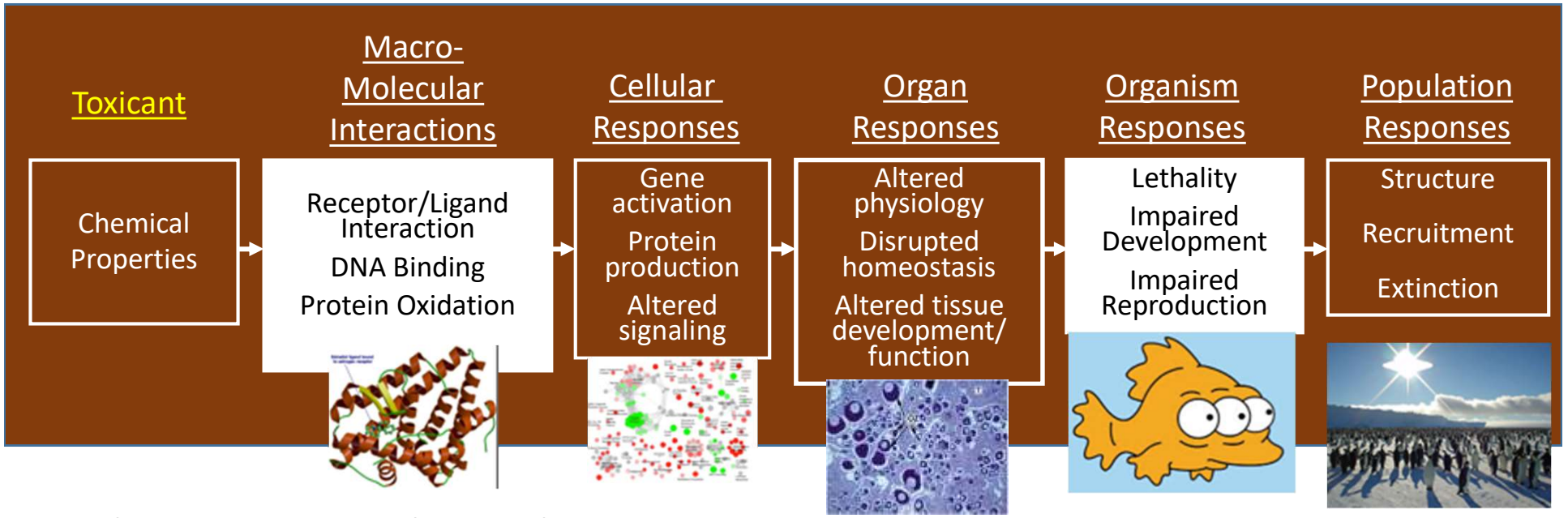
Example



- Can we expect this perturbation lead to an adverse outcome?

Adverse Outcome Pathways … are new

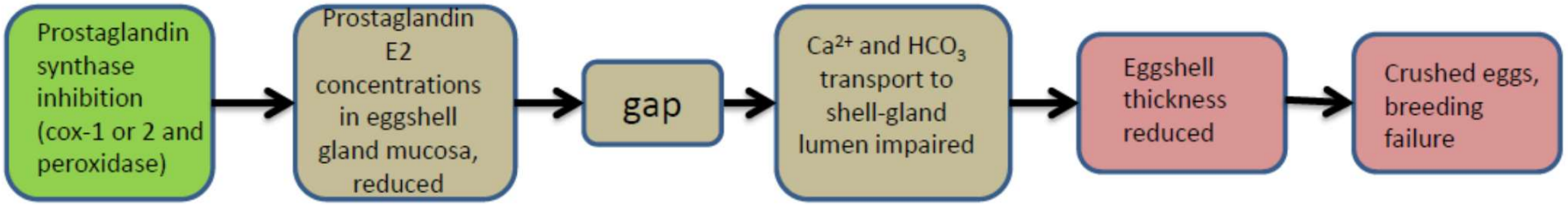
An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment. (Ankley et al. 2010. Environ. Toxicol. Chem., 29(3): 730-741.)

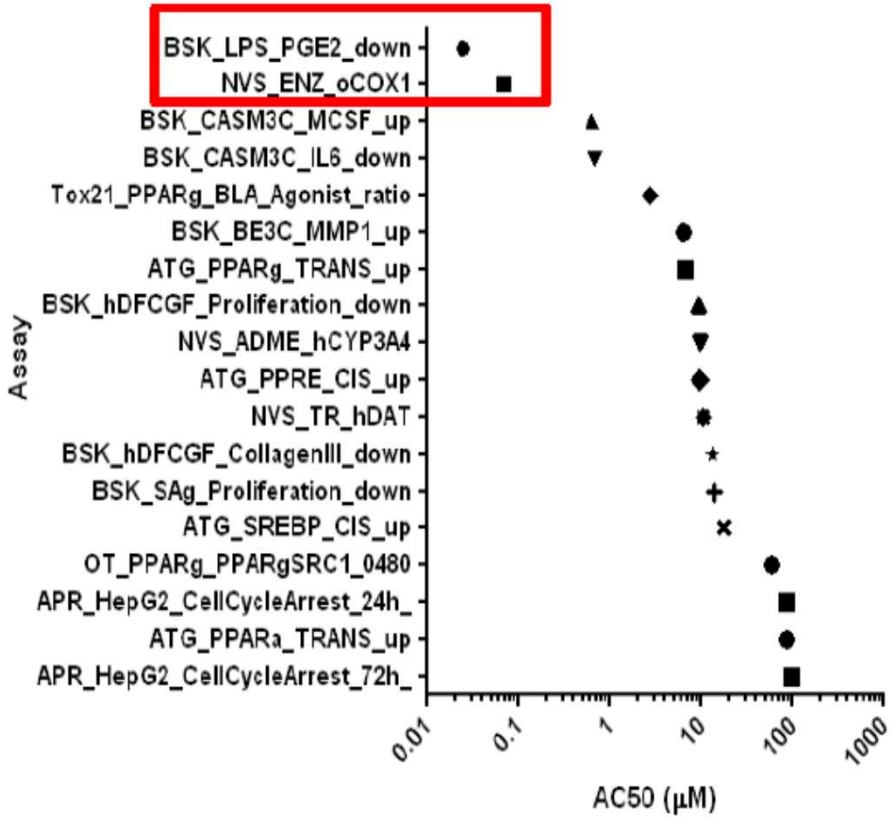


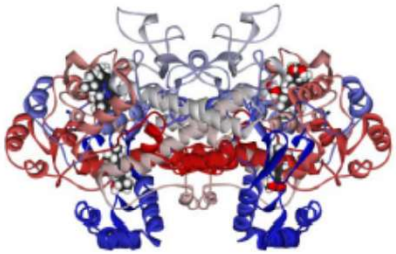
- Helps us organize what we know
- And utilize that knowledge to support risk-based decision-making


stressor

Example

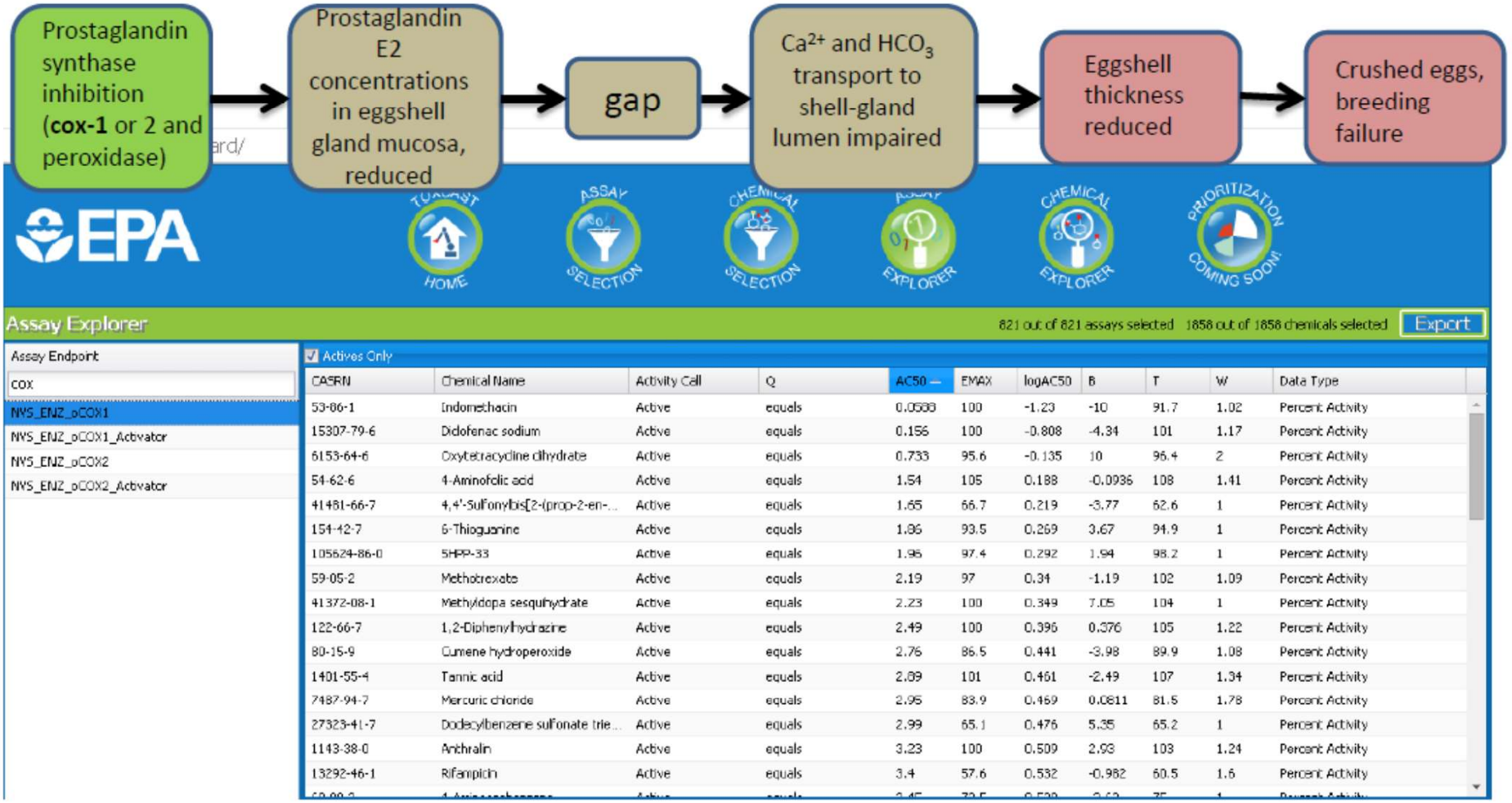

Prostaglandin synthase inhibition (cox-1 or 2 and peroxidase)
Prostaglandin E2 concentrations in eggshell gland mucosa, reduced
gap
Ca^{2+} and HCO_3 transport to shell-gland lumen impaired
Eggshell thickness reduced
Crushed eggs, breeding failure


BSK_LPS_PGE2_down
NVS_ENZ_oCOX1
BSK_CASM3C_MCSF_up
BSK_CASM3C_IL6_down
Tox21_PPARg_BLA_Agonist_ratio
BSK_BE3C_MMP1_up
ATG_PPARg_TRANS_up
BSK_hDFCGF_Proliferation_down
NVS_ADME_hCYP3A4
ATG_PPRE_CIS_up
NVS_TR_hDAT
BSK_hDFCGF_CollagenIII_down
BSK_SAg_Proliferation_down
ATG_SREBP_CIS_up
OT_PPARg_PPARgSRC1_0480
APR_HepG2_CellCycleArrest_24h_
ATG_PPARa_TRANS_up
APR_HepG2_CellCycleArrest_72h_
Assay
0.01
0.1
1
10
100
1000
AC50 (µM)




Ah-ha

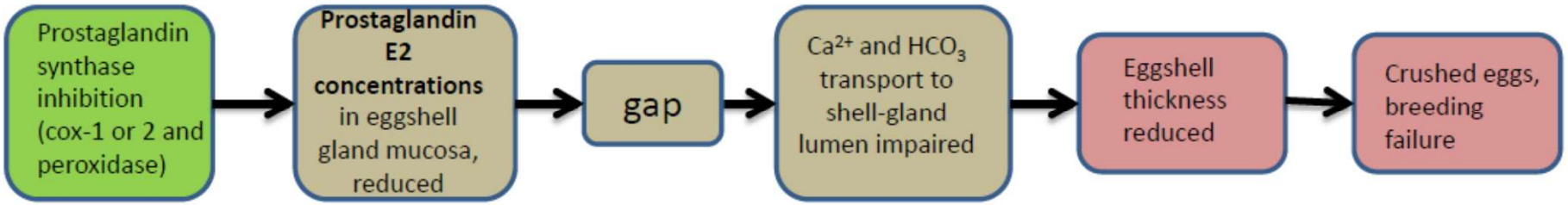
Introduction

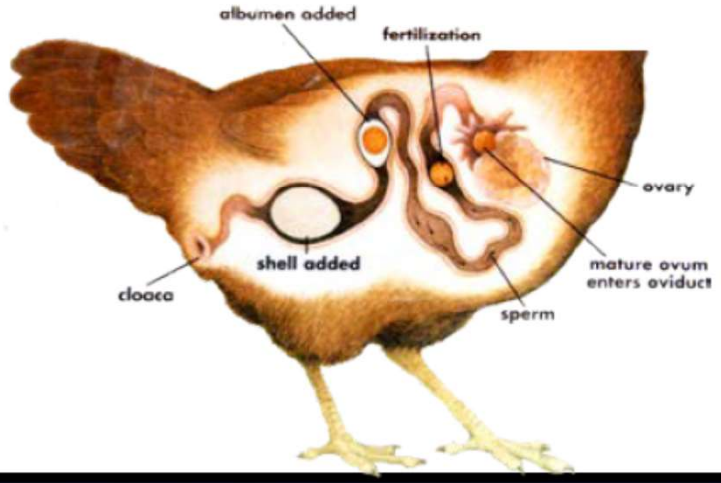


CASRN	Chemical Name	Activity Call	Q	AC50	EMAX	logAC50	B	T	W	Data Type
53-86-1	Indomethacin	Active	equals	0.0588	100	-1.23	-10	91.7	1.02	Percent Activity
15307-79-6	Diclofenac sodium	Active	equals	0.156	100	-0.808	-4.34	101	1.17	Percent Activity
6153-64-6	Oxytetracycline dihydrate	Active	equals	0.733	95.6	-0.135	10	96.4	2	Percent Activity
54-62-6	4-Aminofolic acid	Active	equals	1.54	105	0.188	-0.0936	108	1.41	Percent Activity
41481-66-7	4,4'-Sulfonylbis[2-(prop-2-en-...	Active	equals	1.65	66.7	0.219	-3.77	62.6	1	Percent Activity
154-42-7	6-Thioguanine	Active	equals	1.86	93.5	0.269	3.67	94.9	1	Percent Activity
105624-86-0	SHPP-33	Active	equals	1.96	97.4	0.292	1.94	98.2	1	Percent Activity
59-05-2	Methotrexate	Active	equals	2.19	97	0.34	-1.19	102	1.09	Percent Activity
41372-08-1	Methyldopa sesquihydrate	Active	equals	2.23	100	0.349	7.05	104	1	Percent Activity
122-66-7	1,2-Diphenylhydrazine	Active	equals	2.49	100	0.396	0.376	105	1.22	Percent Activity
80-15-9	Cumene hydroperoxide	Active	equals	2.76	86.5	0.441	-3.98	89.9	1.08	Percent Activity
1401-55-4	Tannic acid	Active	equals	2.89	101	0.461	-2.49	107	1.34	Percent Activity
7487-94-7	Mercuric chloride	Active	equals	2.96	83.9	0.469	0.0811	81.5	1.78	Percent Activity
27323-41-7	Dodecylbenzene sulfonate trie...	Active	equals	2.99	65.1	0.476	5.35	65.2	1	Percent Activity
1143-38-0	Anthralin	Active	equals	3.23	100	0.509	2.93	103	1.24	Percent Activity
13292-46-1	Rifampicin	Active	equals	3.4	57.6	0.532	-0.982	60.5	1.6	Percent Activity

A set of chemicals for which there may be reason expect egg-shell thinning

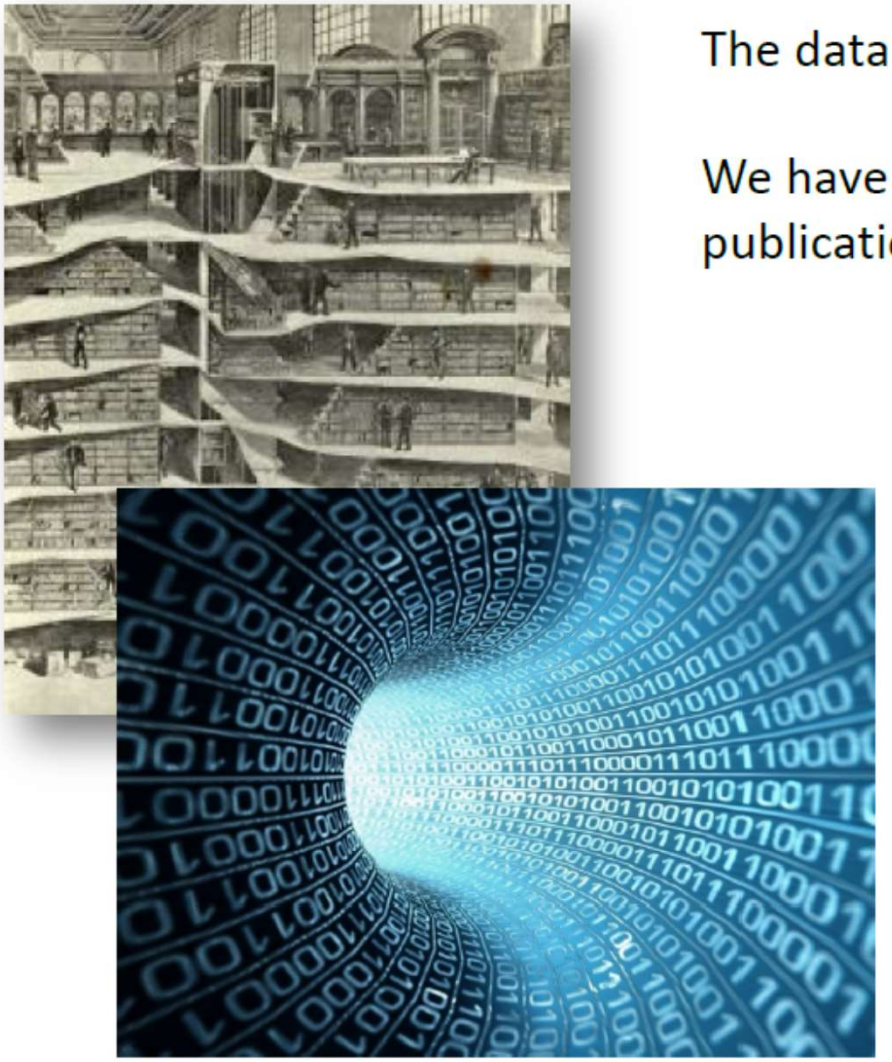
Introduction





- **Alternative tests**: Suggests informative endpoints we may be able to measure more rapidly and cost-effectively in laboratory toxicity tests.
- **Biomarkers**: Suggests biomarkers we could measure in animals from the environment – early warning; diagnostic
- Particularly if we can translate into a quantitative prediction of probability or severity of AO.
- **Diagnostic potential**: Suggests an etiology for observed patterns of biological response – may help trace back to causative agents and/or sources.

21st Century Regulatory Decision-Making



The data are there….

We have entire libraries of scientific publications available at our fingertips…..

Scientific challenge of the 21st C….

How do we make effective use of those data and our wealth of existing scientific knowledge to support regulatory decision-making?

AOPs are part of the solution.

Mode of Action (MOA) Concept in HH

Critical Reviews in Toxicology, 36:781–792, 2006
ISSN: 1040-8444 print / 1547-6898 online
DOI: 10.1080/10408440600977677

informa
Healthcare

IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans

Alan R. Boobis

Critical Reviews in Toxicology, 38:87–96, 2008
ISSN: 1040-8444 print / 1547-6898 online
DOI: 10.1080/10408440701749421

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Healthcare

IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans

Alan R. Boobis
Section of Experimental Medicine and Toxicology, Division of Medicine, Imperial College London,

Review Article

Journal of Applied Toxicology

Received: 18 September 2013, Accepted: 19 September 2013 Published online in Wiley Online Library: 25 October 2013

(wileyonlinelibrary.com) DOI 10.1002/jat.2949

New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis†

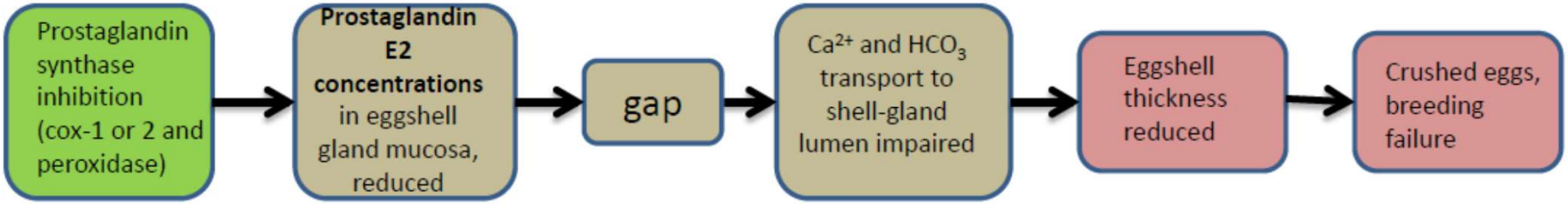
M. E. Meek[a], A. Boobis[b], I. Cote[c], V. Dellarco[d], G. Fotakis[e], S. Munn[f], J. Seed[g] and C. Vickers[h]*

ABSTRACT: The World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework has been updated to reflect the experience acquired in its application and extend its utility to emerging areas in toxicity testing and non-testing methods. The underlying principles have not changed, but the framework's scope has been extended to enable integration of information at different levels of biological organization and reflect evolving experience in a much broader range of potential applications. Mode of action/species concordance analysis can also inform hypothesis-based data generation and research priorities in support of risk assessment. The modified framework is incorporated within a roadmap, with feedback loops encouraging continuous refinement of fit-for-purpose testing strategies and risk assessment. Important in this construct is consideration of dose–response relationships and species concordance analysis in weight of evidence. The modified Bradford Hill considerations have been updated and additionally articulated to reflect increasing experience in application for cases where the toxicological outcome of chemical exposure is known. The modified framework can be used as originally intended,

- In many respects synonymous with AOP concept.
- Only major difference was the dominant applications to which they were applied.
- Strong focus on systematic documentation of WoE (Bradford-Hill considerations).

Regulatory Application

Until recently, AOP development has been an ad hoc process.



- When can you trust that the relationships depicted provide a sound/defensible foundation for regulatory decision-support?

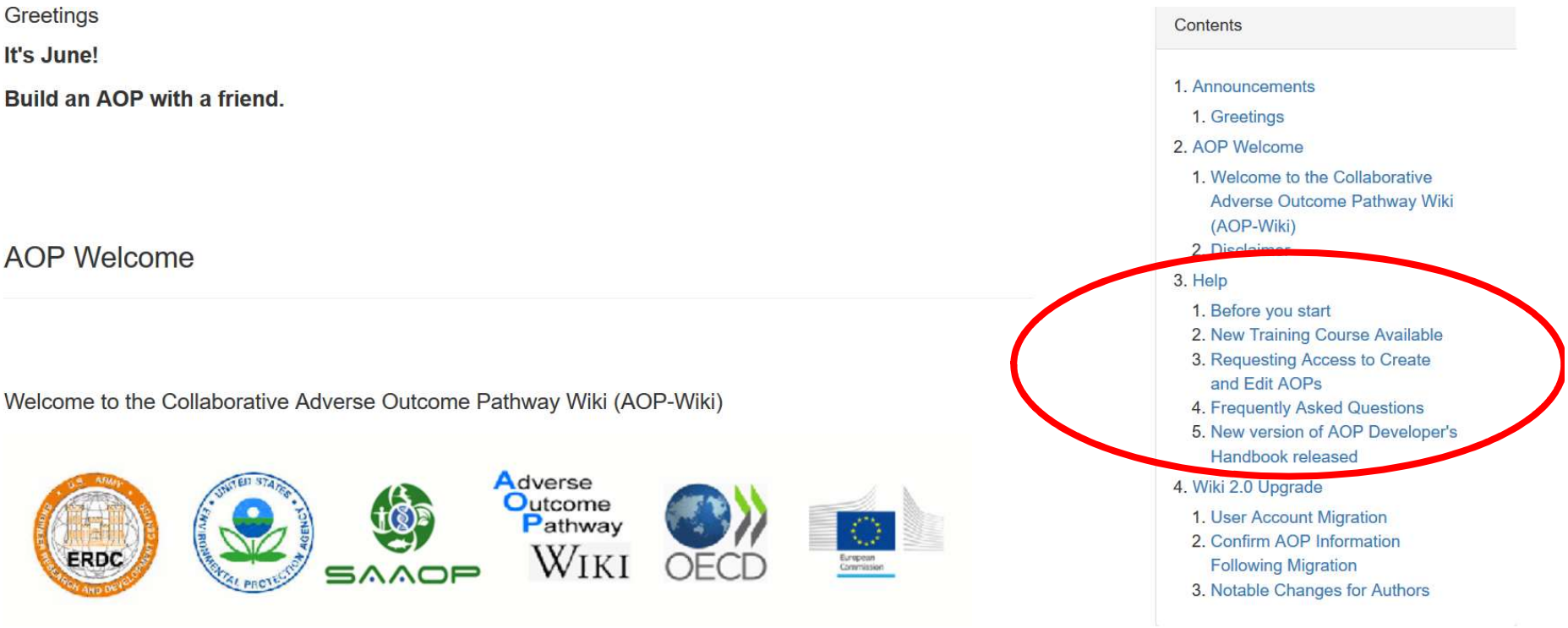
- What is the biological/toxicological applicability domain?
  - Taxa, life stage, target organ(s), route of exposure

- How do we present in a systematic and transparent manner?

Trainings on AOPs

AOPWiki → Training courses + Handbook

https://aopwiki.org/training/wiki/



Greetings

It's June!

Build an AOP with a friend.

AOP Welcome

Welcome to the Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki)

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Contents Notes

Welcome...

to the Adverse Outcome Pathway (AOP)

AOP-Wiki Course

This course works best on Google Chrome, Safari or Internet Explorer

Click on icons below to visit sites

AOP Training committee

- Subgroup of OECD/EAGMST
- Established to share recent developments and solicit input from potential developers and users of AOPs
- Started in 2014

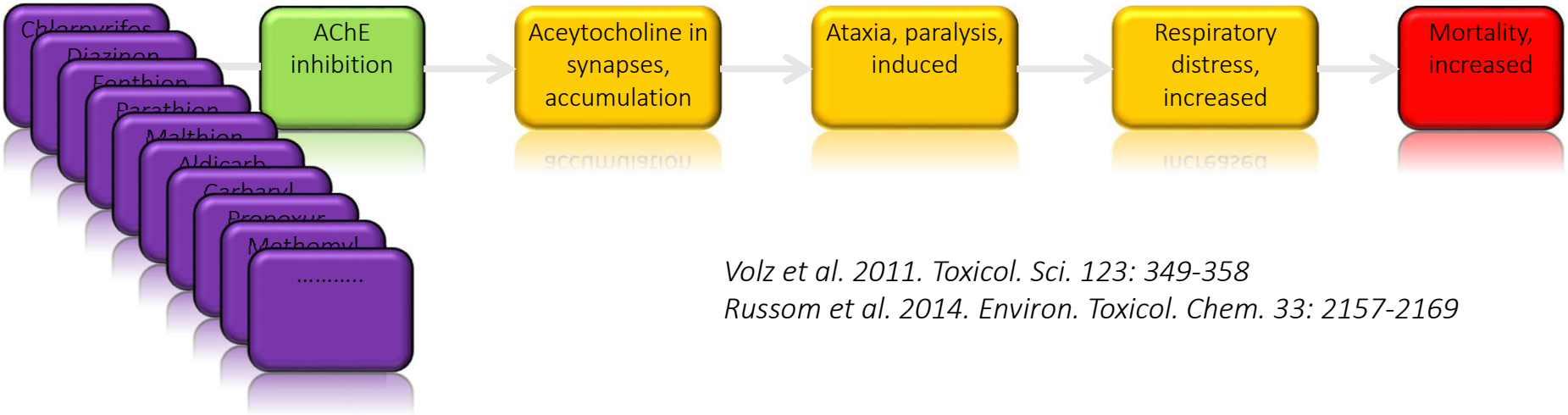
Training group members

Dan Villeneuve, Bette Meek, Steve Edwards, Kristie Sullivan, Brigitte Landesmann, Magdalini Sachana, Sharon Munn, Kate Willett, Kate Goyak, Sabina Halappanavar, Hristo Aladjov & Mirjam Luijten (chair)

Principles of AOP Development

1. AOPs are not chemical-specific

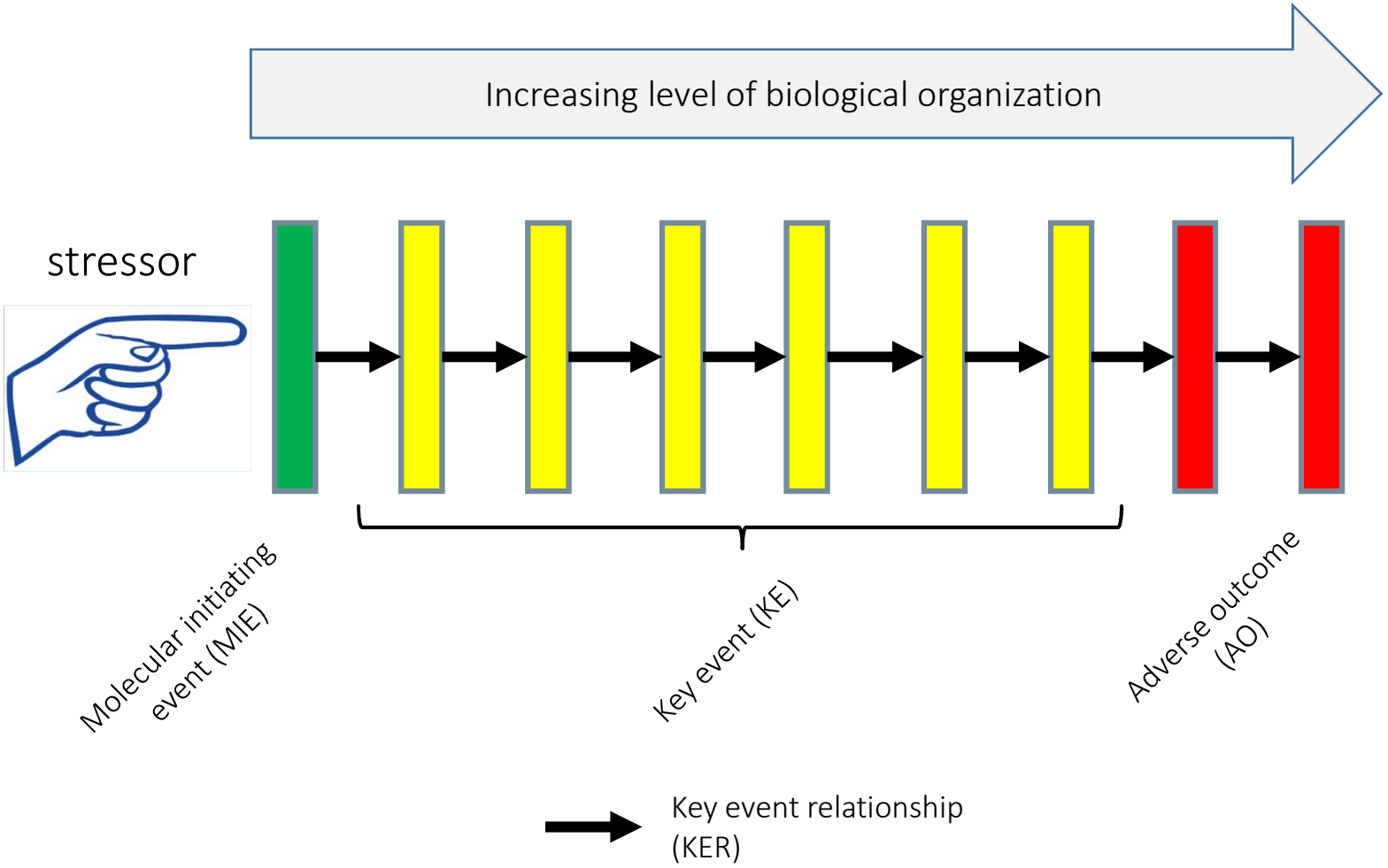
- Not trying to describe what a single chemical does
- Trying to describe what ANY chemical that perturbs the MIE with sufficient potency and duration is likely to do- Biological motifs of failure
- *Describing* AOP does not require chemical-specific information.
- *Applying* those motifs in a predictive 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

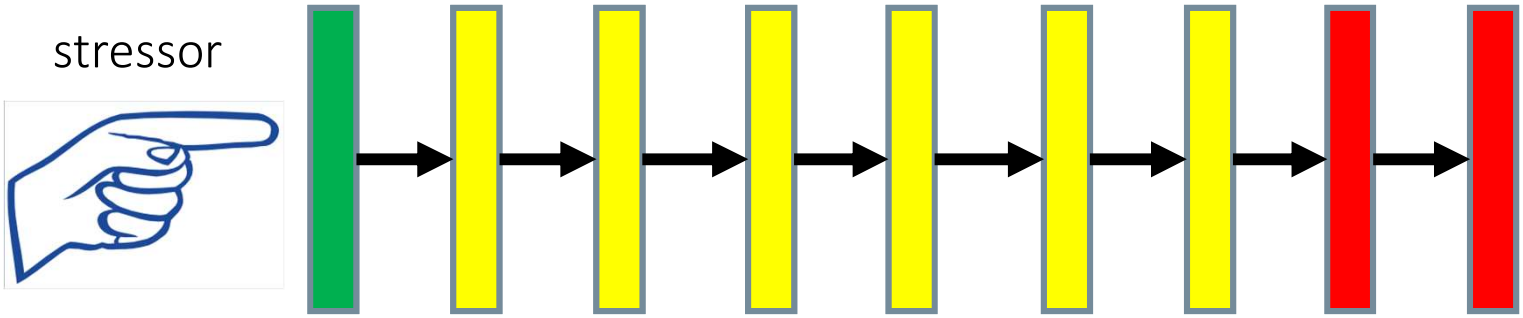
Principles of AOP Development

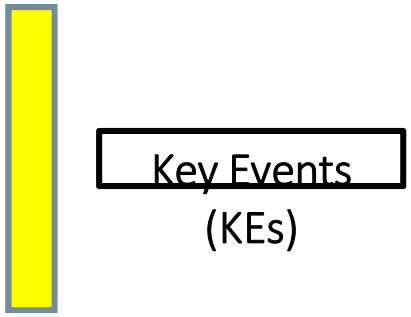
2. AOPs are Modular



Principles of AOP Development

Two Primary Building Blocks





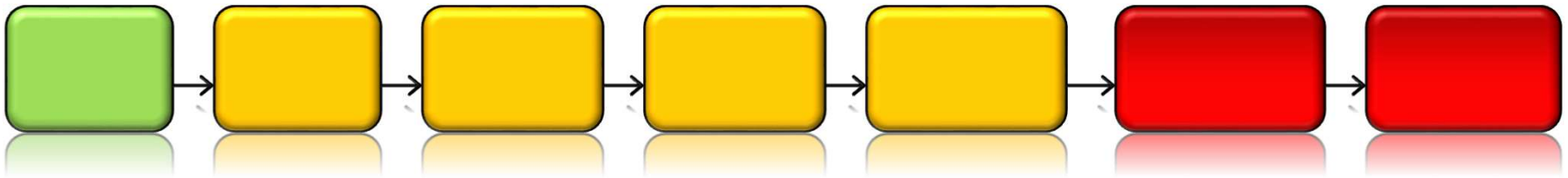
Functional unit of observation/verification

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

Description

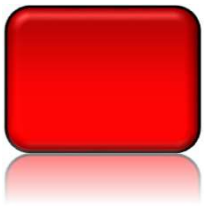
- Methods for observing/measuring
- Taxonomic applicability

Principles of AOP Development



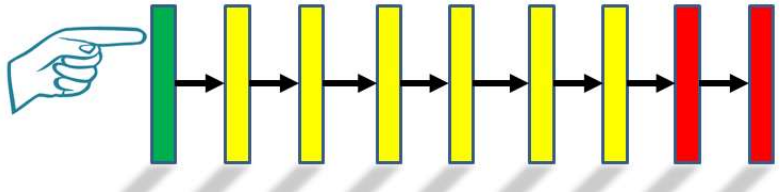
Molecular initiating event (MIE) – A specialized type of KE that represents the initial point of chemical interaction, on the molecular level, within an organism, that results in a perturbation that starts the AOP.

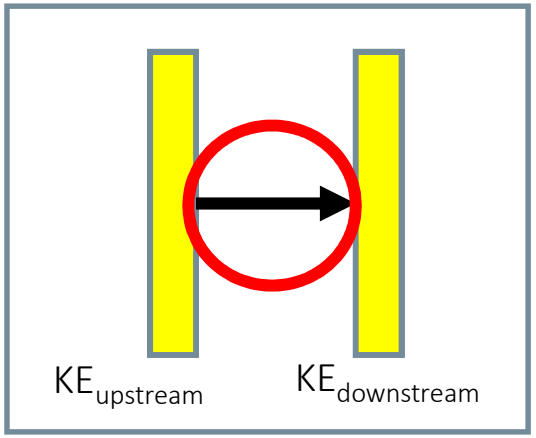
Adverse Outcome (AO) – A specialized type of KE that is generally accepted as being of regulatory significance on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test.



Principles of AOP Development

Two Primary Building Blocks





Key Event Relationships (KERs):

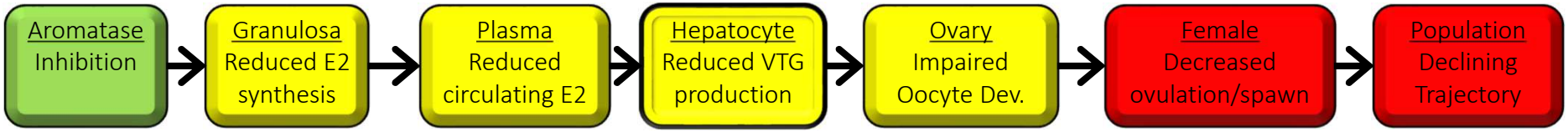
Functional unit of inference/extrapolation

- 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}

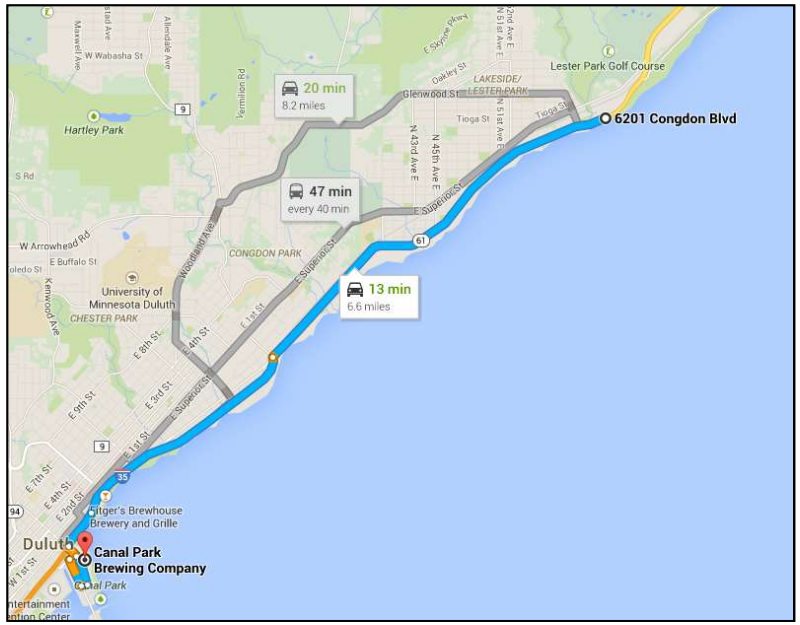
- Supported by plausibility and evidence
- Quantitative understanding

Principles of AOP Development

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



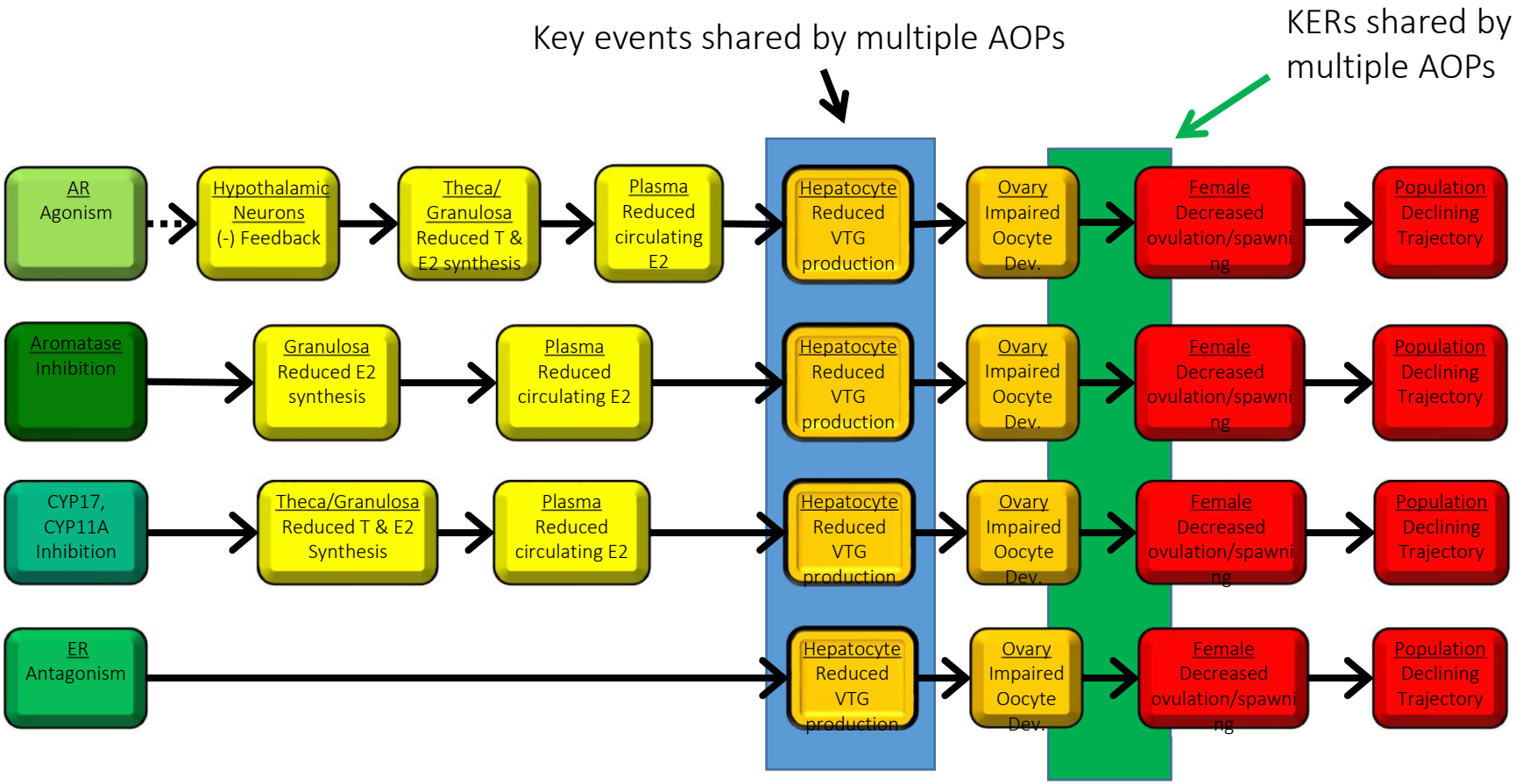
- By convention AOP consists of a single sequence of key events connecting an MIE to AO (no branches)
- AOP is a pragmatic simplification of complex biology
- For a “pure ligand” – functional unit of prediction



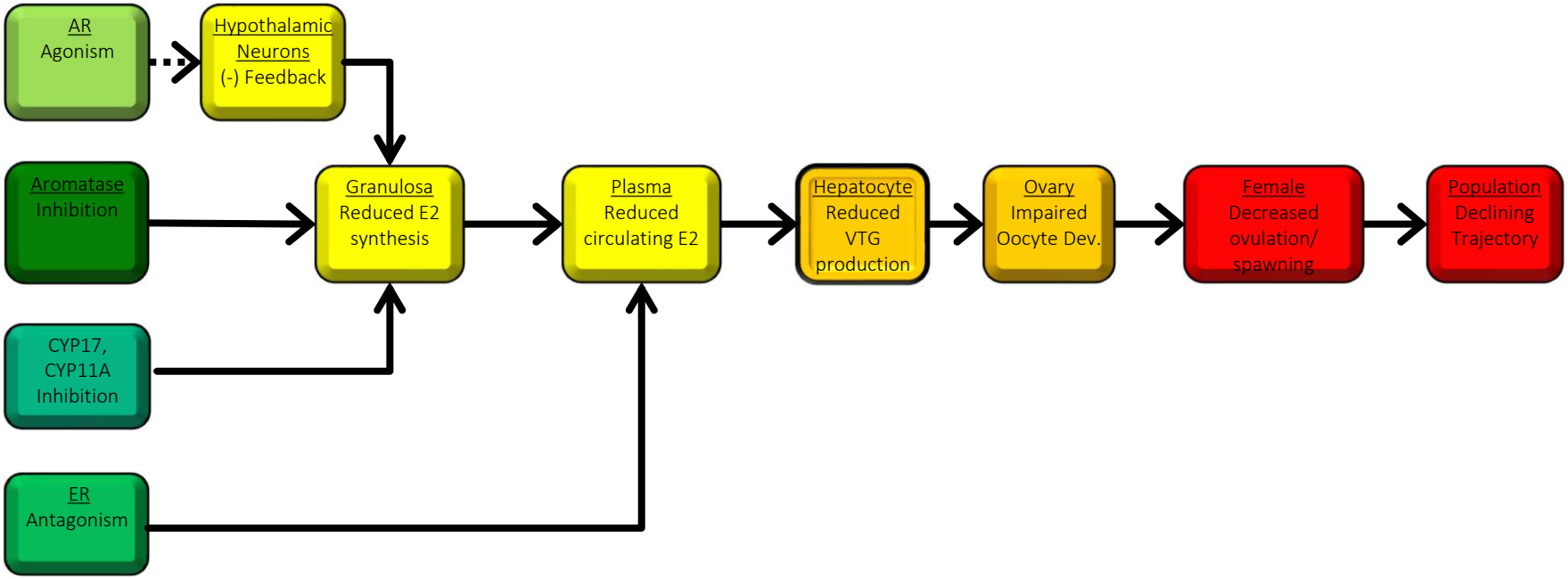
One set of directions from point A to point B, not the map of all possible routes

Principles of AOP Development

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

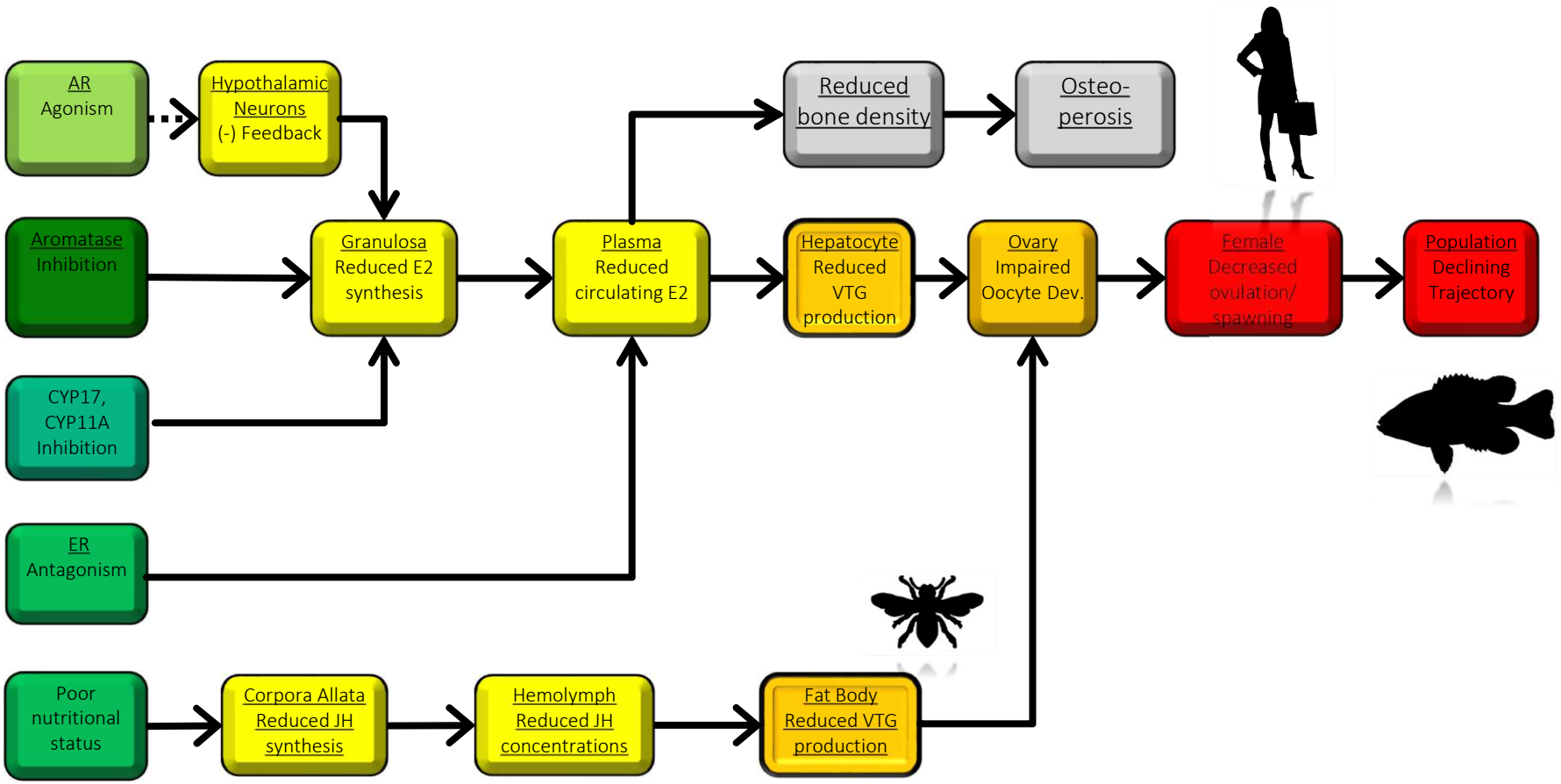


Principles of AOP Development



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

Principles of AOP Development



AOP networks also a way to represent conservation and divergence of toxicological responses across taxa, life stages, etc.

Principles of AOP Development

5. AOPs are living documents

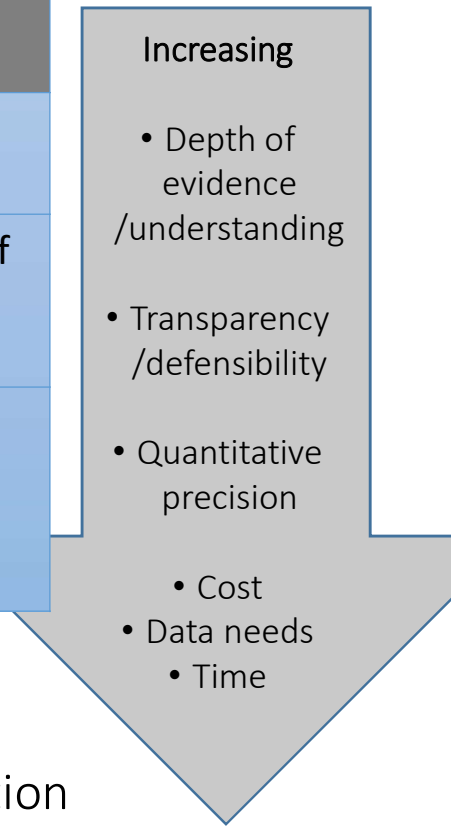
- AOPs are a way of organizing existing knowledge
- As methods for observing biology evolve:
  - ✓ New possibilities for KEs
  - ✓ Ability to measure KEs with greater precision/accuracy
- As new experiments are published:
  - ✓ Weight of evidence supporting (or rejecting) KERs grows
  - ✓ New AOPs and new branches in AOP networks discovered
- There is no objective “complete”

Principles of AOP Development

5. AOPs are living documents

Operationally-defined “stages” of AOP development

Stages of AOP Development	Characteristics
Putative AOPs:	Hypothesized set of KEs and KERs primarily supported by biological plausibility and/or statistical inference
Formal AOPs:	Include assembly and evaluation of the supporting weight of evidence – developed in AOP knowledgebase in accordance with internationally-harmonized OECD guidance
Quantitative AOPs:	Supported by quantitative relationships and/or computational models that allow quantitative translation of key event measurements into predicted probability or severity of adverse outcome



- All stages have potential utility
- Level of development desired/required depends on the application

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