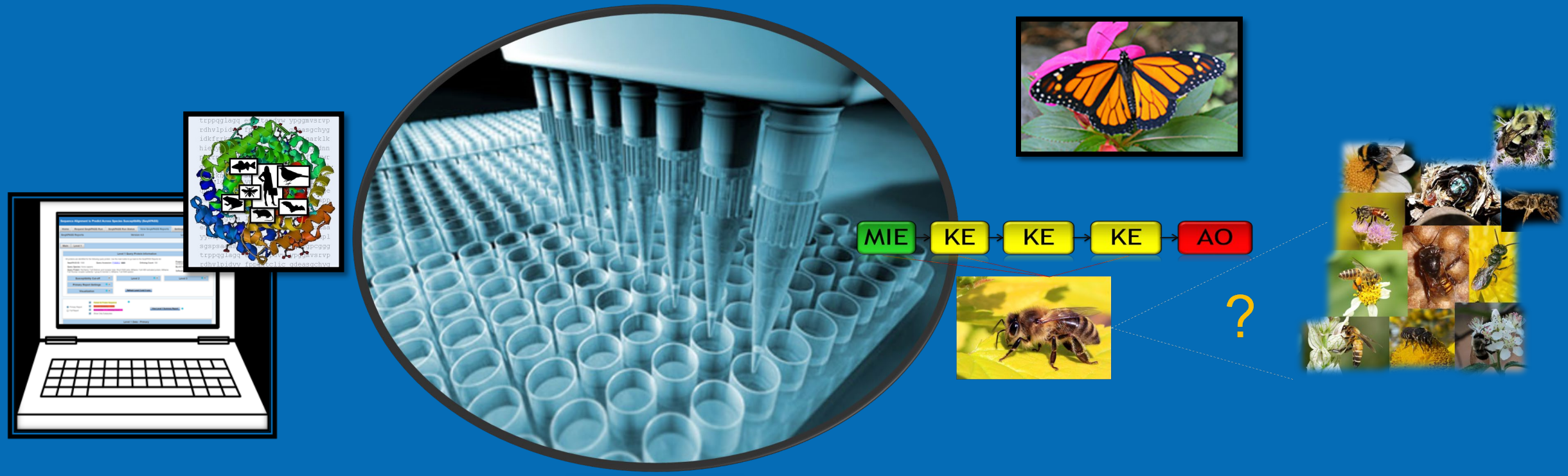
EPA
United States
Environmental Protection
Agency

Practical application of new approach methods to enhance the understanding of chemical effects on pollinators

Carlie A. LaLone, Ph.D.
Research Bioinformaticist



Office of Research and Development
Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA

Aug. 26th, 2021

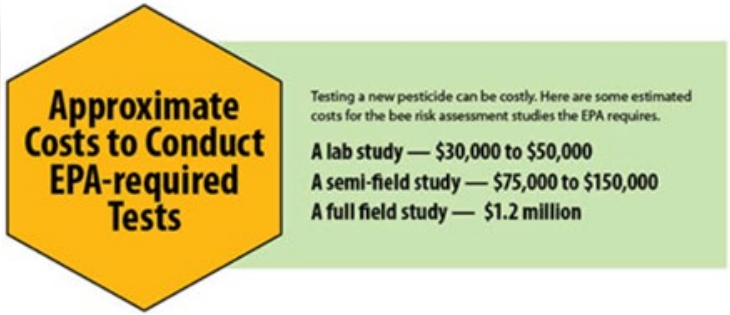
Transformation of Toxicity Testing

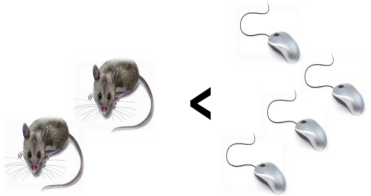
Historically:

Whole animal test

- **Observe Toxic Outcome**
  - **Examples**
    - **mortality**

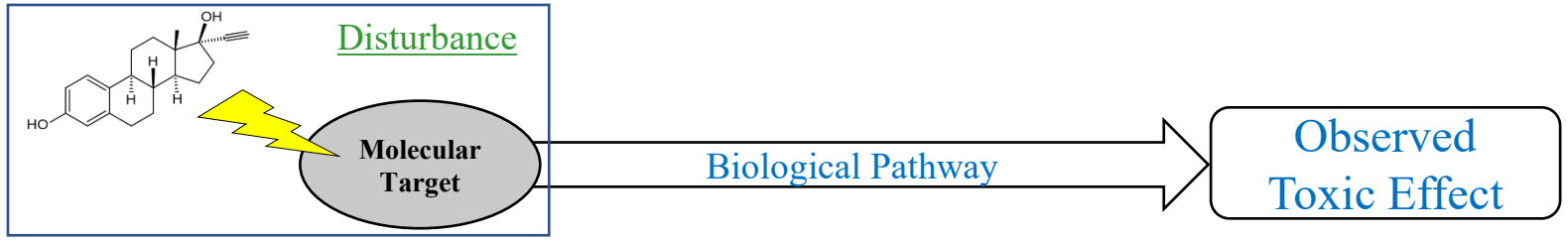
Resource intensive





Toxicity Testing in the 21st Century:

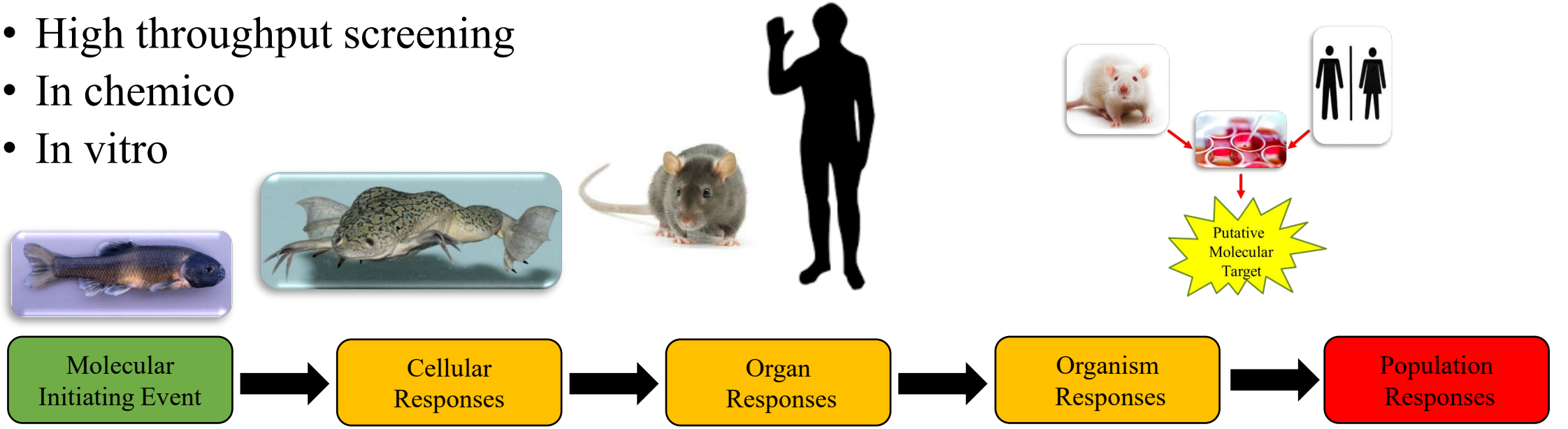
- ***In vitro* and *in silico* methods**
  - **Pathway-based approaches**
  - **Focus on disturbance of the biological pathway**
    - **Predictive of the observable toxic effects**



Enabled by evolution of the science and technology

New Approach Methods for Regulatory Decision-Making

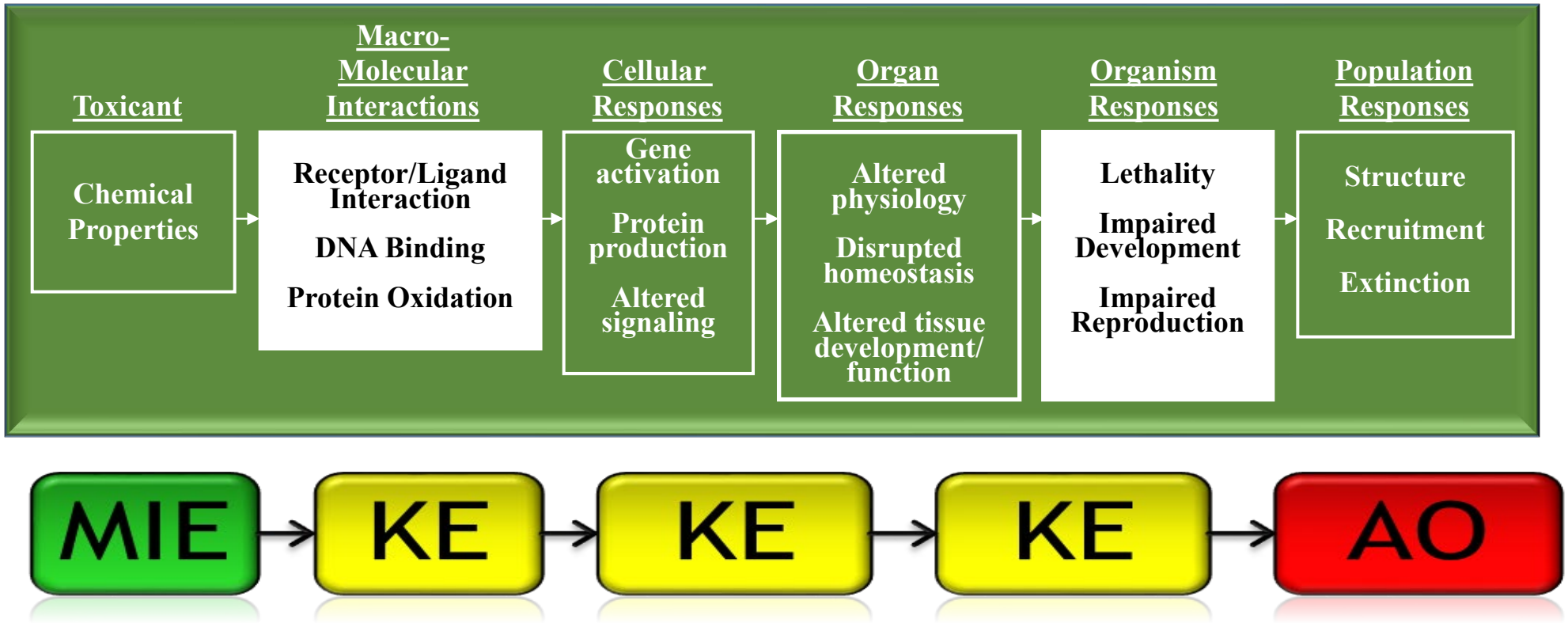
- An umbrella term
  - Systems biology
  - In silico and bioinformatics
  - Omics
  - High throughput screening
  - In chemico
  - In vitro



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

Bioinformatics

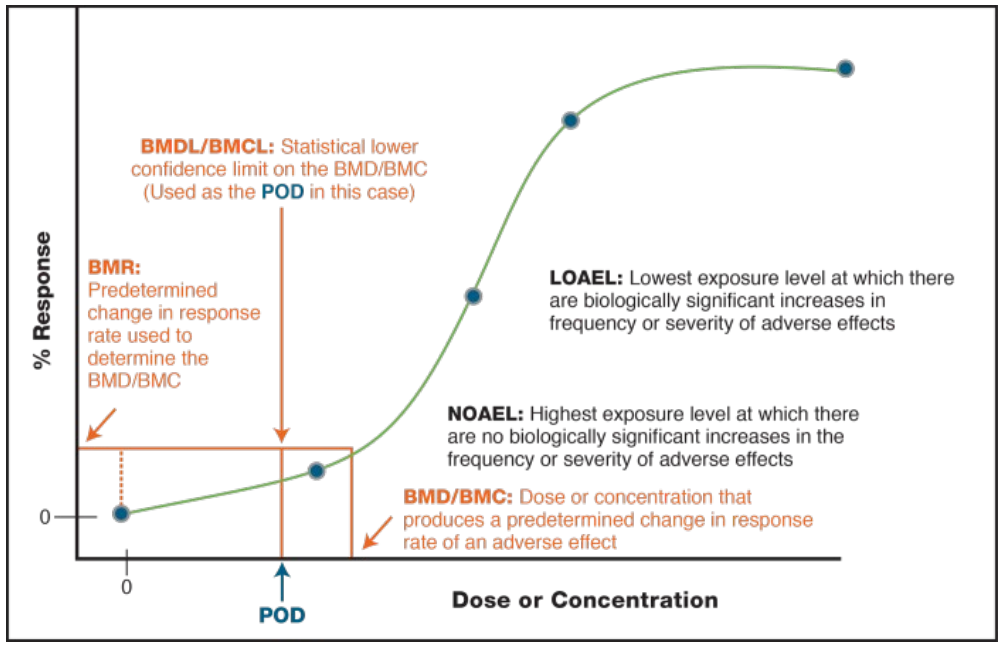
- Combines mathematics, information science, and biology to answer biological questions

- Developing methodology and analysis tools to explore large volumes of biological data
  - Query, extract, store, organize, systematize, annotate, visualize, mine, and interpret complex data
    - Usually pertains to DNA and amino acid sequences

Let the computers do the work

High Throughput Transcriptomics

- Uses gene expression profiling as an endpoint for rapidly evaluating the effects of large numbers of chemicals
  - Hundreds or thousands of genes using targeted transcriptomic panels (i.e. L1000, S1500+), or the whole transcriptome using microarrays, RNA-Seq or targeted RNA-Seq
- Performed in concentration-response mode can provide potency estimates for the concentrations of chemicals that produce perturbations in cellular response pathways



Traditional Toxicity Testing

Direct observation of the concentration at which adverse effects occur

HTTr

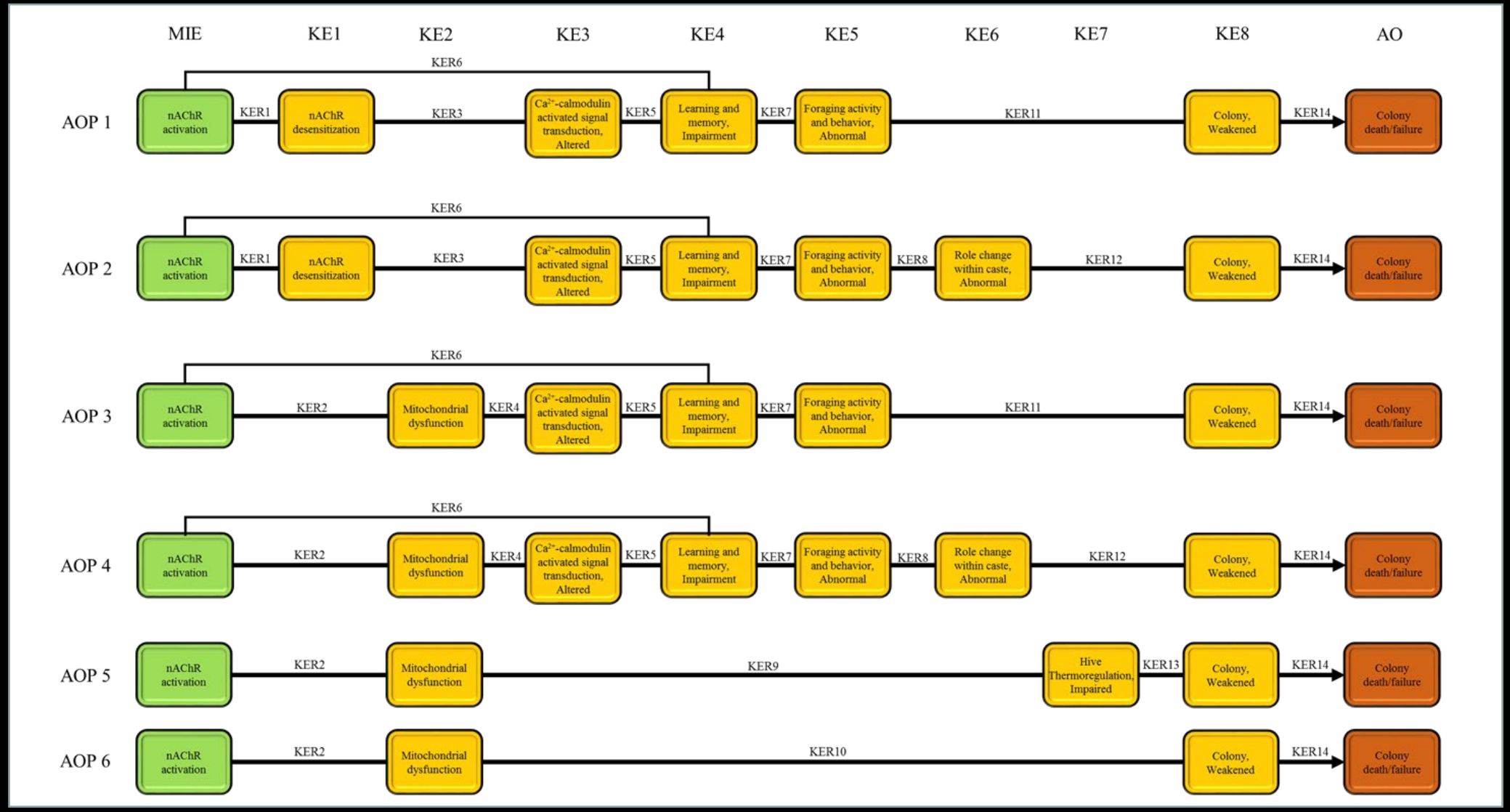
The dose-response relationship of gene expression data provides an estimate of the lowest dose of a chemical to induce a significant change in biological activity

Multiple Chemical and Non-chemical Stressors

Neonicotinoids have received a great deal of attention
Is there a link to colony death/failure?
What is the mechanism?

fppt.com

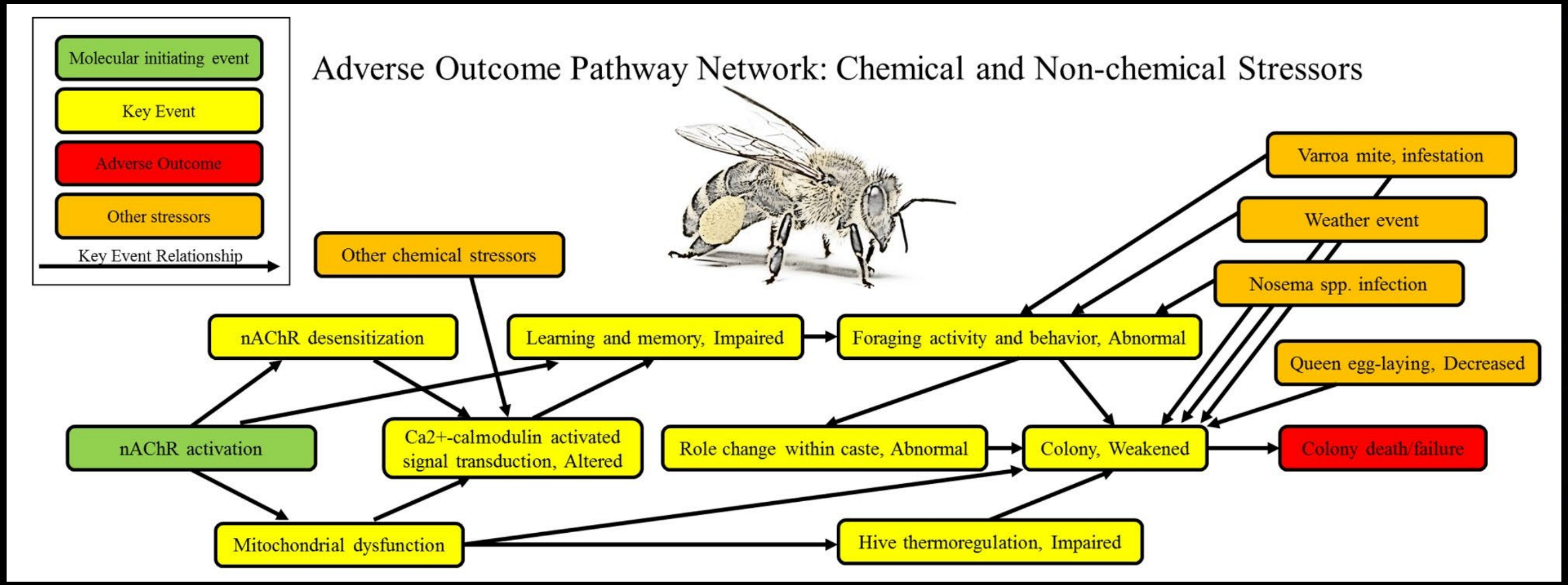
AOP Descriptions



LaLone et al., 2017. STOTEN 584-585, 751-775

fppt.com

LaLone et al., 2017. STOTEN 584-585, 751-775



Define Knowledge Gaps

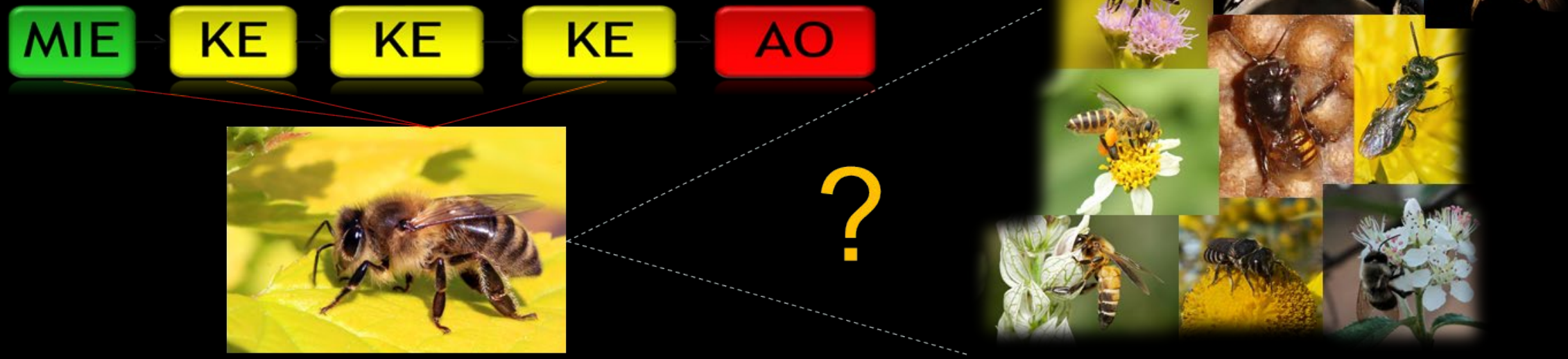
Understand nodes that may be impacted by multiple stressors

Assists in development of mitigation strategies

How to define the taxonomic relevance of the AOP network?

fppt.com

AOP Network developed with empirical studies focused in Apis mellifera

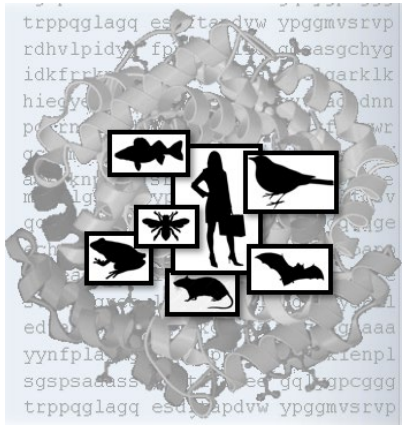


Empirical toxicity data do not exist for the majority of species

- Bioinformatics approaches for cross species extrapolation

fppt.com

EPA United States Environmental Protection Agency



TOXICOLOGICAL SCIENCES, 153(2), 2016, 228–245

doi: 10.1093/toxsci/kfw119
Advance Access Publication Date: June 30, 2016
Research article

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS): A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity

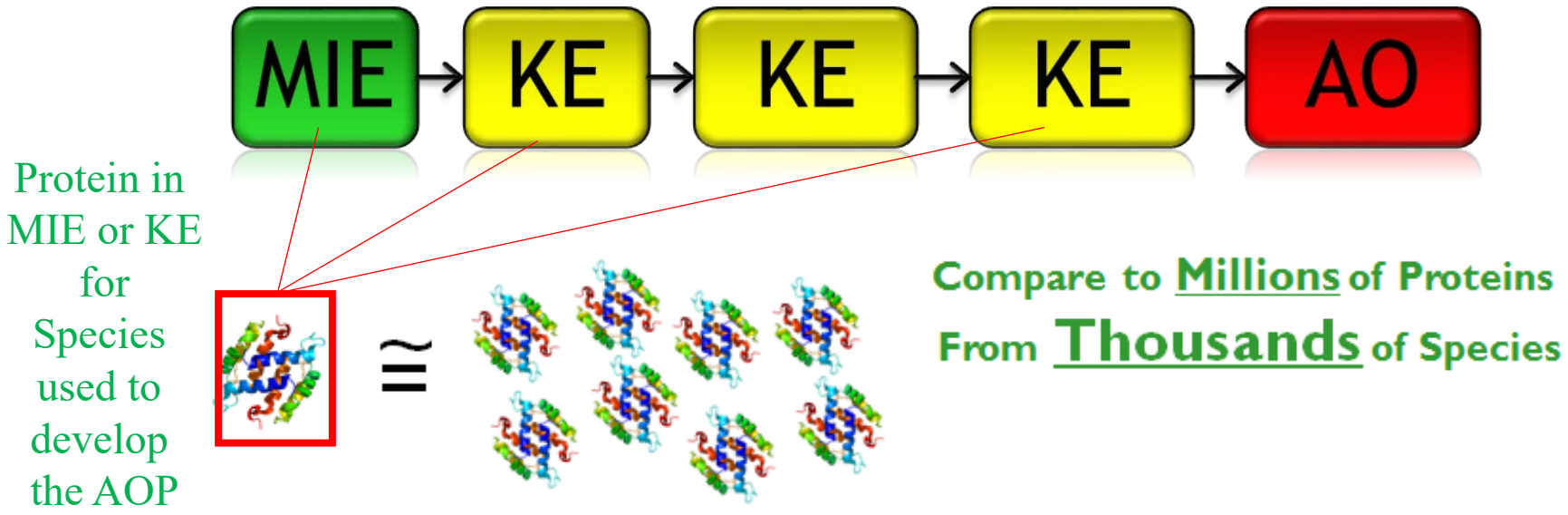
Carlie A. LaLone,*,[1] Daniel L. Villeneuve,* David Lyons,† Henry W. Helgen,‡ Serina L. Robinson,§,[2] Joseph A. Swintek,¶ Travis W. Saari,* and Gerald T. Ankley*

https://seqapass.epa.gov/seqapass/

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool

Evaluation of MIE and KE conservation across species



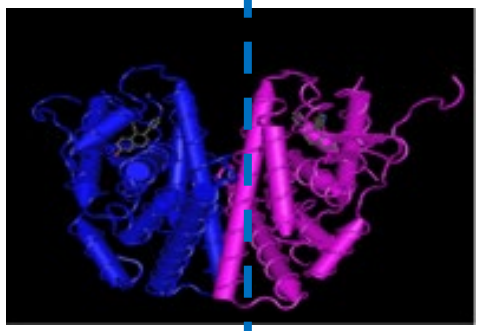
- Greater similarity = Greater likelihood that chemical can act on the protein
- Line of Evidence: Predict Potential Chemical Susceptibility Across Species
  - Receptor/enzyme available for the chemical to act upon

- Conservation of MIE and early KEs: Extrapolate across taxa

Sequence

MTMTLHTKASGMALLHQIQGNELEPLNRPQLKIPLERPLGE
VYLDSSKPAVYNYPEGAAYEFNAAAAANAQVYGQTGLPYG
PGSEAAAFGSNGLGGFPPLNSVSPSPLMLLHPPPQLSPFLQ
PHGQQVPYYLENEPSGYTVREAGPPAFYRPNSDNRRQGGR
ERLASTNDKGSMAMESAKETRYCAVCNDYASGYHYGVWSC
EGCKAFFKRSIQGHNDYMCPATNQCTIDKNRRKSCQACRLR
KCYEVGMMKGGIRKDRRGGRMLKHKRQRDDGEGRGEVG
SAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMVSALLA
EPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKV
PGFVDLTLHDQVHLLECAWLEILMIGLVWRSMEHPGKLLFA
PNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEF
VCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLM

Structure



Function

Bioinformatics

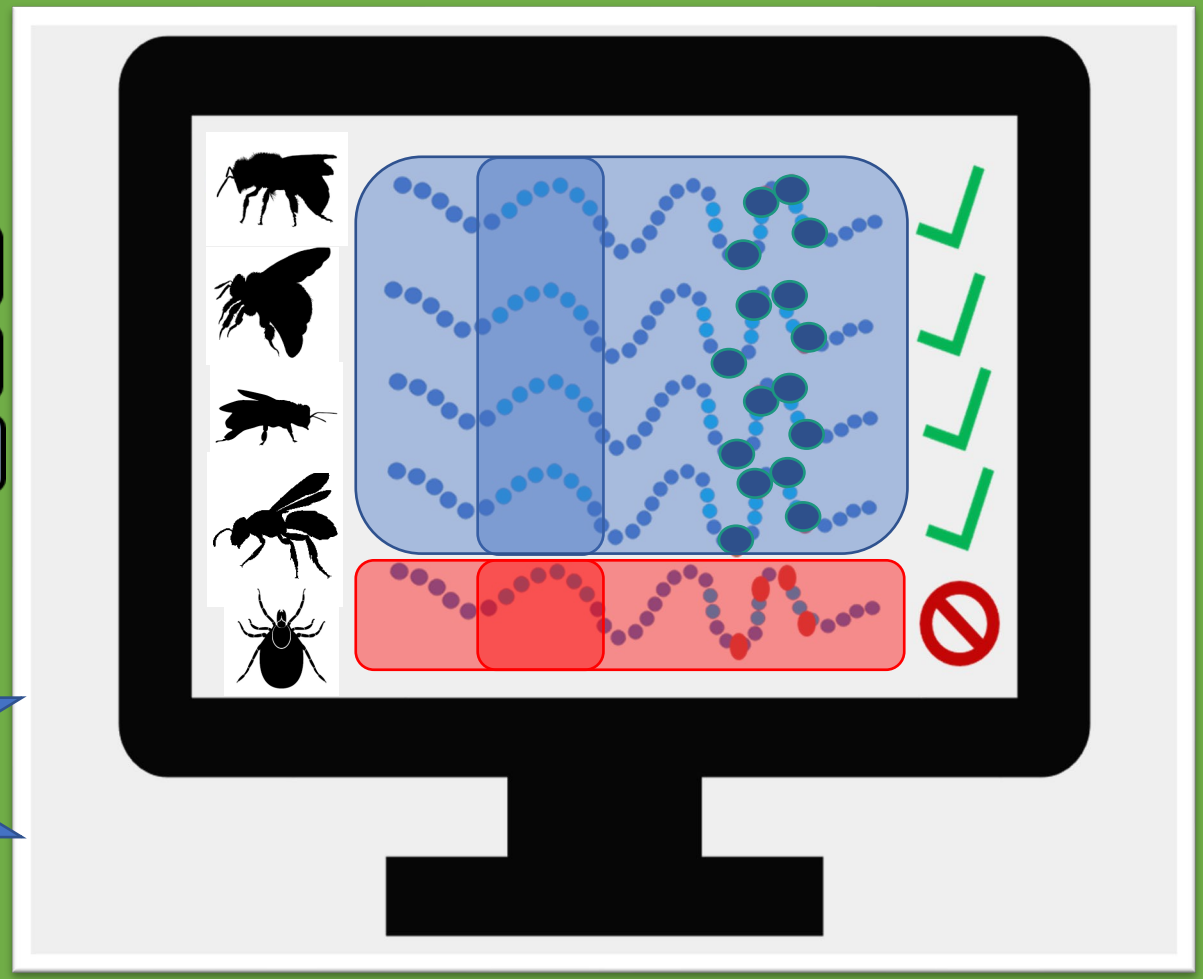
Flexible Analysis Based On Available Data

Level 1 Primary Amino Acid Sequence Alignments

Level 2 Conserved Functional Domain Alignments

Level 3 Critical (Close Contact) Amino Acid Conservation

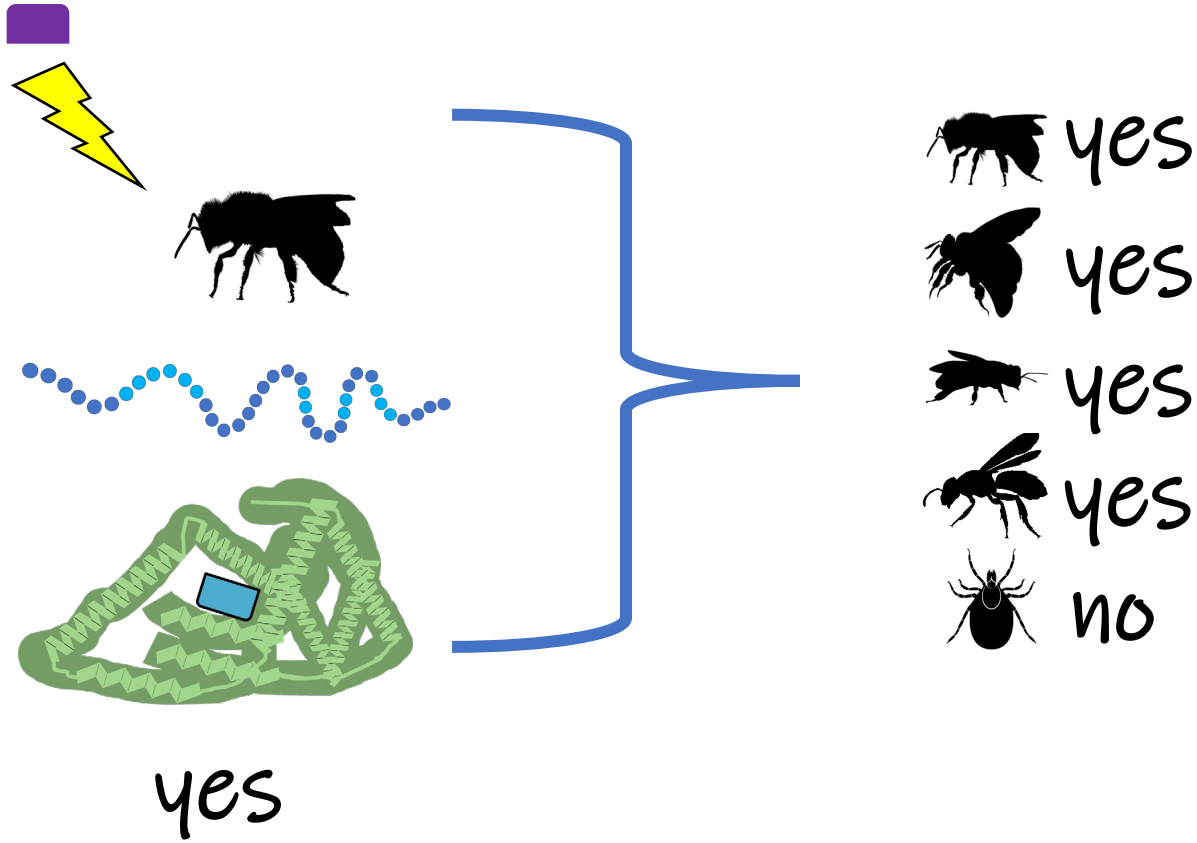
seqapass.epa.gov/seqapass/



Gather Lines of Evidence Toward Protein Conservation

Apis ≈ *Non-Apis*

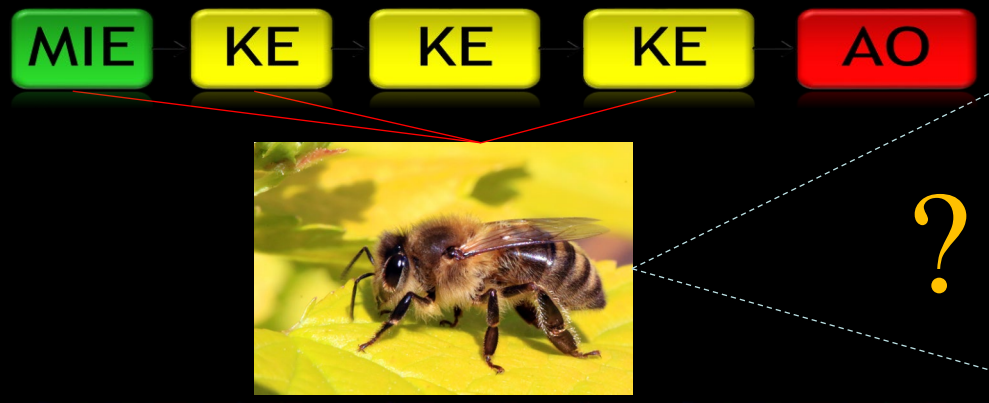
SeqAPASS Predicts Likelihood of Similar Susceptibility based on Sequence Conservation:



Line(s) of evidence indicate
- The protein is conserved
- The protein is NOT conserved

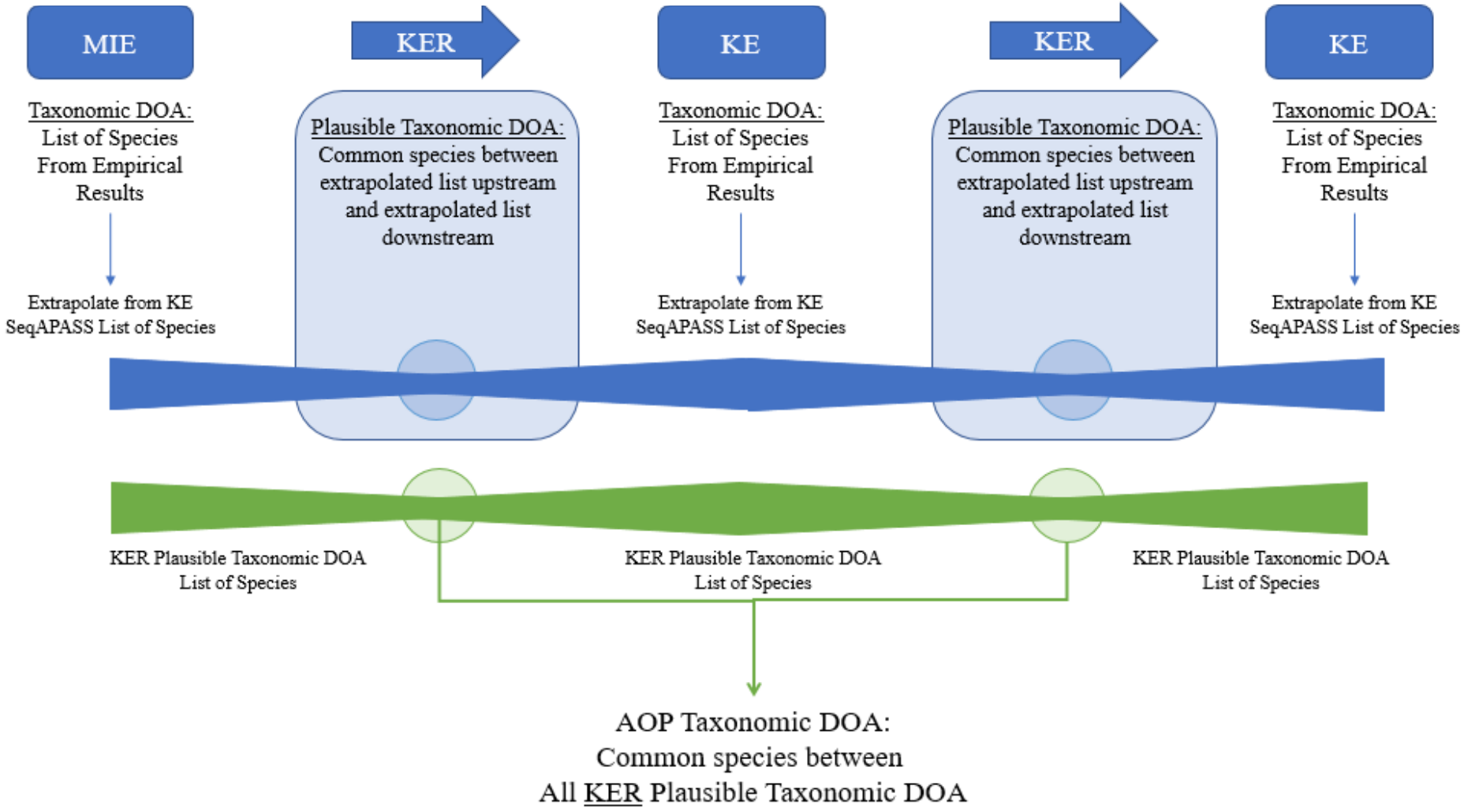
Define the Taxonomic Domain with SeqAPASS

- Nicotinic acetylcholine receptor – MIE, KE1
  - Multiple subunits (LaLone et al., Tox. Sci. 2016)
- ATP Synthase (mitochondrial) – KE2 and KE3
- Calmodulin – KE3
- Adenylate cyclase – KE3
- Protein kinase C – KE3
- Calcium calmodulin dependent protein kinase II – KE3
- cAMP-responsive element-binding protein – KE3
- Vitellogenin precursor – KE6
- Juvenile hormone esterase – KE6
- Methyl farnesoate epoxidase – KE6

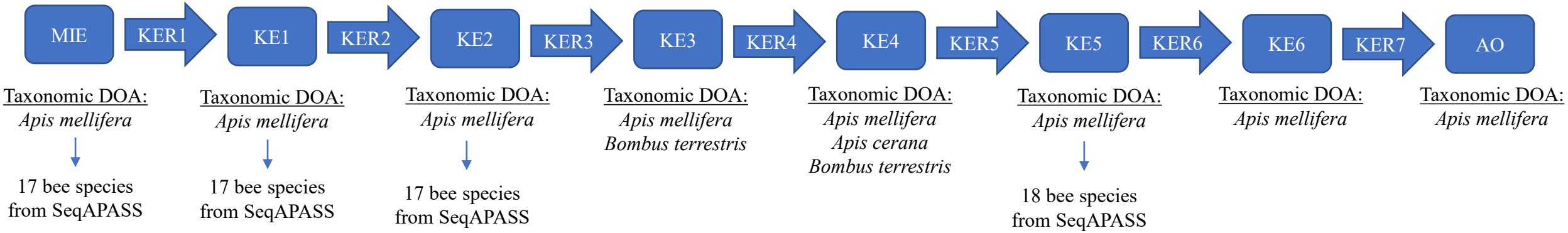


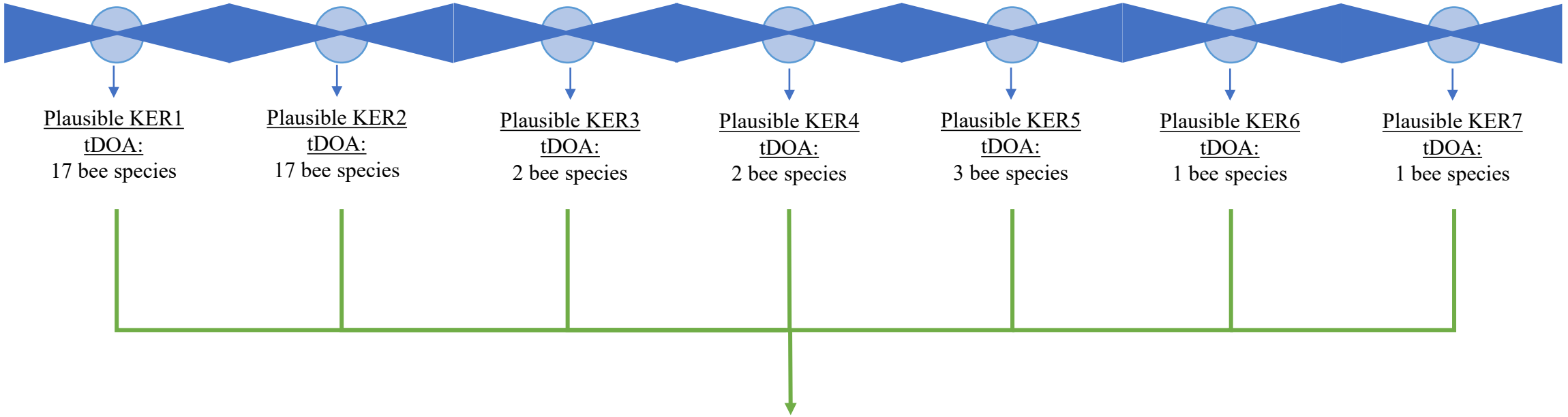
fppt.com

Define the Taxonomic Domain of Applicability:

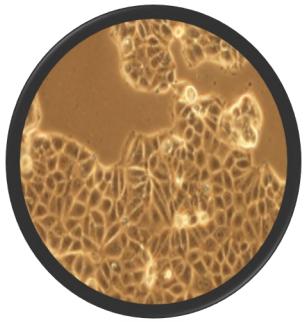



MIE
nAChR activation
KER1
KE1
nAChR desensitization
KER2
KE2
Ca2+-calmodulin activated signal transduction, Altered
KER3
KE3
Learning and memory, Impairment
KER4
KE4
Foraging activity and behavior, Abnormal
KER5
KE5
Role change within caste, Abnormal
KER6
KE6
Colony, Weakened
KER7
AO
Colony death/failure


MIE
KER1
KE1
KER2
KE2
KER3
KE3
KER4
KE4
KER5
KE5
KER6
KE6
KER7
AO
Taxonomic DOA:
Apis mellifera
17 bee species from SeqAPASS
Taxonomic DOA:
Apis mellifera
17 bee species from SeqAPASS
Taxonomic DOA:
Apis mellifera
17 bee species from SeqAPASS
Taxonomic DOA:
Apis mellifera
Bombus terrestris
Taxonomic DOA:
Apis mellifera
Apis cerana
Bombus terrestris
Taxonomic DOA:
Apis mellifera
18 bee species from SeqAPASS
Taxonomic DOA:
Apis mellifera
Taxonomic DOA:
Apis mellifera


Plausible KER1 tDOA:
17 bee species
Plausible KER2 tDOA:
17 bee species
Plausible KER3 tDOA:
2 bee species
Plausible KER4 tDOA:
2 bee species
Plausible KER5 tDOA:
3 bee species
Plausible KER6 tDOA:
1 bee species
Plausible KER7 tDOA:
1 bee species
AOP tDOA: Common species between all KER plausible tDOA
Apis mellifera

Toxicogenomic Approach



Whole human transcriptome

NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Research Report on
National Toxicology Program Approach to Genomic Dose-Response Modeling

NTP RR 5

APRIL 2018

- Number of mammalian studies have shown short-term transcriptomics-based PODs are predictive of apical potency.
- Generally, within ½ log.
- Health protective points of departure.

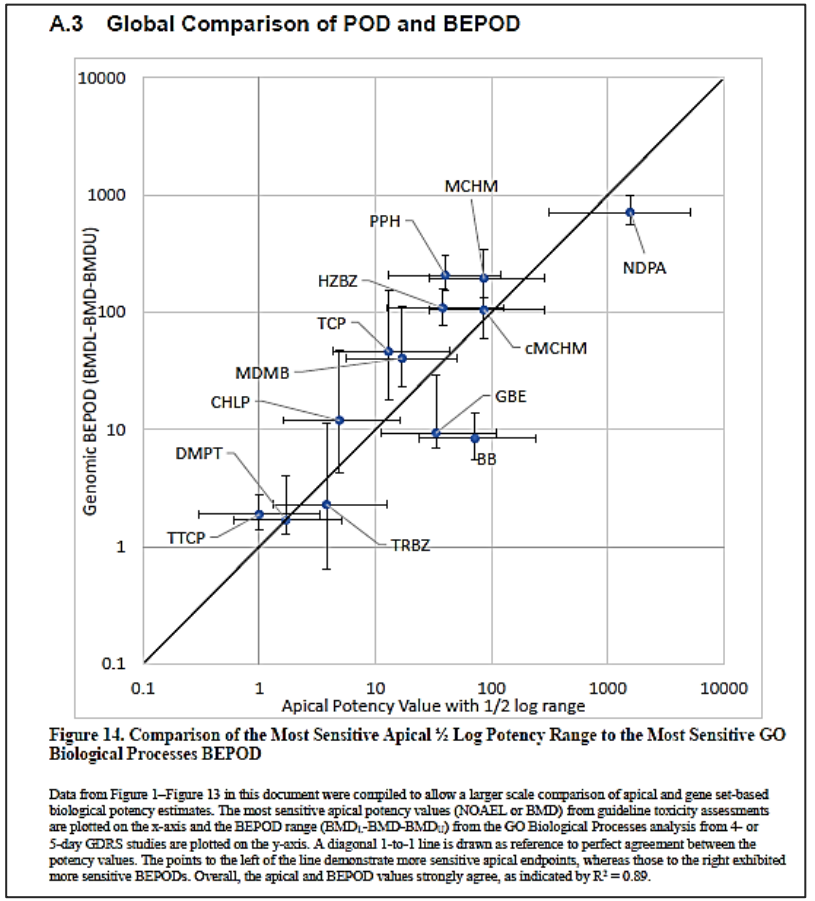


Figure 14. Comparison of the Most Sensitive Apical ½ Log Potency Range to the Most Sensitive GO Biological Processes BEPOD

Data from Figure 1–Figure 13 in this document were compiled to allow a larger scale comparison of apical and gene set-based biological potency estimates. The most sensitive apical potency values (NOAEL or BMD) from guideline toxicity assessments are plotted on the x-axis and the BEPOD range (BMD_L-BMD-BMD_U) from the GO Biological Processes analysis from 4- or 5-day GDRS studies are plotted on the y-axis. A diagonal 1-to-1 line is drawn as reference to perfect agreement between the potency values. The points to the left of the line demonstrate more sensitive apical endpoints, whereas those to the right exhibited more sensitive BEPODs. Overall, the apical and BEPOD values strongly agree, as indicated by $R^2 = 0.89$.

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 181(1), 2021, 68–89

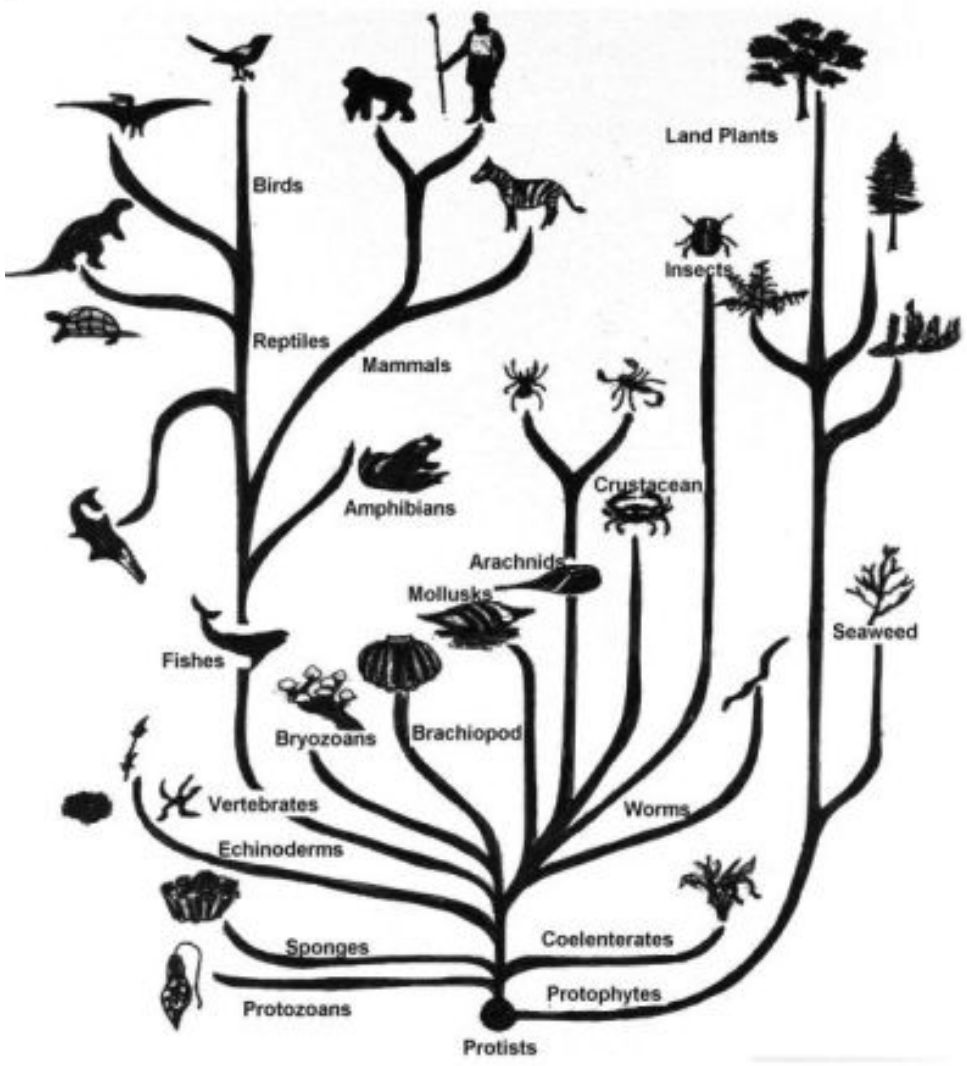
doi: 10.1093/toxsci/kfab009
Advance Access Publication Date: 4 February 2021
Research Article

High-Throughput Transcriptomics Platform for Screening Environmental Chemicals

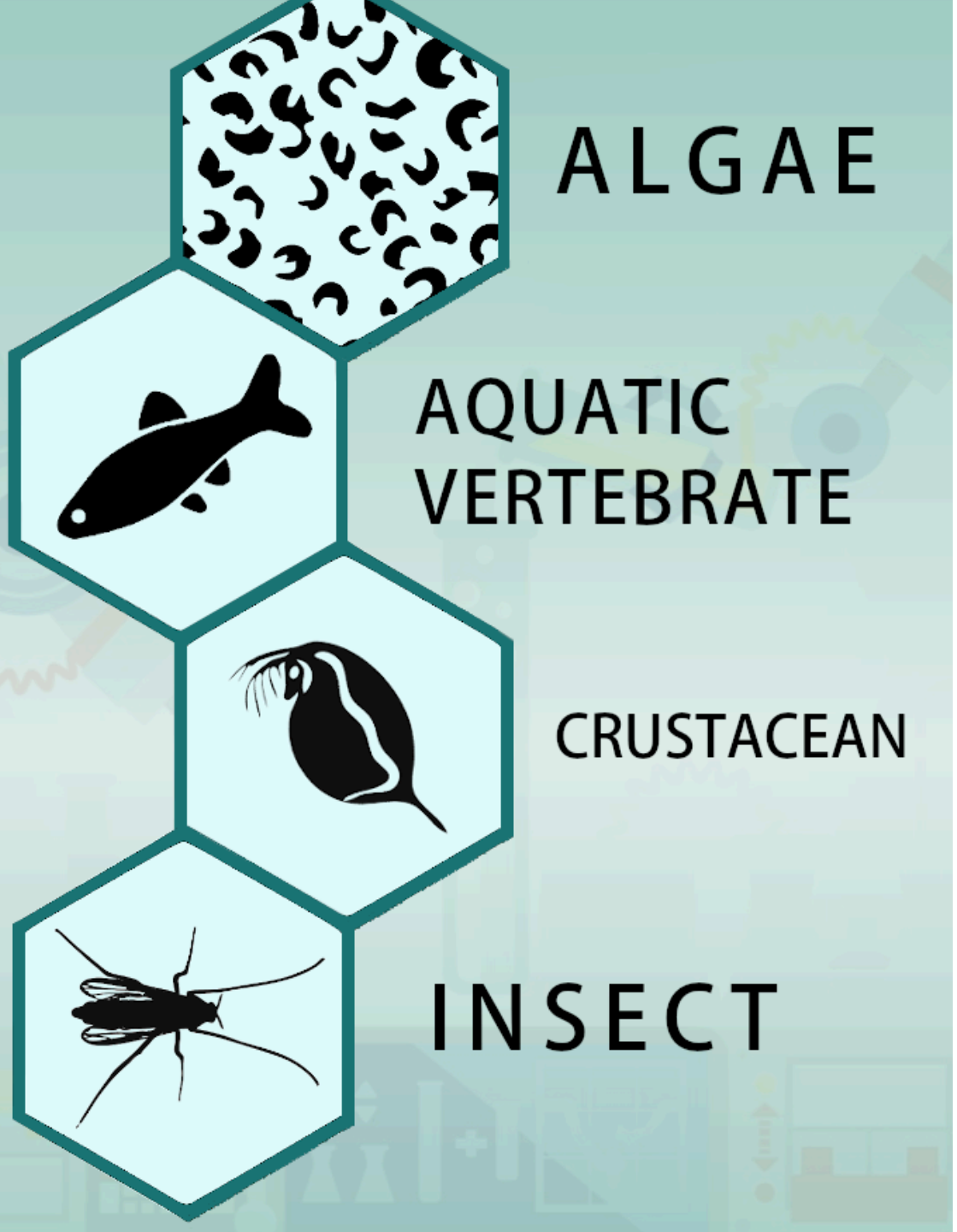
Joshua A. Harrill,*,1 Logan J. Everett,* Derik E. Haggard,*,† Thomas Sheffield,*,† Joseph L. Bundy,* Clinton M. Willis,*,‡ Russell S. Thomas,* Imran Shah,* and Richard S. Judson*

Toxicological Sciences, Volume 181, Issue 1, May 2021, Pages 68–89, https://doi.org/10.1093/toxsci/kfab009

Ecotoxicology Perspective



- Humans are just a tiny fraction of the biological diversity we are charged to protect.
- Many genes/pathways are conserved
- Unique physiology in other kingdoms, phyla, classes…
- How do we assure those pathways are covered?



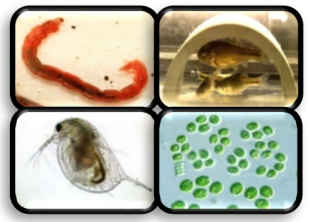
High throughput assays for three major trophic levels of aquatic ecosystems

- Primary producers (e.g., algae)
- Primary consumers (e.g., zooplankton, aquatic inverts)
- Secondary consumers (e.g., fish)

Commonly used for GHS classification and labeling of chemicals for environmental hazard

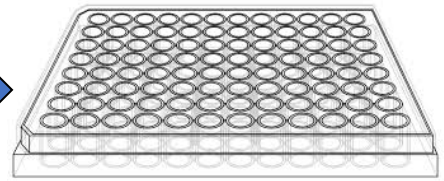
Aquatic organisms highly vulnerable to exposure

Incorporating transcriptomics as assessment endpoint



Load organisms

24 h exposure

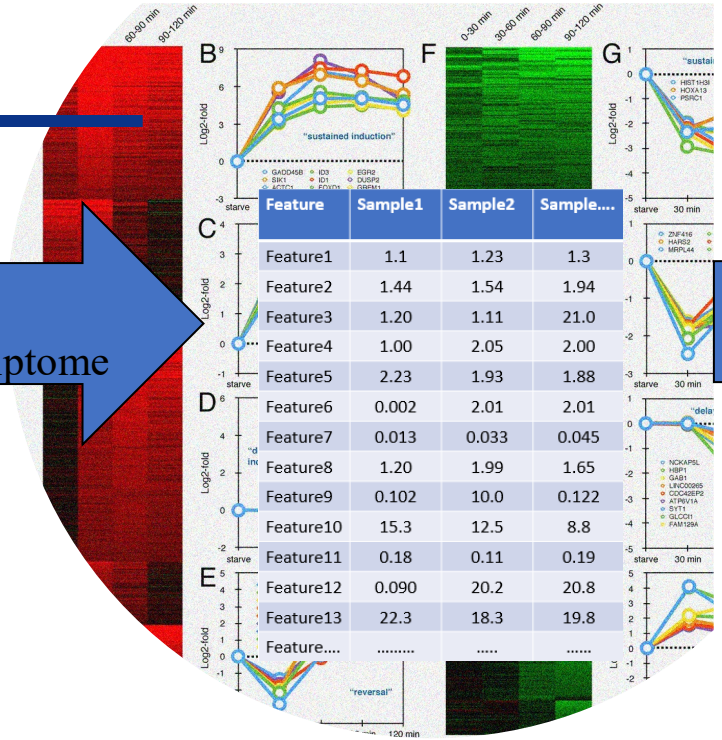


Phenotypic anchoring

Homogenate /extract

RNA

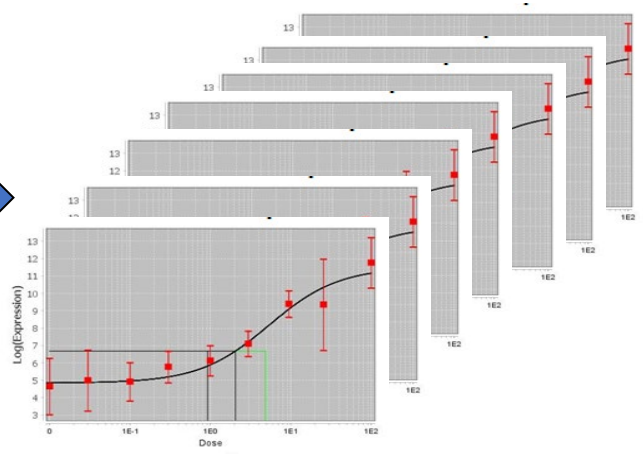
Whole transcriptome



Feature	Sample1	Sample2	Sample....
Feature1	1.1	1.23	1.3
Feature2	1.44	1.54	1.94
Feature3	1.20	1.11	21.0
Feature4	1.00	2.05	2.00
Feature5	2.23	1.93	1.88
Feature6	0.002	2.01	2.01
Feature7	0.013	0.033	0.045
Feature8	1.20	1.99	1.65
Feature9	0.102	10.0	0.122
Feature10	15.3	12.5	8.8
Feature11	0.18	0.11	0.19
Feature12	0.090	20.2	20.8
Feature13	22.3	18.3	19.8
Feature....	.....	.....	.....

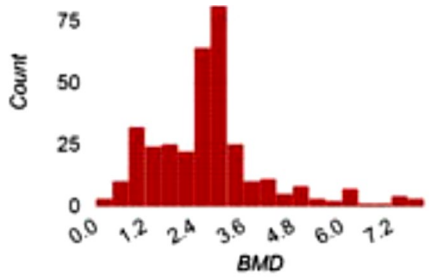
Curv

ve fitting



BMD Express

Distribution of BMDs



10th centile BMD

Point of departure

[tPOD]

Rough estimate per chemical ~$5K for one species

Evaluating the approach

[tPOD] ≤ [Most sensitive chronic endpoint]

[tPOD] > [Most sensitive chronic endpoint]

[tPOD] <<<< [Most sensitive chronic endpoint]

Approach is being explored with cell-lines for eco species as well

Where do we go from here?

- NAMs can enhance our mechanistic understanding of chemical effects
- AOP information can identify research gaps to guide focused studies and aid in the identification of mitigation strategies to eliminate or reduce impact of chemicals (https://aopwiki.org/ is freely available)
- Bioinformatics can inform taxonomic domain of applicability
  - SeqAPASS (https://seqapass.epa.gov/seqapass/) is freely available
  - Lines of evidence toward structural conservation
  - Useful for cross species extrapolation to predict chemical susceptibility
- High-throughput transcriptomics and derivation of tPOD
  - Being explored for eco-species
    - Do tPODs provide a protective estimate of chemical toxicity in comparison to PODs?
    - Rapid and cost-effective

Acknowledgements

U.S. EPA, ORD

Donovan Blatz (ORISE)
Marissa Jensen (Univ. Minnesota Duluth)
Dan Villeneuve
Kevin Flynn

GDIT

Thomas Transue
Cody Simmons
Audrey Wilkinson

SeqAPASS v5.1

LaLone.Carlie@epa.gov

https://seqapass.epa.gov/seqapass/