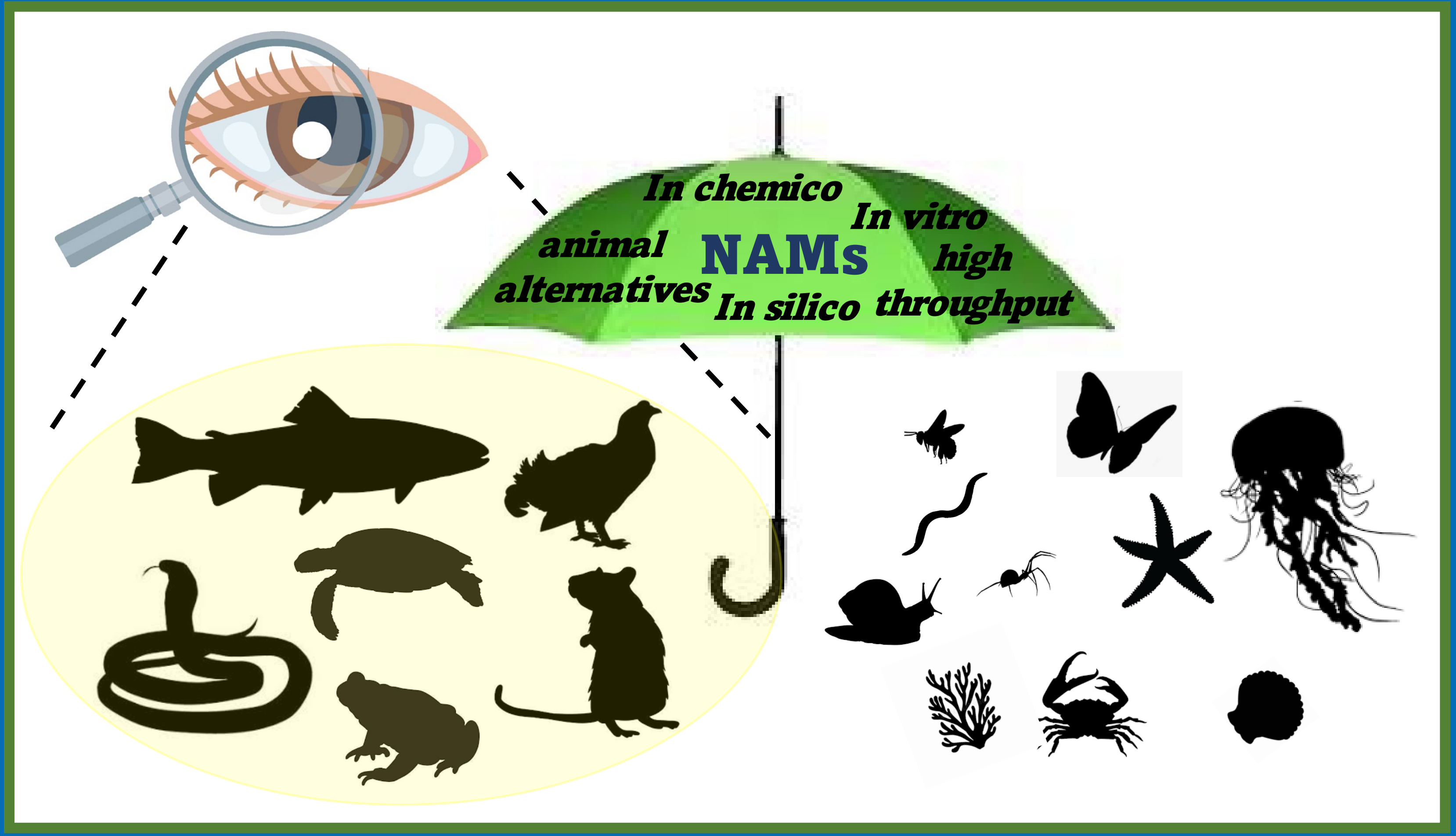
# Unleashing the Influence of Invertebrates through Application of New Approach Methods



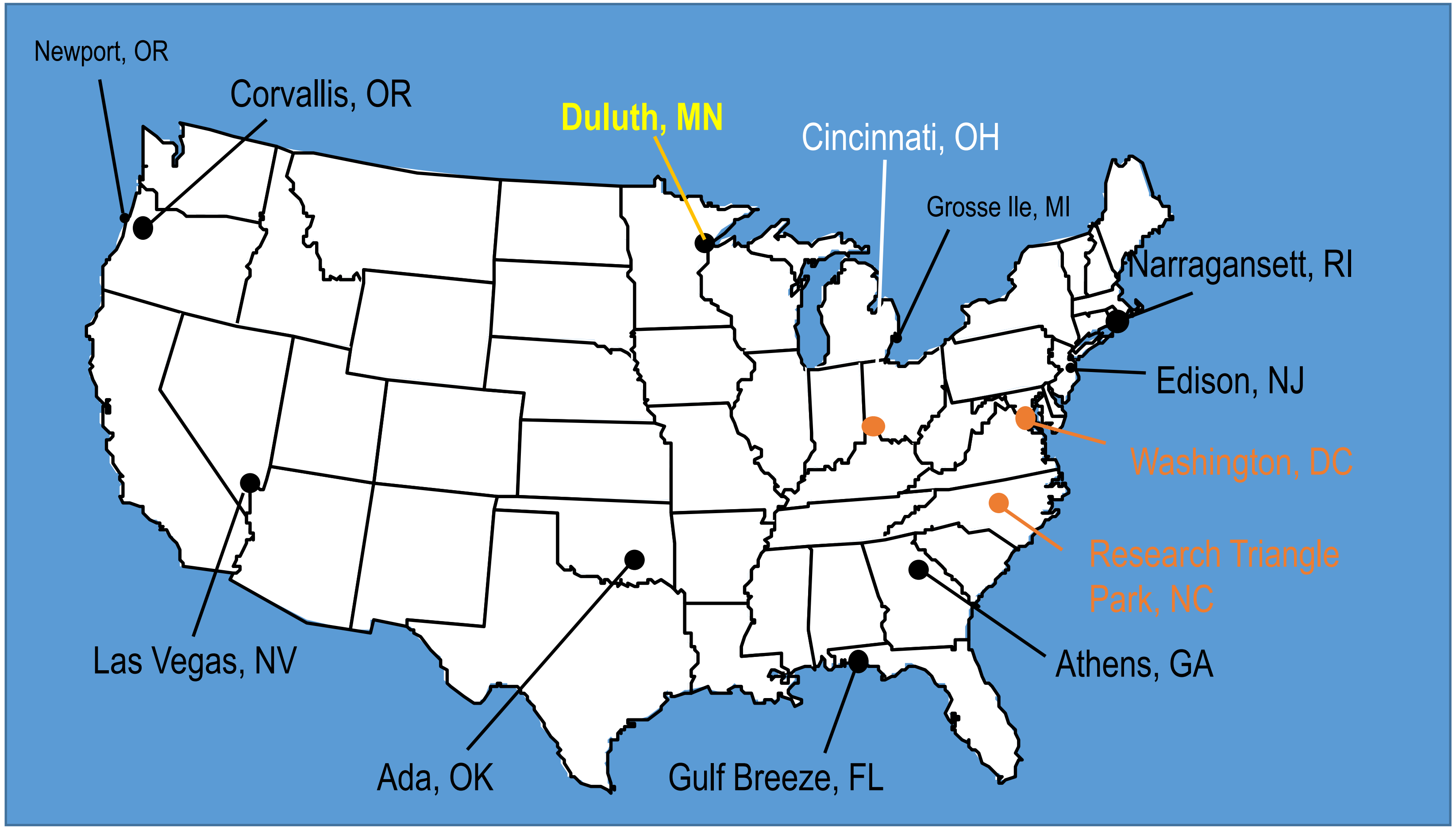

Presenter: Carlie A. LaLone, Ph.D.

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

# Outline


- Introduction
- Chemicals and EPA
- Toxicity Testing in the 21$^{st}$ Century
- New Approach Methods
  - Adverse Outcome Pathways (AOPs)
    - Understanding the biology
  - US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)
    - Cross Species Extrapolation
  - High throughput transcriptomics

# Office of Research and Development



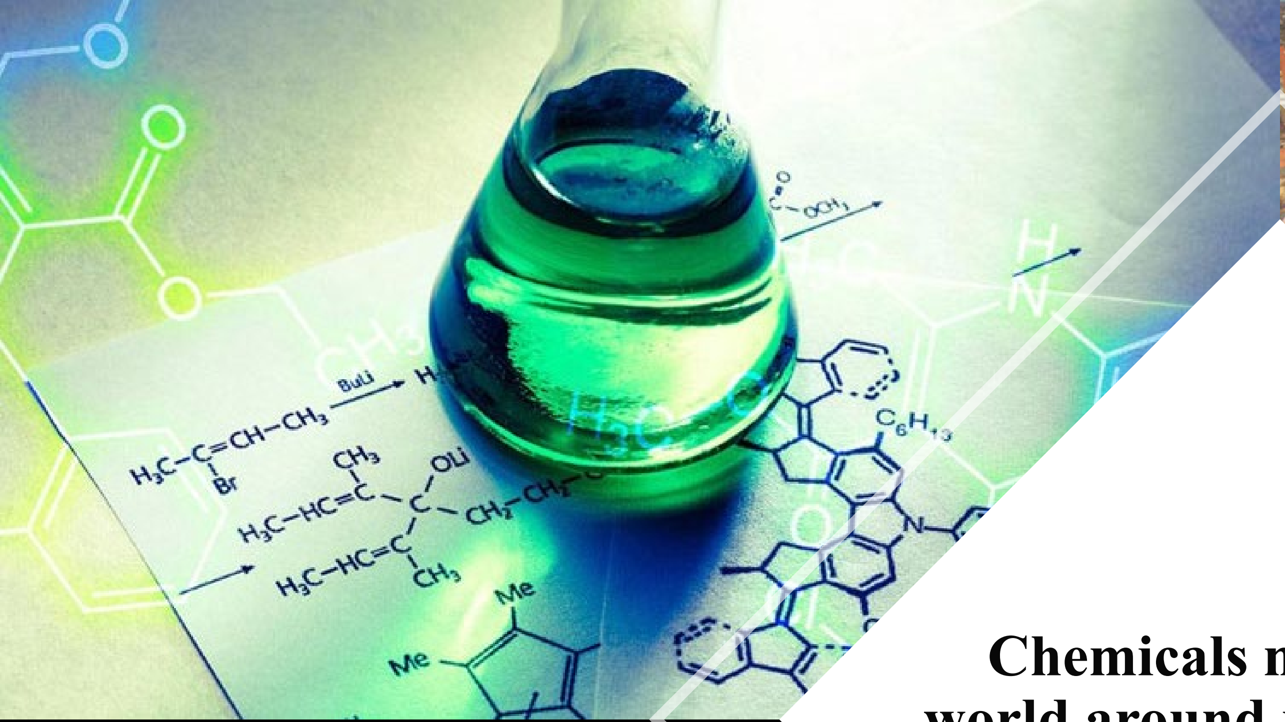
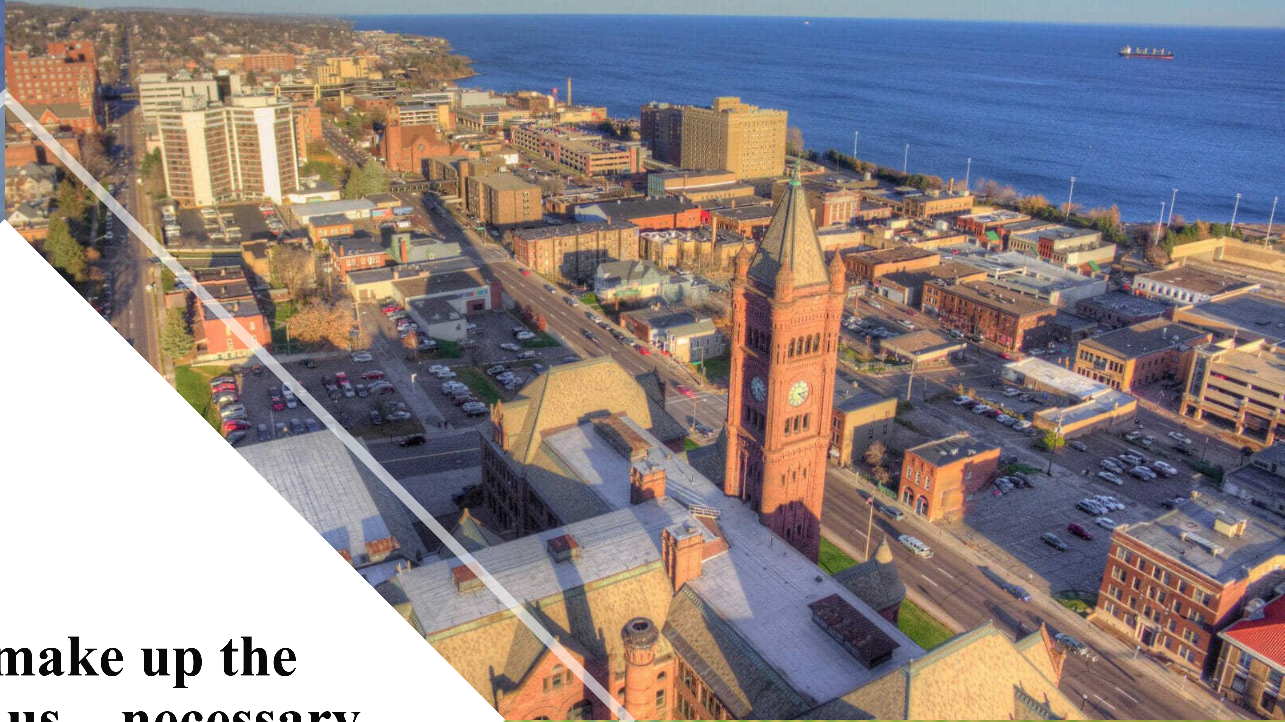
Office of Research and Development
Center for Computational Toxicology and Exposure

# Great Lakes Toxicology and Ecology Division

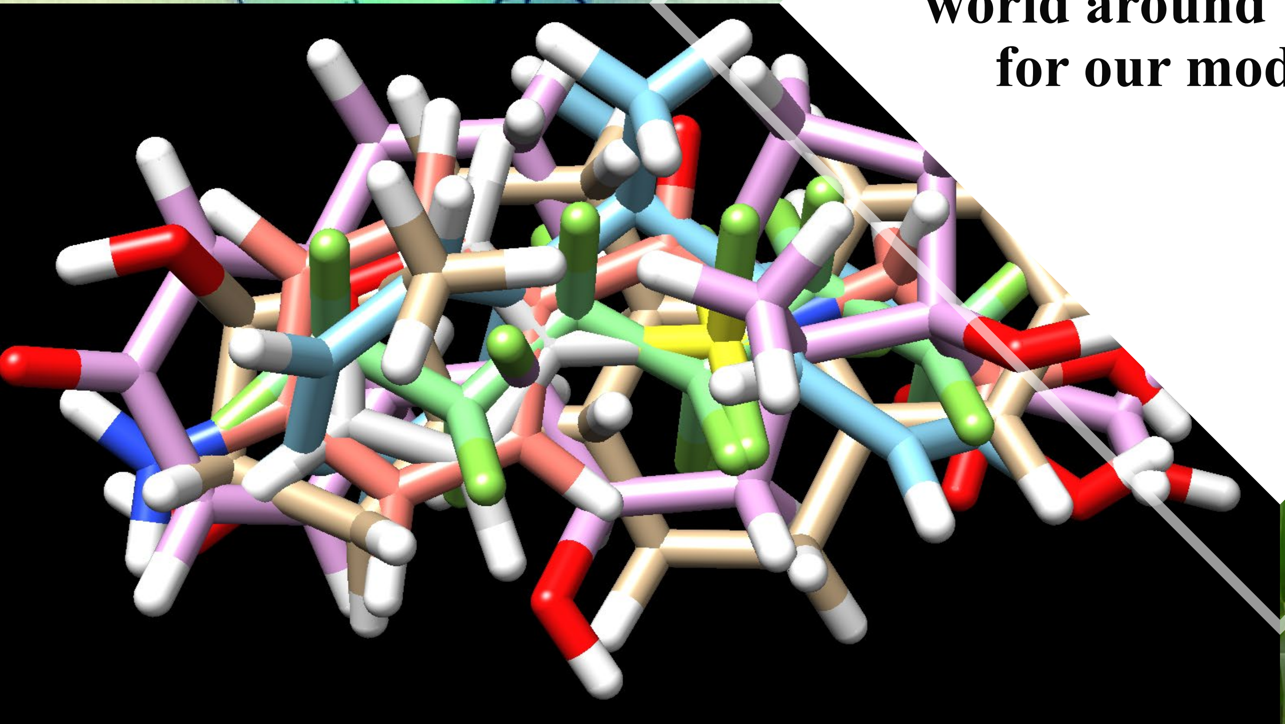
- Freshwater ecotoxicology
- Freshwater ecology

EPA
United States
Environmental Protection
Agency

# Protect Human Health and the Environment

**Chemicals make up the world around us – necessary for our modern society**




EPA United States Environmental Protection Agency

# Toxicity Testing to Understand Chemical Safety

- Regulatory decision-making

**US EPA Examples:**

*Clean Air Act*

*Clean Water Act*

*Resource Recovery Act*

*Endangered Species Act*

*Food Quality Protection Act*

*Endocrine Disruptor Screening Program*

*Federal Insecticide, Fungicide, and Rodenticide Act*

*Frank R. Lautenberg Chemical Safety for the 21st Century Act*

*Comprehensive Environmental Response, Compensation, and Liability Act*

*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*

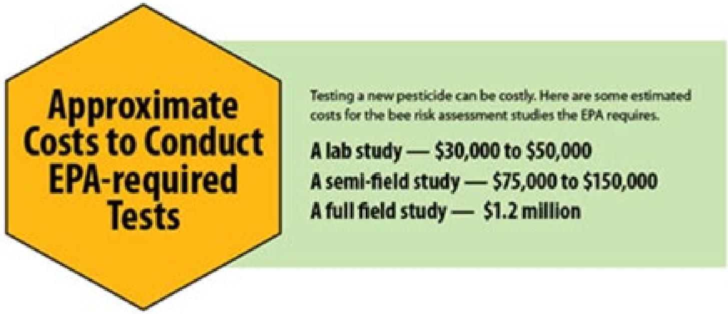
# Transformation of Toxicity Testing

<u>Historically:</u>

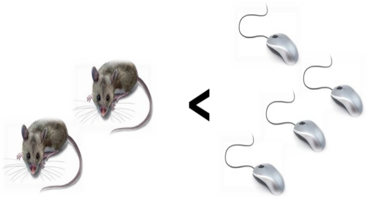
**Whole animal test**

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

**Resource intensive**

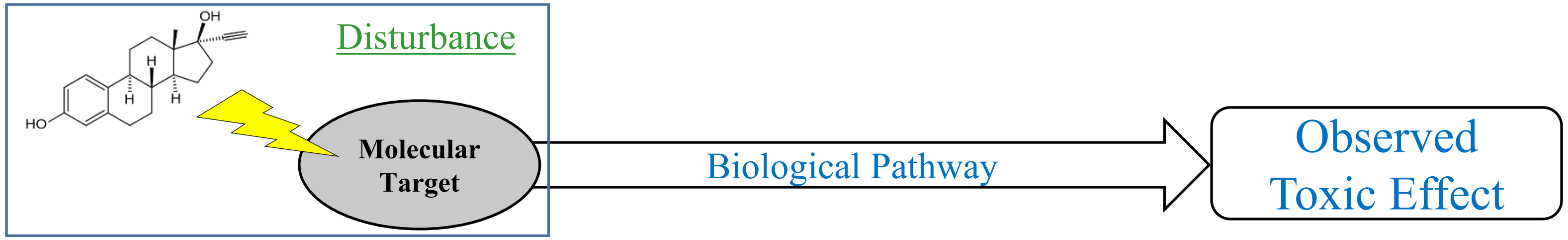






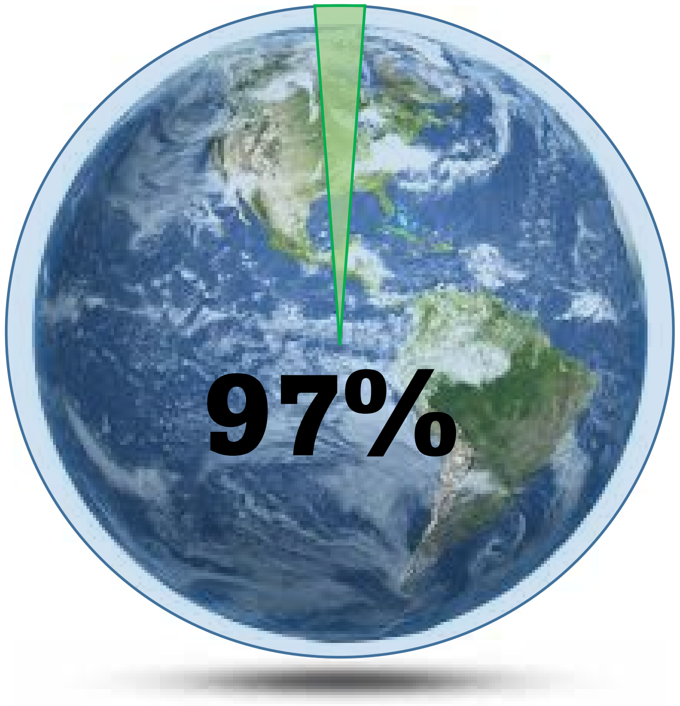

<u>Toxicity Testing in the 21st Century:</u>

- ***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

# ~97% of the animal species on Earth are invertebrates
# ~1.25 million invertebrate species are now known



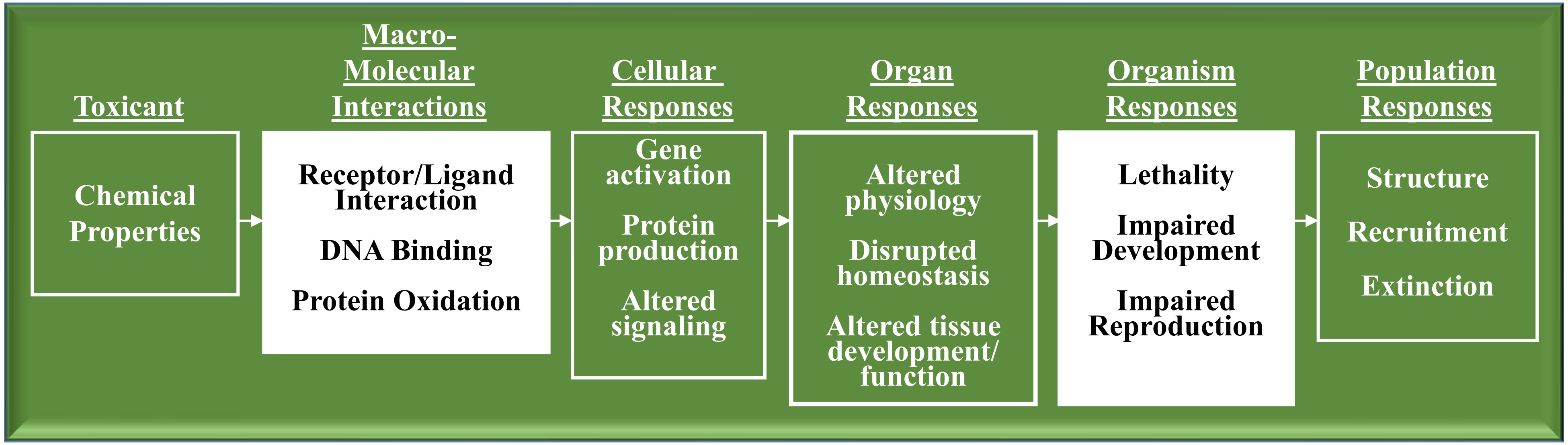

Handful of invertebrate species as model organisms for understanding chemical effects


short lifespans and rapid life cycles
cheap and readily available
ability to control diet and surroundings
Surrogate
easy maintenance and good breeding capabilities
requires least space and time-consuming care

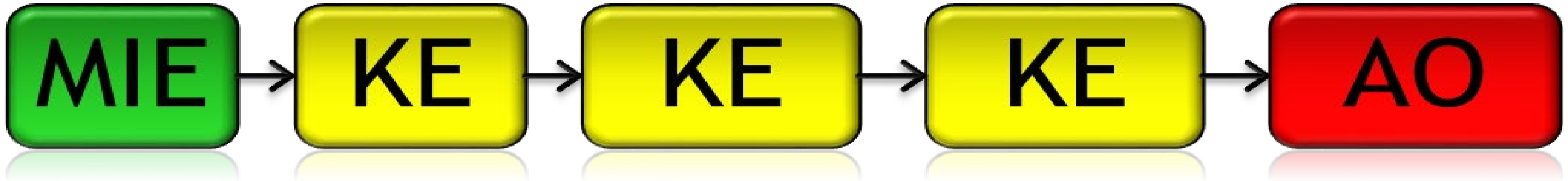
# The Adverse Outcome Pathway Framework

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.

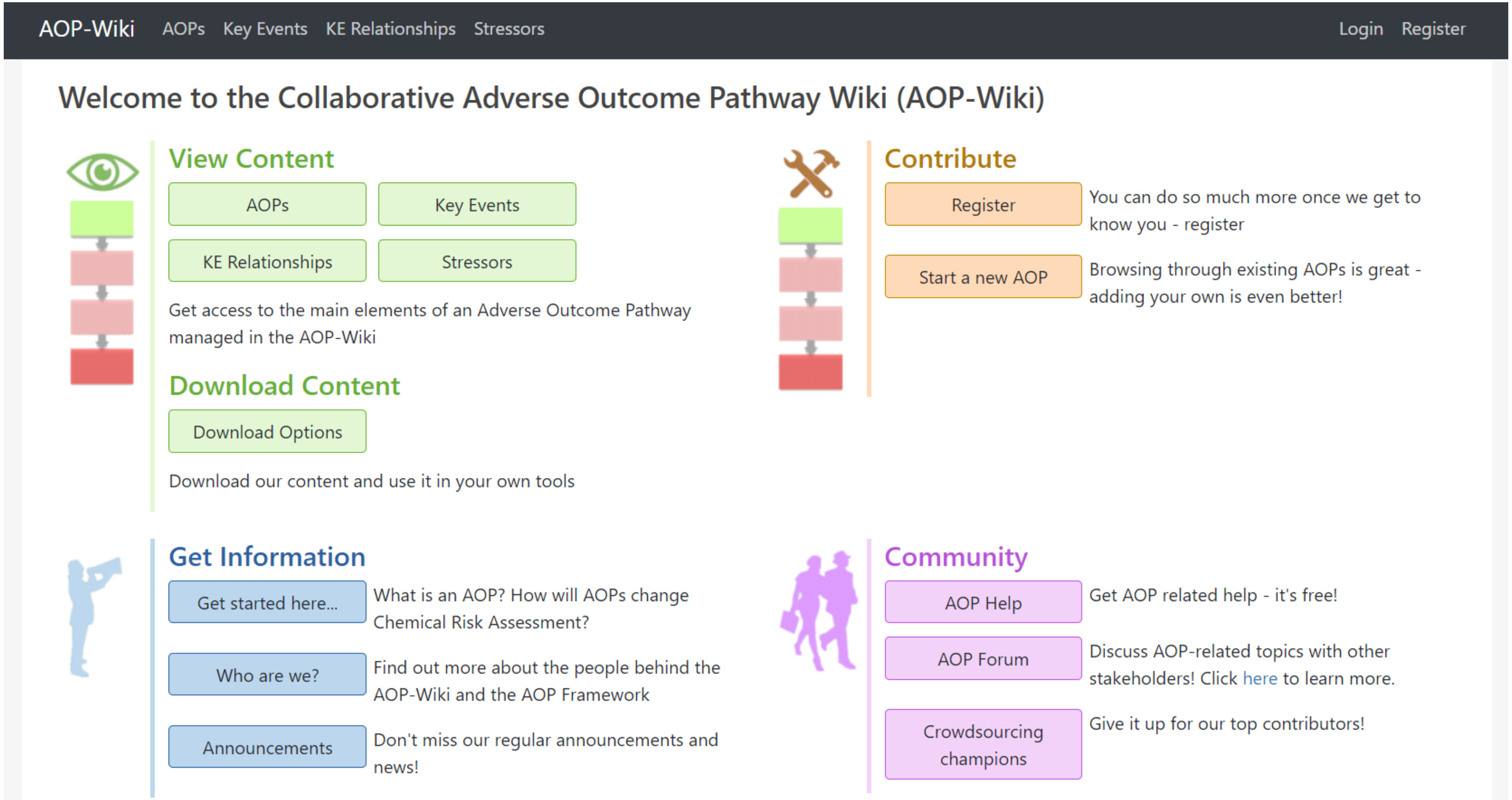
*(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741)*




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



# AOP-Wiki: AOP Development



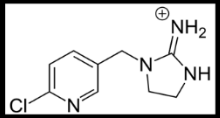
# Multiple Chemical and Non-chemical Stressors

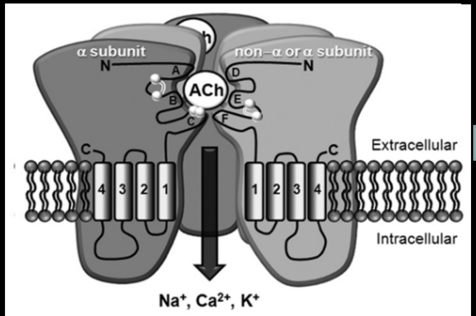

Is there a link to colony death/failure?
What is the mechanism?

# Neonicotinoids

- Target leaf-chewing and plant-sucking insects:
  - aphids, whiteflies, thrips, leafhoppers, scales, weevils, beetles, leaf miners, flies, cockroaches, sweet potato whitefly biotype, rice stink bug, brown marmorated stink bug, mealybug, sawflies, mole crickets, white grubs, lacebugs, billbugs, beetles, weevils, termites, turf insects, soil insects
- Molecular target: nicotinic acetylcholine receptor (nAChR)

Molecular Initiating Event






Picture from: Jones and Sattelle, 2010

Studies have linked neonicotinoids to impacts on individual bees:

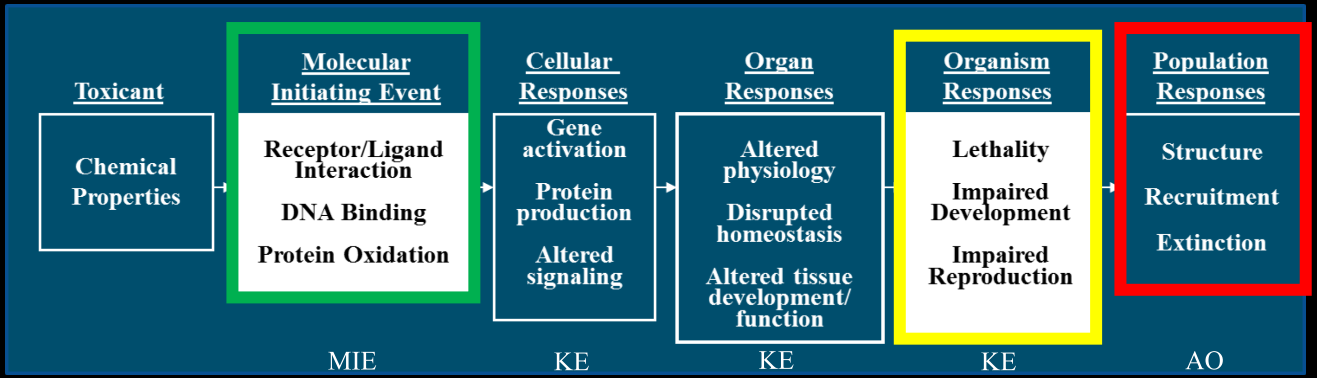
- Foraging: Homing, efficiency, behavior
- Learning and memory: Proboscis extension reflex

# Can activation of the nAChR lead to colony death/failure?

Foraging
Learning and Memory

Colony
Death/failure

nAChR



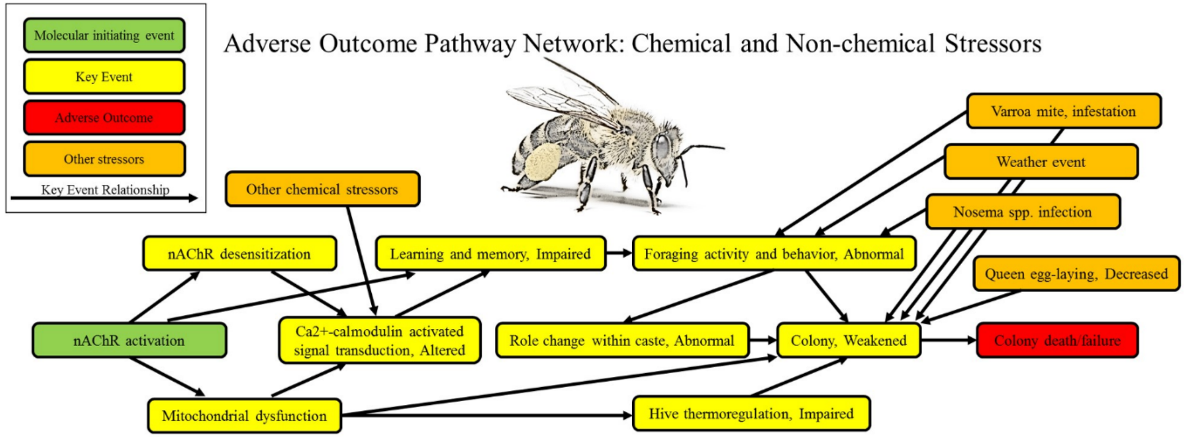

Identify Key Knowledge Gaps for Future Research Initiatives

## Evaluating Weight of Evidence

- Biological plausibility
- Empirical evidence

# The Adverse Outcome Pathway Framework:
## Application beyond the model organisms

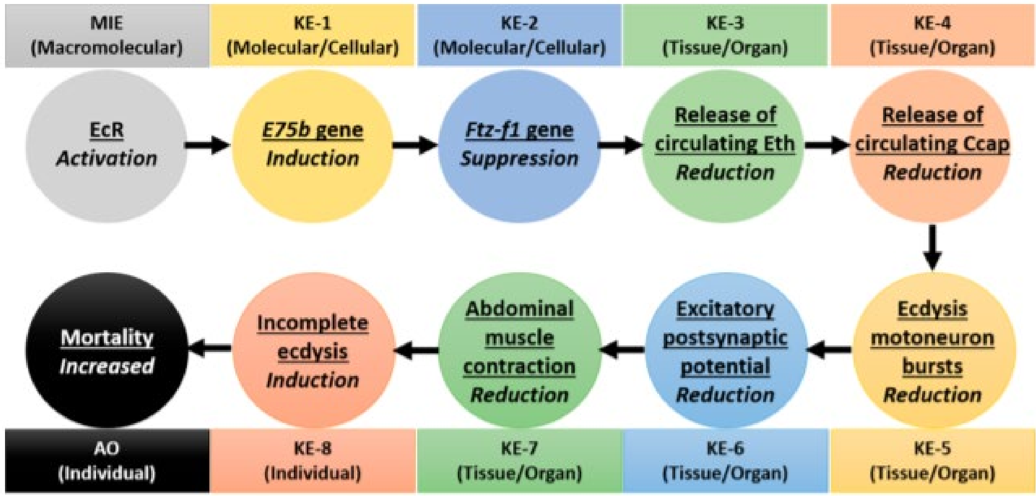
*LaLone et al., 2017. STOTEN 584-585, 751-775*




**AOP development**

*Apis mellifera (*European honey bee)
*Apis cerana* (Asian honey bee)
*Bombus terrestris* (Buff-tailed bumblebee)

*Song et al., 2017. Environ. Sci. Technol. 51, 4142−4157*




**AOP development**

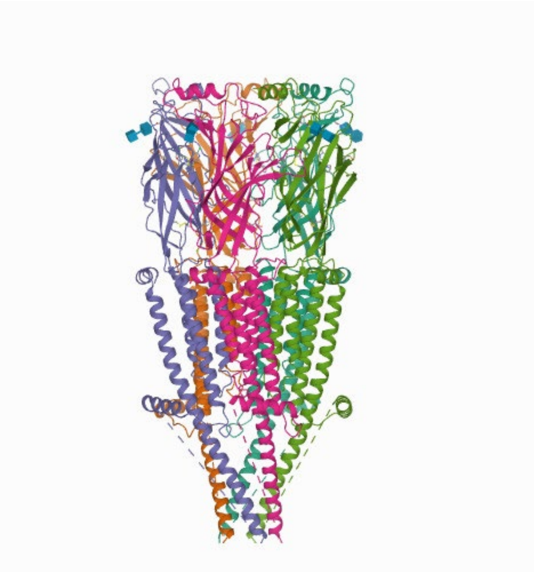
*Daphnia magna* (Water flea)
*Tribolium castaneum* (Red flour beetle)
*Bombyx mori* (Domestic silk moth)
*Blattella germanica* (German cockroach)
*Ostrinia nubilalis* (European corn borer)
*Drosophila (Fruit fly)*

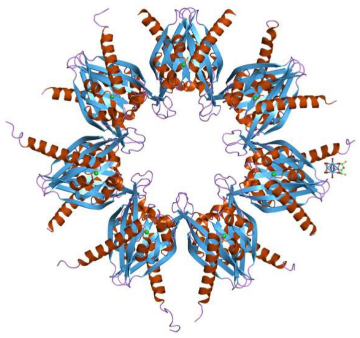
*Locusta migratoria* (migratory locus)
*Nilaparvata lugens* (brown planthopper)
*Lepeophtheirus salmonis* (Salmon louse)
*Spodoptera littoralis* (African cotton leafworm)
*Rhithropanopeus harrisii* (Harris mud crab)
*Choristoneura fumiferana* (Eastern spruce budworm)

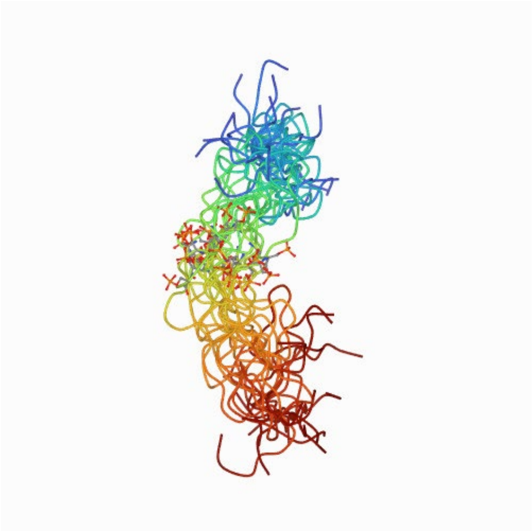
Define the taxonomic domain of applicability – How broadly could we anticipate extrapolating AOP information across species?

# Enhance the definition of taxonomic domain of applicability for AOPs

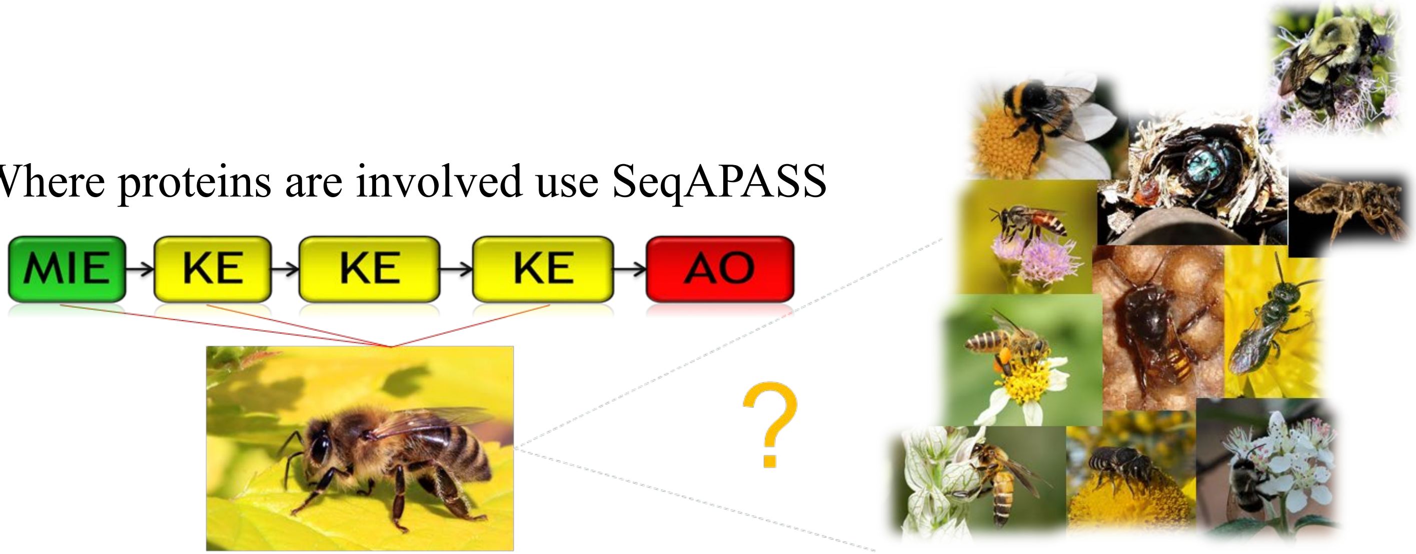
MIE → KER → KE → KER → KE







Where proteins are involved use SeqAPASS



# Bioinformatics

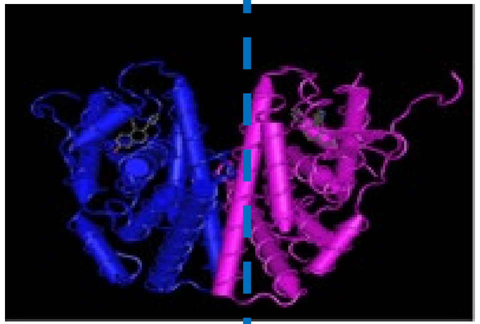
- Combines mathematics, information science, and biology to answer biological questions
- Developing methodology and analysis tools to explore large volumes of biological data
  - Query, extract, store, organize, systematize, annotate, visualize, mine, and interpret complex data
    - Usually pertains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

**Let the computers do the work**

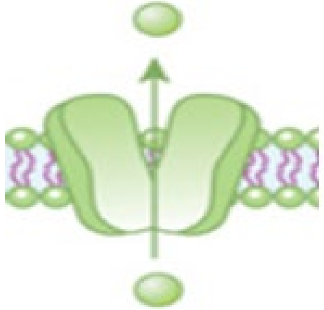

Sequence

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

Structure



Function



# Bioinformatics

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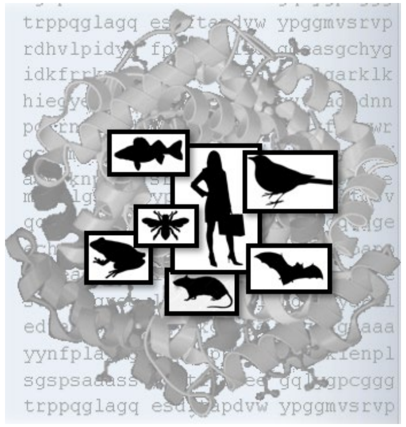
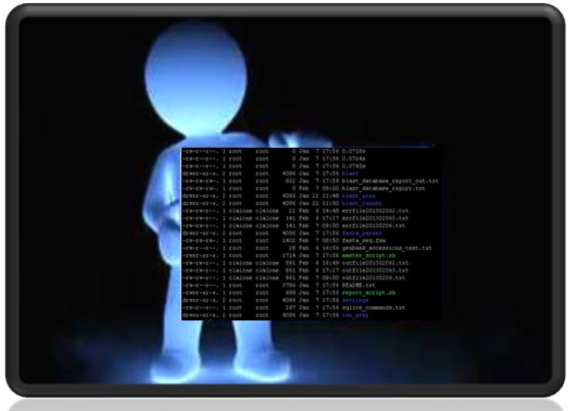


https://seqapass.epa.gov/seqapass/

# Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)




OXFORD

SOT Society of Toxicology

www.toxsci.oxfordjournals.org

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

doi: 10.1093/toxsci/kfw119

Advance Access Publication Date: June 30, 2016

Research article

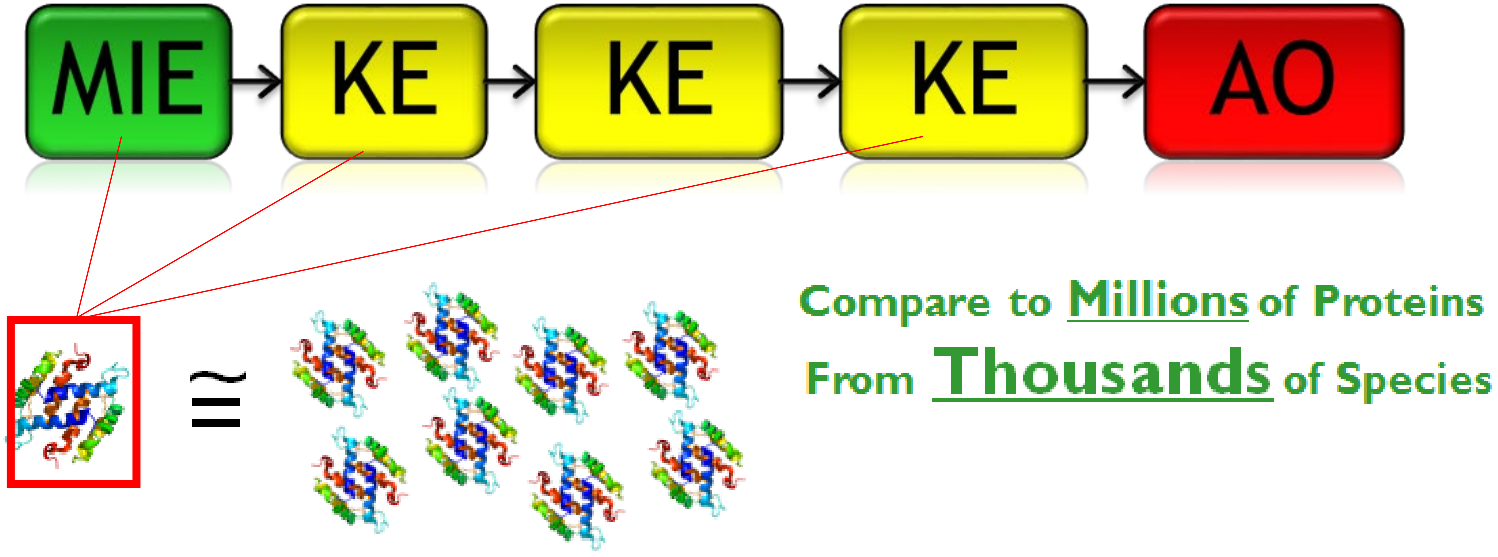

## Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS): A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity


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[*]


# Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool

Evaluation of MIE and KE conservation across species

Protein in MIE or KE for Species used to develop the AOP

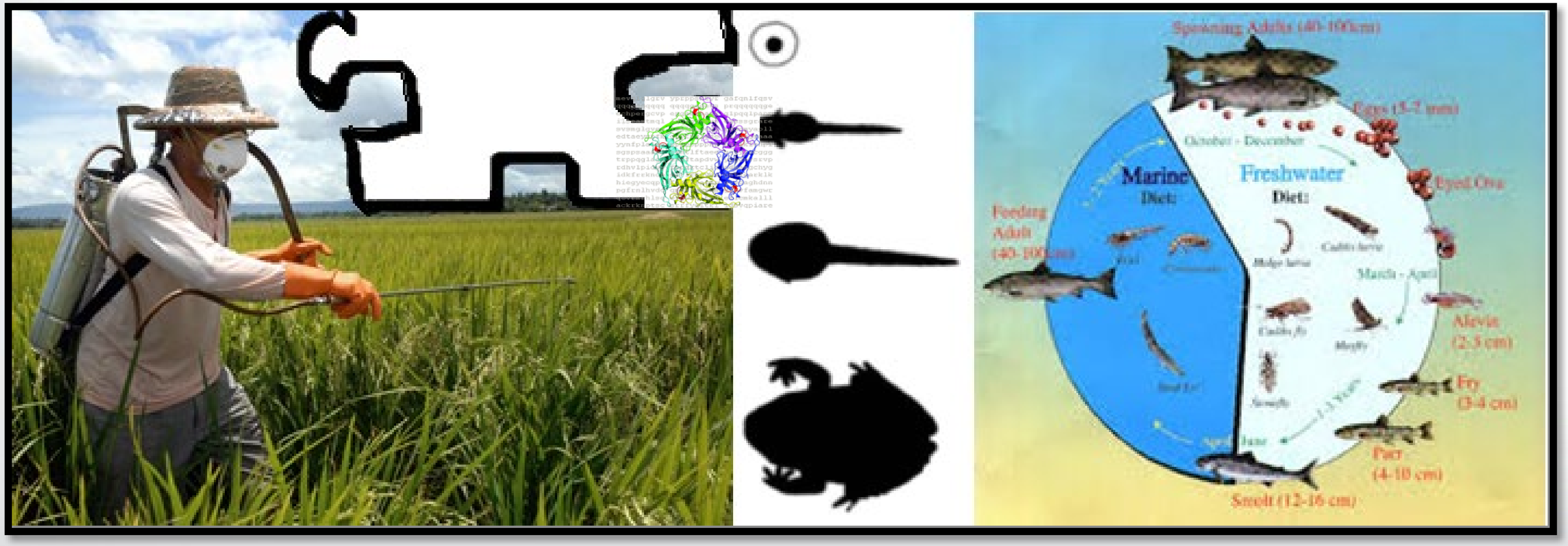



- Greater similarity = Greater likelihood that chemical can act on the protein
- Line of Evidence: Predict Potential Chemical Susceptibility Across Species
    - Receptor/enzyme available for the chemical to act upon

- Conservation of MIE and early KEs: Extrapolate across taxa

# Predict Relative Intrinsic Susceptibility

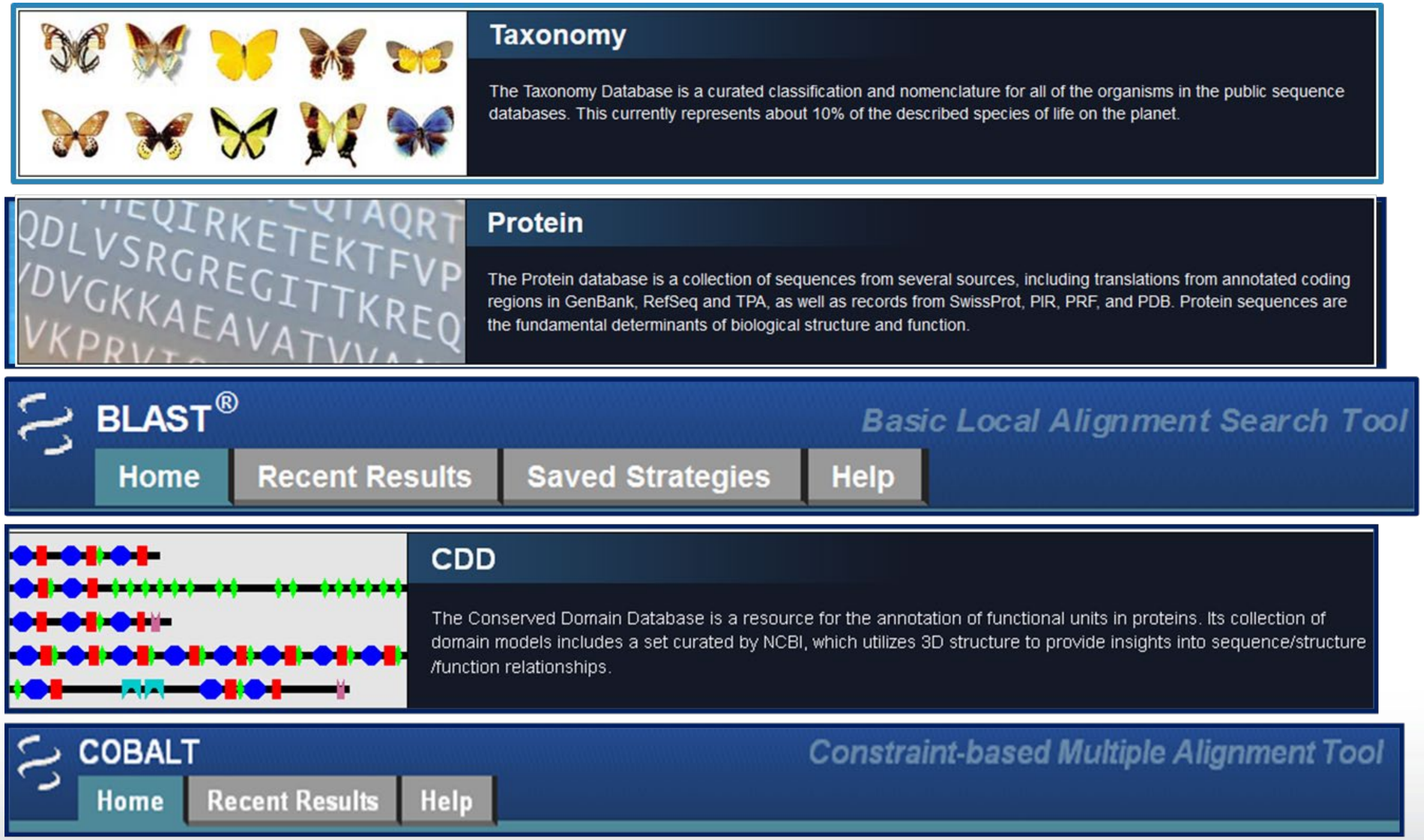
- **Intrinsic susceptibility can be defined as the vulnerability (or lack thereof) of an organism to chemical insult due to its inherent biological composition**
  - **Receptor/enzyme (protein) available for the chemical to act upon**
- **Relative: based on comparisons to a query protein**
  - **Molecular target conservation is but a component of multiple determinants of species susceptibility**




# Developing SeqAPASS:

# Available Databases and Tools

- **National Center for Biotechnology Information**
- **Established in 1988: a division of National Library of Medicine at NIH**



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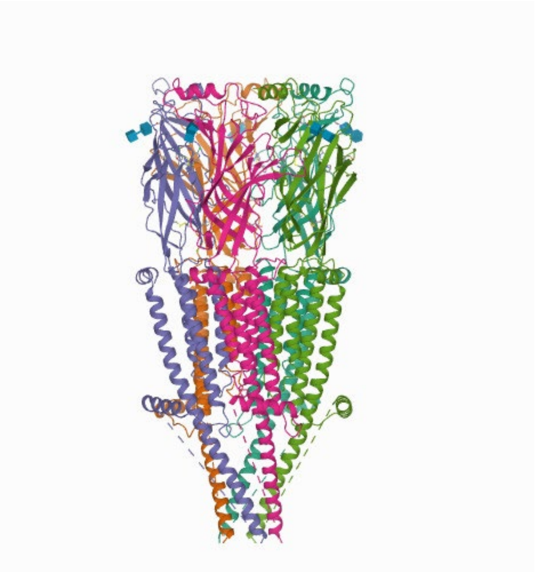
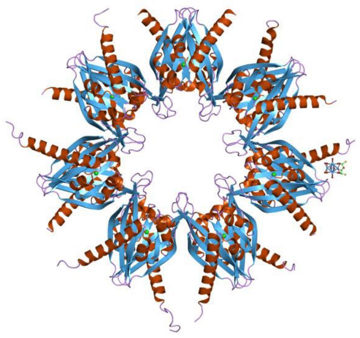
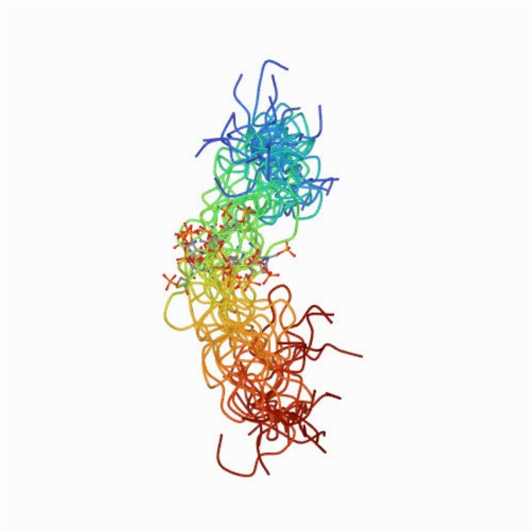
# Enhance the definition of taxonomic domain of applicability for AOPs

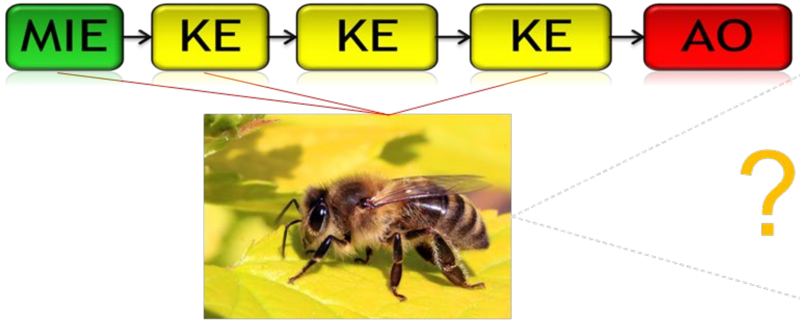
MIE → KER → KE → KER → KE







Where proteins are involved use SeqAPASS



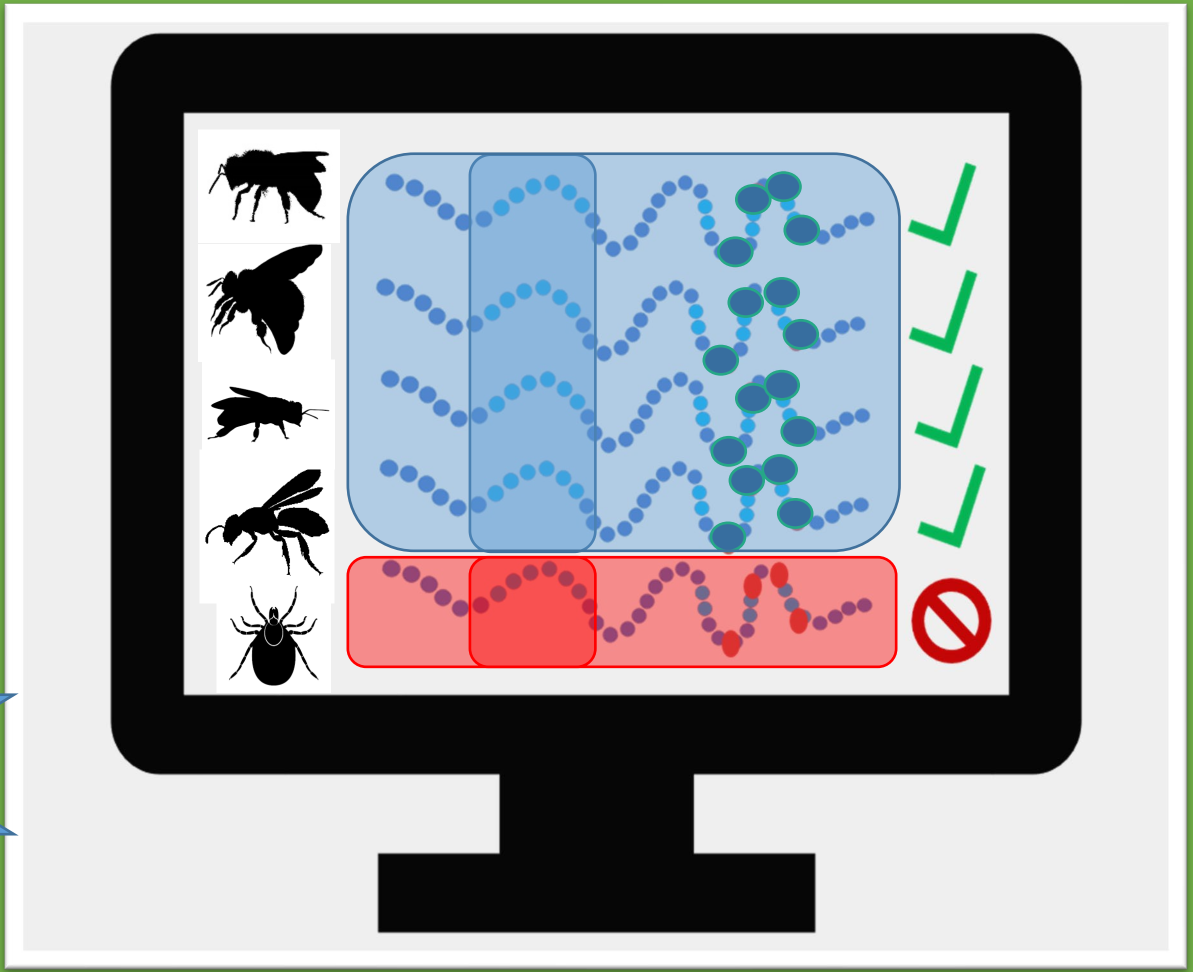

**Flexible Analysis Based On Available Data**

**Level 1** Primary Amino Acid Sequence Alignments

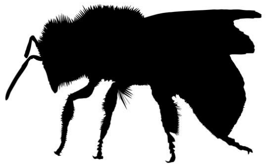
**Level 2** Conserved Functional Domain Alignments

**Level 3** Critical (Close Contact) Amino Acid Conservation

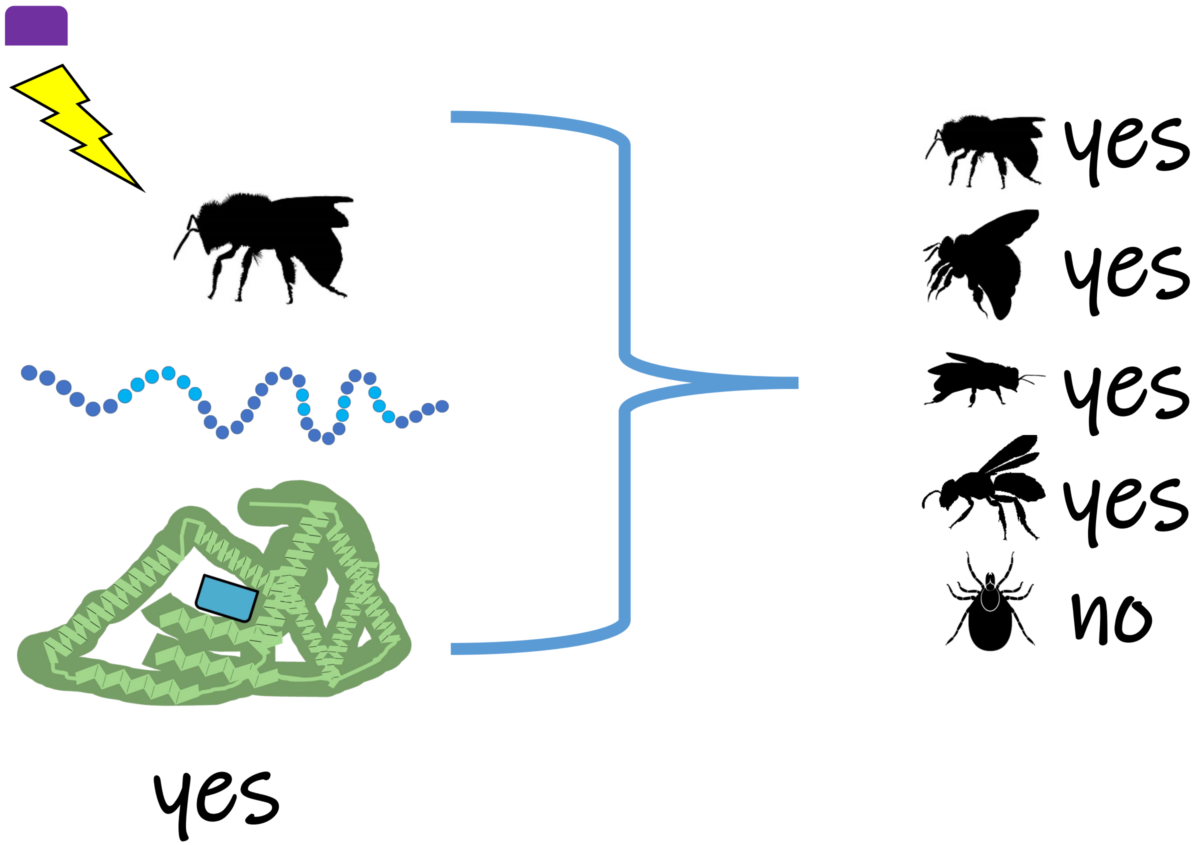
seqapass.epa.gov/seqapass/



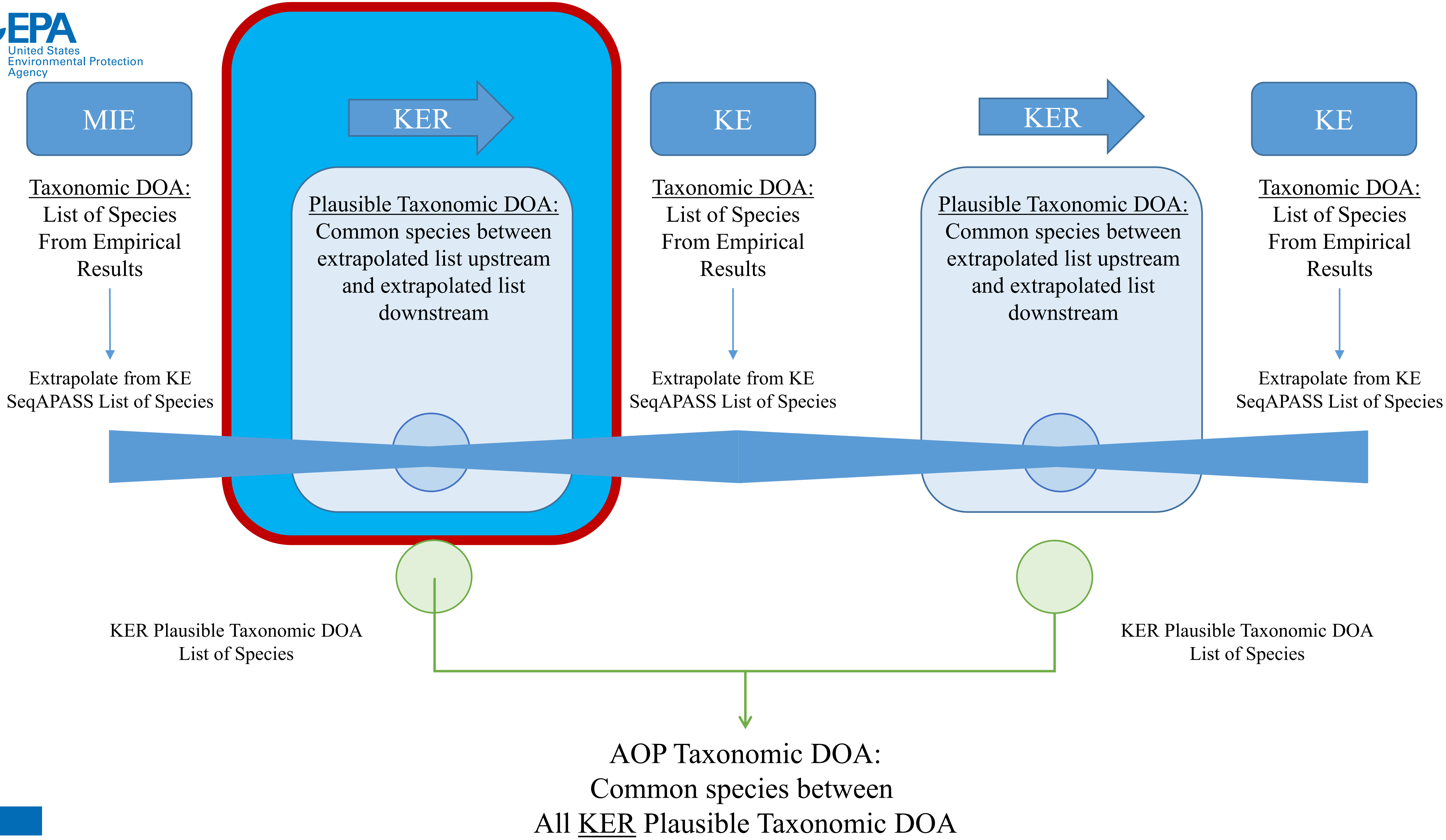
# Gather Lines of Evidence Toward Protein Conservation

*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



MIE
KER
KE
KER
KE
Taxonomic DOA:
List of Species
From Empirical
Results
Extrapolate from KE
SeqAPASS List of Species
Plausible Taxonomic DOA:
Common species between
extrapolated list upstream
and extrapolated list
downstream
Taxonomic DOA:
List of Species
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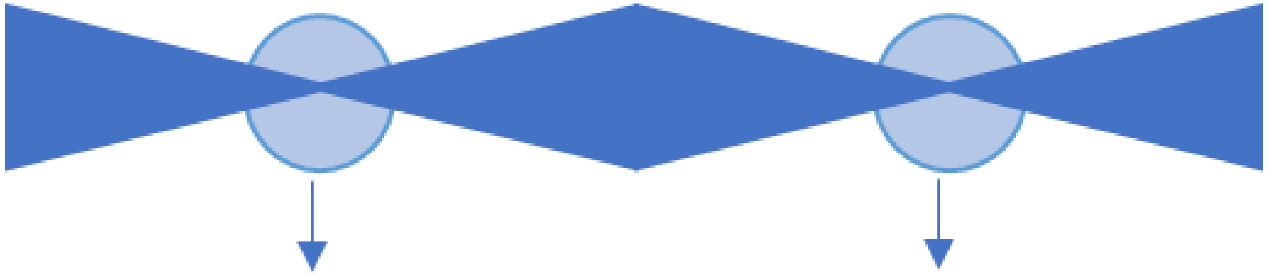
From Empirical
Results
Extrapolate from KE
SeqAPASS List of Species
KER Plausible Taxonomic DOA
List of Species
KER Plausible Taxonomic DOA
List of Species
AOP Taxonomic DOA:
Common species between
All KER Plausible Taxonomic DOA



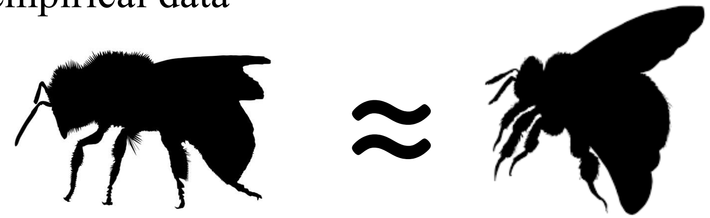
<u>Plausible KER1</u>
<u>tDOA:</u>
17 bee species

<u>Plausible KER2</u>
<u>tDOA:</u>
17 bee species

Individual tested species with empirical data

17 Untested species through extrapolation

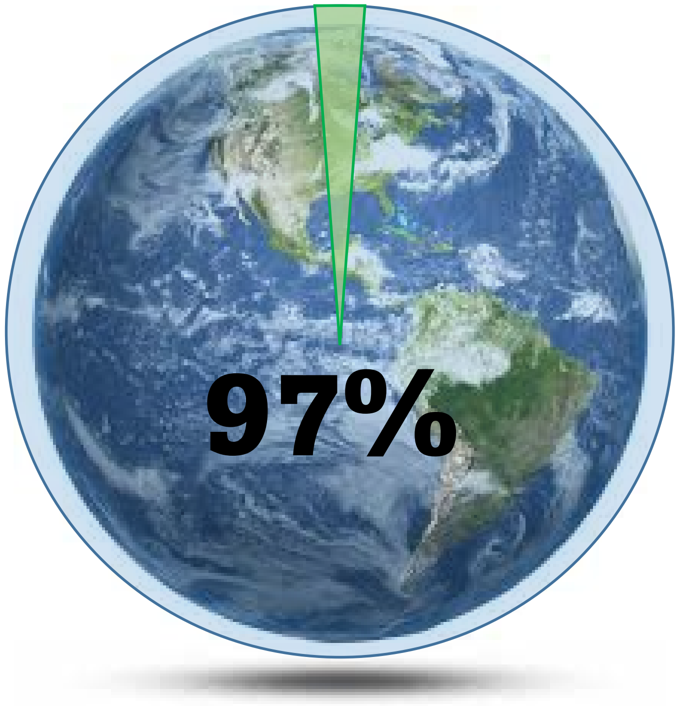
*Apis*



*Non-Apis*

Bioinformatics approaches expand the applicability of the AOP beyond the model organism

# ~97% of the animal species on Earth are invertebrates
# ~1.25 million invertebrate species are now known




Handful of invertebrate species as model organisms for understanding chemical effects


# Recommended Needs

Better understanding of unique biology of invertebrate species

Development of invertebrate relevant AOPs

More quality sequence data with better annotation among invertebrate species

United States Environmental Protection Agency

## Genomes Sequenced with Annotation

174 Insects
59 Other Invertebrates

0.02 % of all invertebrates

(https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/)

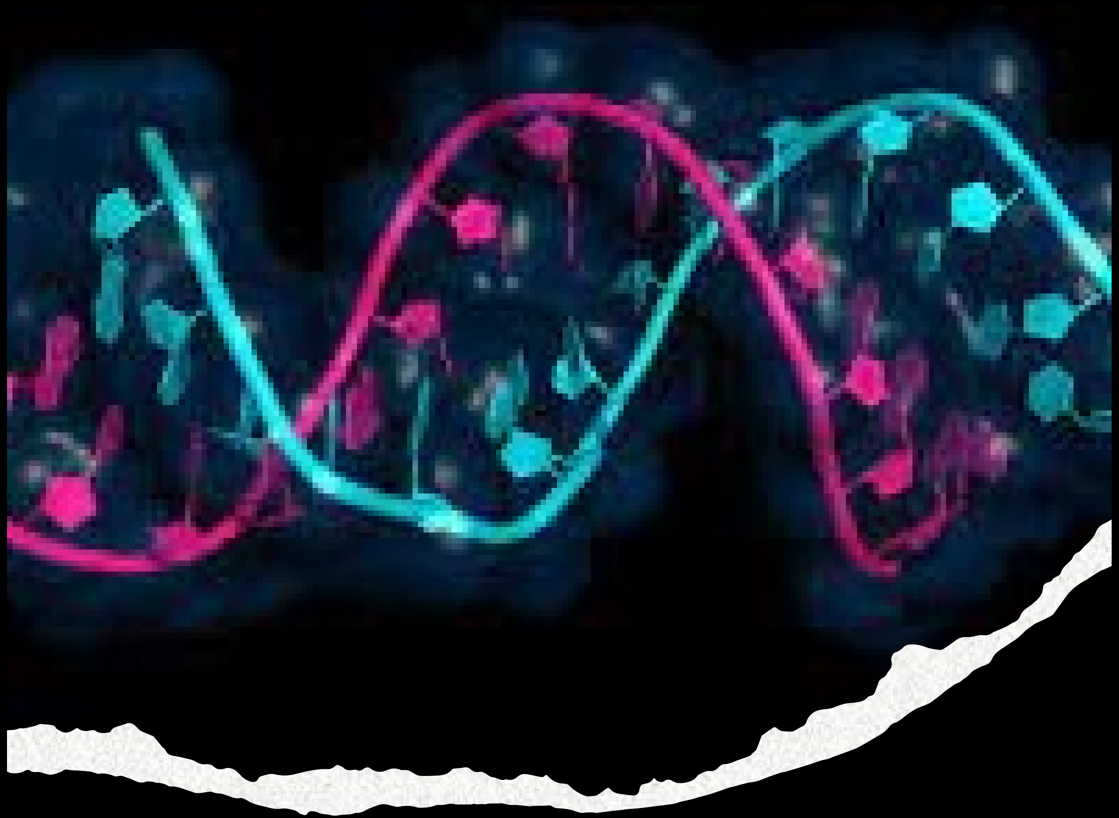
## Bee Genomes

1. Apis cerana (Asiatic honeybee)
2. Apis dorsata (giant honeybee)
3. Apis florea (little honeybee)
4. Apis mellifera (honey bee)
5. Bombus bifarius (Two Form Bumble Bee)
6. Bombus impatiens (common eastern bumble bee)
7. Bombus terrestris (buff-tailed bumblebee)
8. Bombus vancouverensis nearcticus (Nearctic Bumble Bee)
9. Bombus vosnesenskii (yellow-faced bumblebee)
10. Ceratina calcarata (Spurred Small Carpenter bee)
11. Colletes gigas (plasterer bee)
12. Dufourea novaeangliae (Pickerelweed Shortface Bee)
13. Eufriesea mexicana (Orchid bee)
14. Habropoda laboriosa (Southeastern blueberry bee)
15. Megachile rotundata (alfalfa leafcutting bee)
16. Megalopta genalis ( Sweet bee)
17. Nomia melanderi (Alkali bee)
18. Osmia bicornis bicornis (red mason bee)
19. Osmia lignaria (orchard mason bee)

## Butterfly Genomes

1. Danaus plexippus plexippus (monarch butterfly)
2. Papilio machaon (common yellow swallowtail)
3. Papilio polytes (common Mormon)
4. Papilio xuthus (Asian swallowtail)
5. Pararge aegeria (specked wood butterfly)
6. Pieris rapae (cabbage white)
7. Vanessa tameamea (butterflies)
8. Zerene cesonia (dogface butterfly)

# High-throughput transcriptomics



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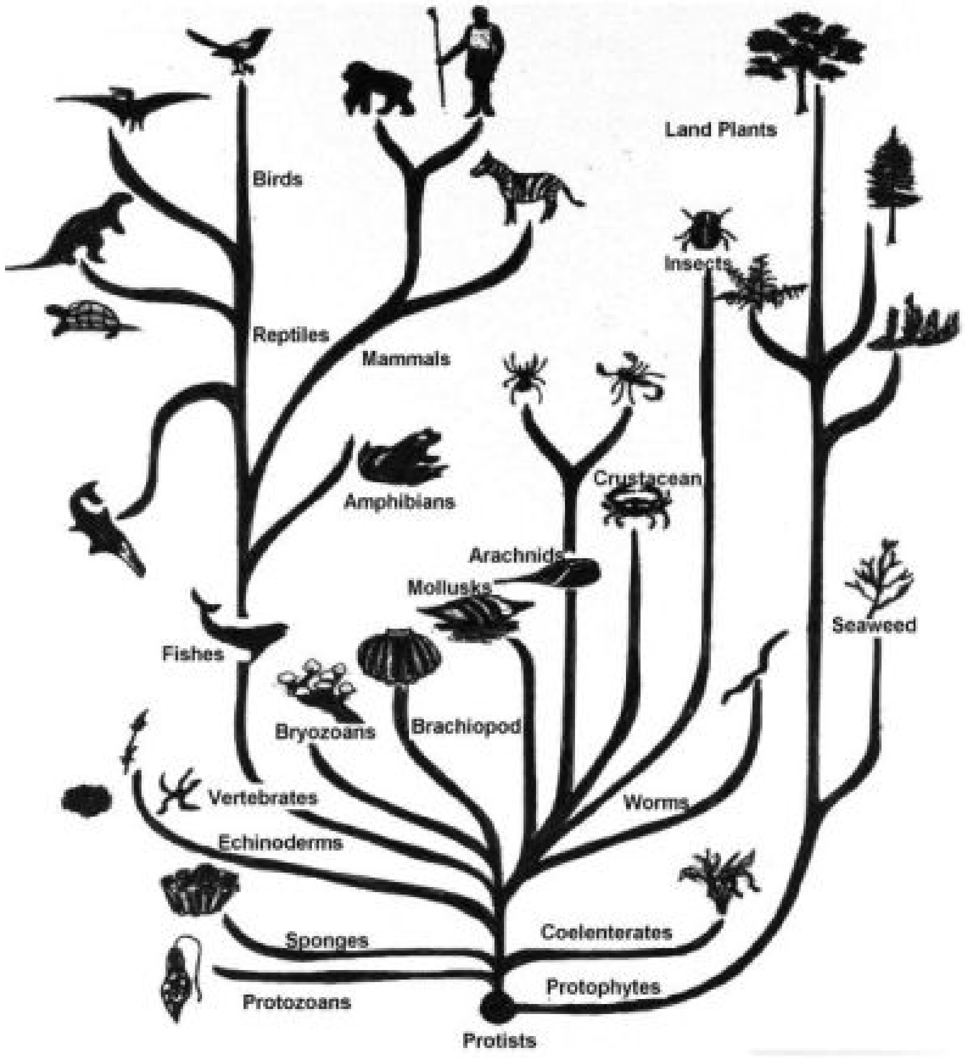
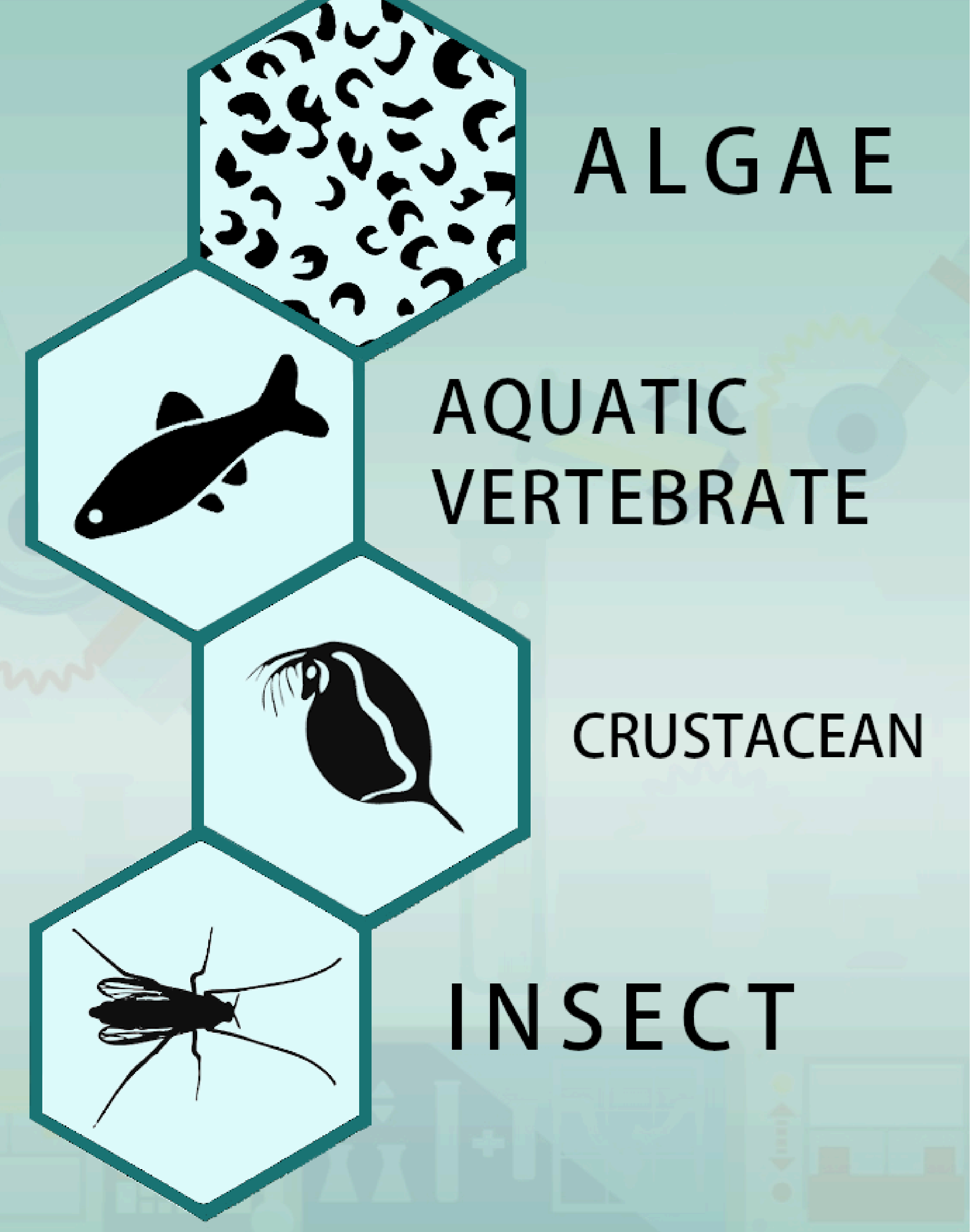
# Ecotoxicology Perspective




- 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?



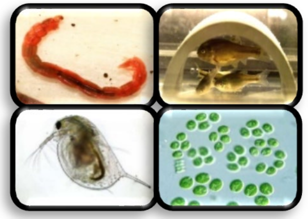

High throughput assays for three major trophic levels of aquatic ecosystems

- 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

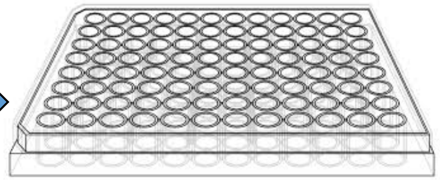
Aquatic organisms highly vulnerable to exposure

# Incorporating transcriptomics as assessment endpoint



Load organisms

24 h exposure

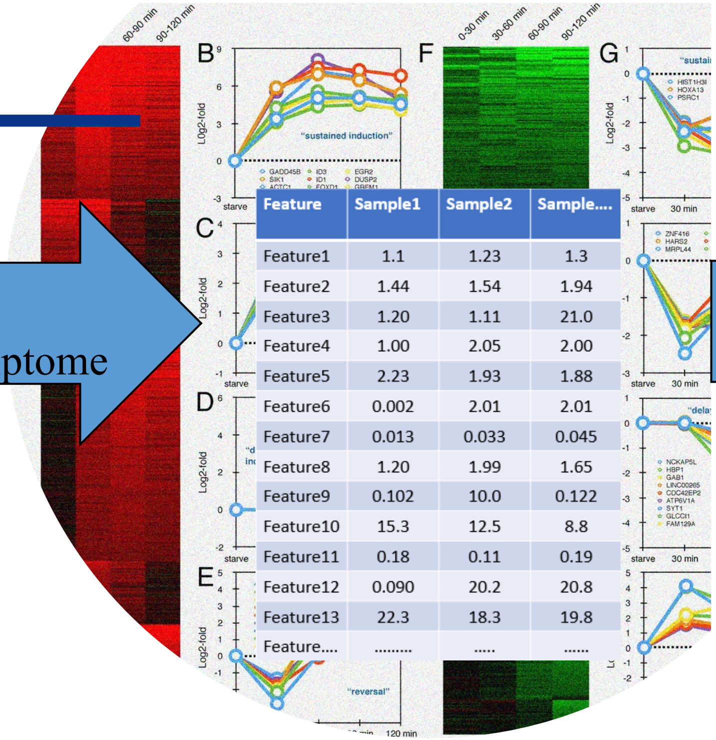


Phenotypic anchoring

Homogenate /extract

**RNA**

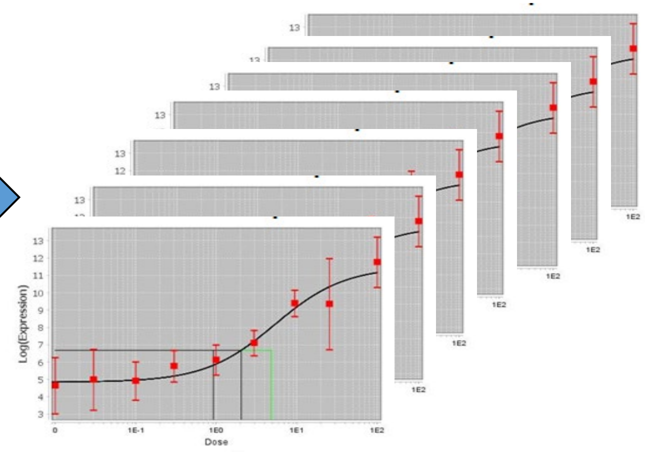
Whole transcriptome




| 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.... | ......... | ..... | ...... |

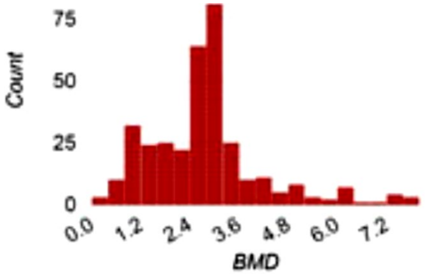
Curv

ve fitting




BMD Express

Distribution of BMDs




10th centile BMD

Point of departure

[tPOD]

Rough estimate per chemical ~$5K for one species

# 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

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SeqAPASS v6.0 (Released Sept. 2021)



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