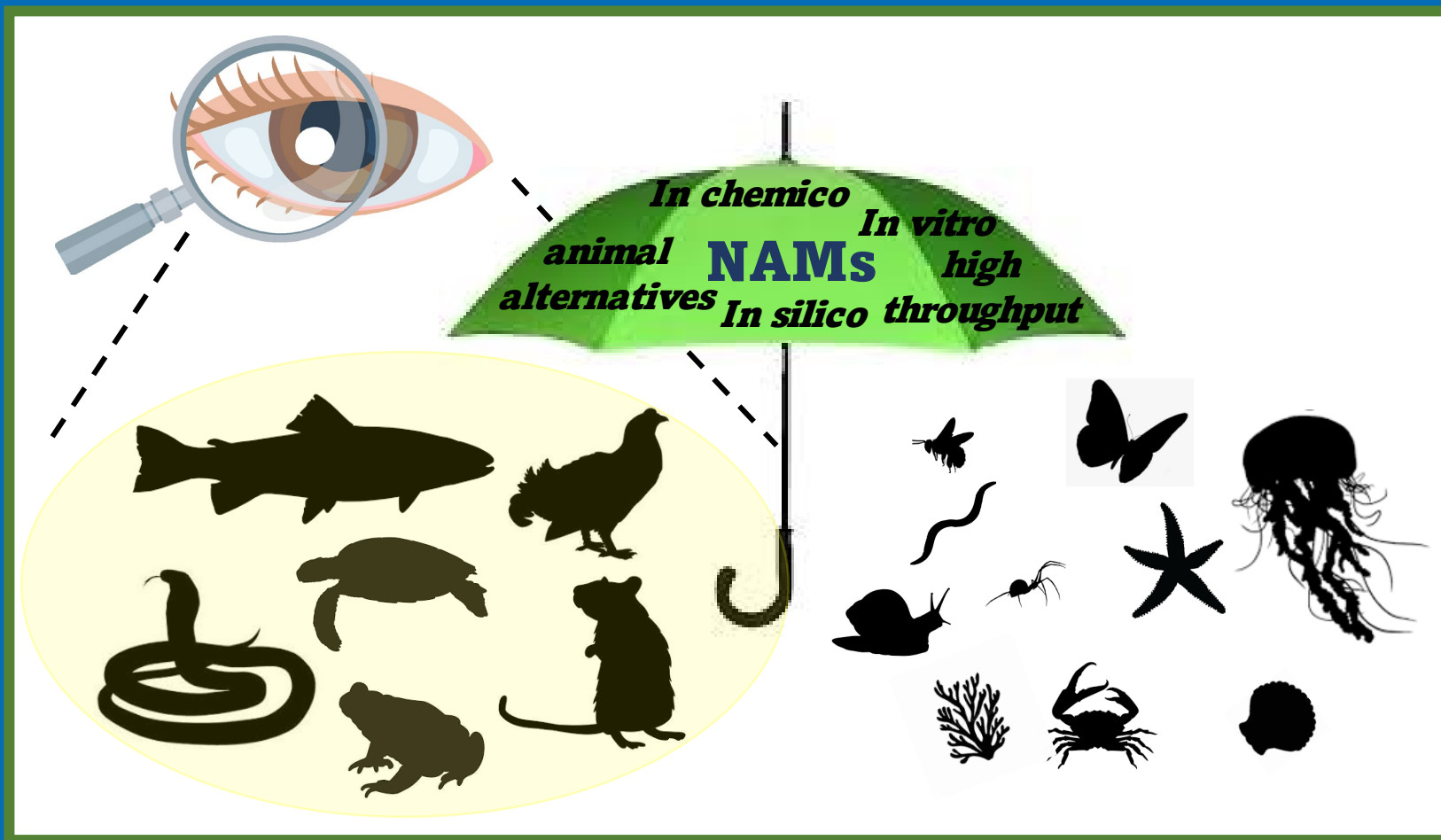


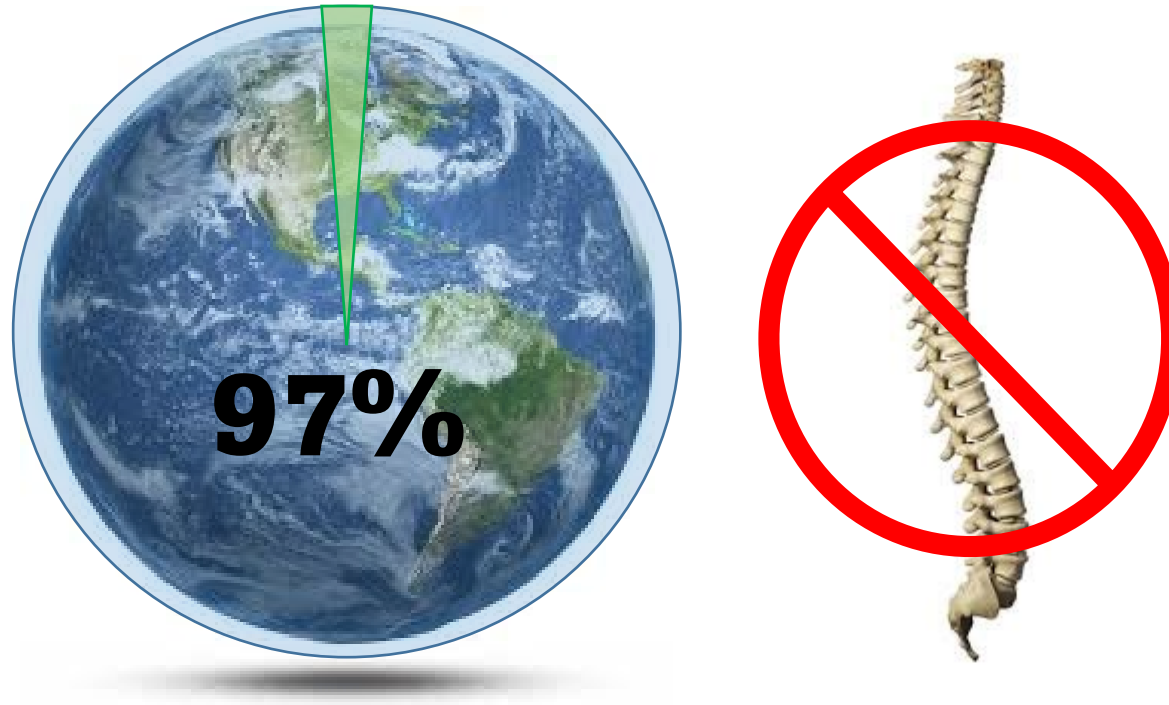
Application of New Approach Methods for Invertebrate Risk Assessment



Presenter: Carlie A. LaLone, Ph.D.



~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



cheap and readily available



short lifespans and rapid life cycles



ability to control diet and surroundings



easy maintenance and good breeding capabilities



requires least space and time-consuming care



Transformation of Toxicity Testing

Historically:

Whole animal test

- Observe Toxic Outcome
 - Examples
 - mortality
- Resource intensive

Approximate Costs to Conduct EPA-required Tests

Testing a new pesticide can be costly. Here are some estimated costs for the bee risk assessment studies the EPA requires.

A lab study — \$30,000 to \$50,000

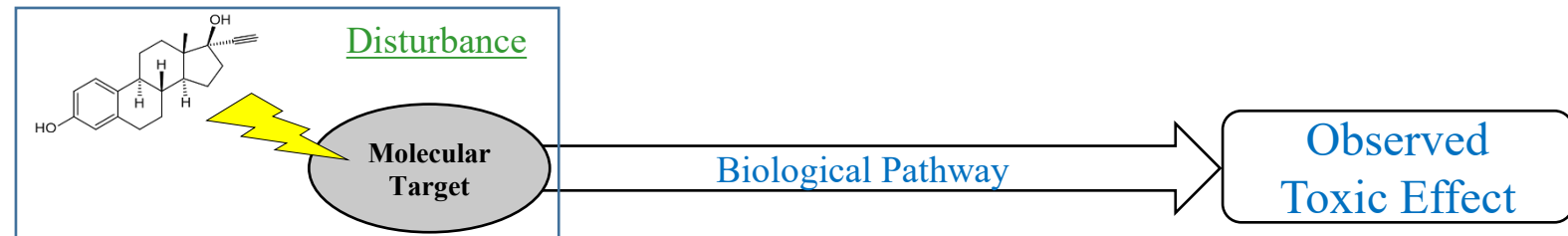
A semi-field study — \$75,000 to \$150,000

A full field study — \$1.2 million



Toxicity Testing in the 21st Century:

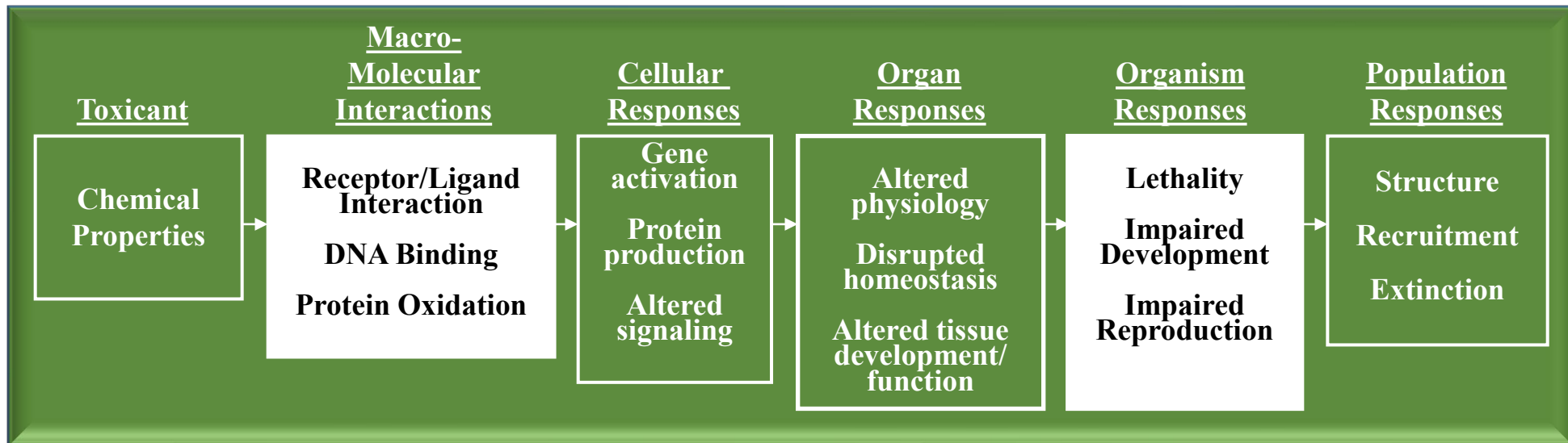
- *In vitro* and *in silico* methods
 - Pathway-based approaches
 - Focus on disturbance of the biological pathway
 - Predictive of the observable toxic effects



Enabled by evolution of the
science and technology

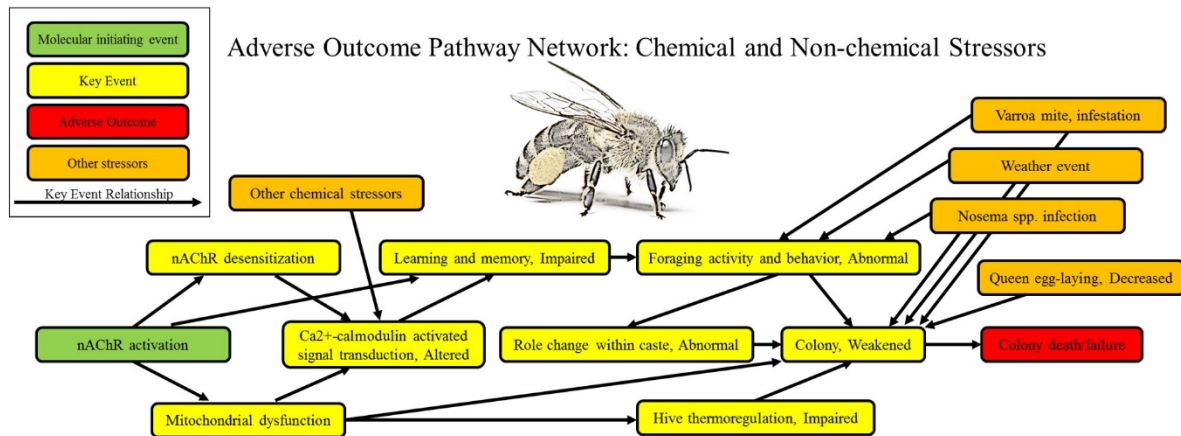
The Adverse Outcome Pathway Framework

Take advantage of existing toxicity data to inform Risk Assessment and understand knowledge gaps



The Adverse Outcome Pathway Framework: Application beyond the model organisms

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



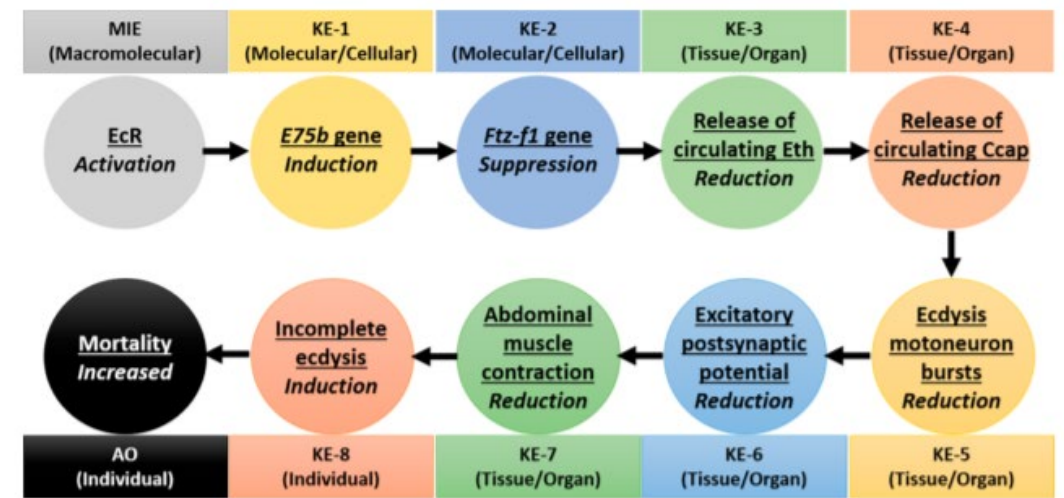
AOP development

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 locust)

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

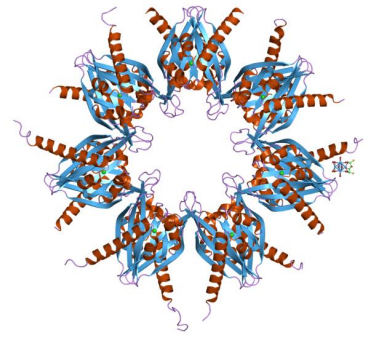
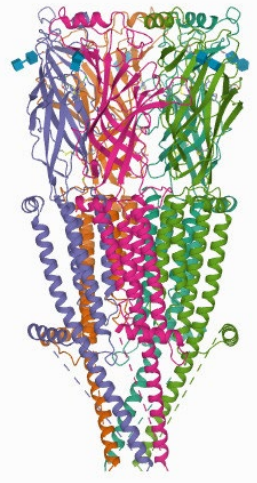
MIE

KER →

KE

KER →

KE

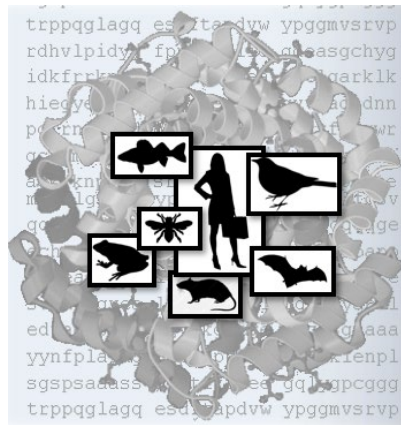


Where proteins are involved use SeqAPASS



?



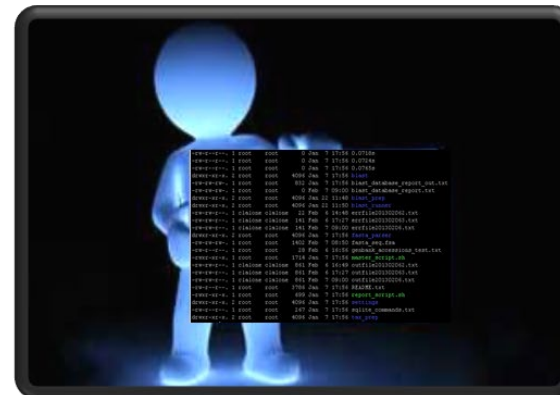
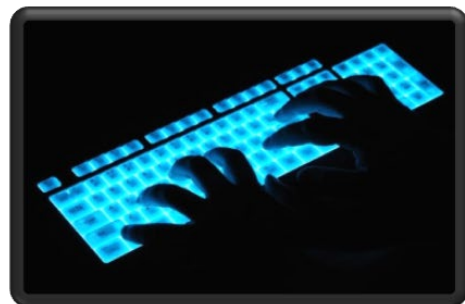


Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS): A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity

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^{*}

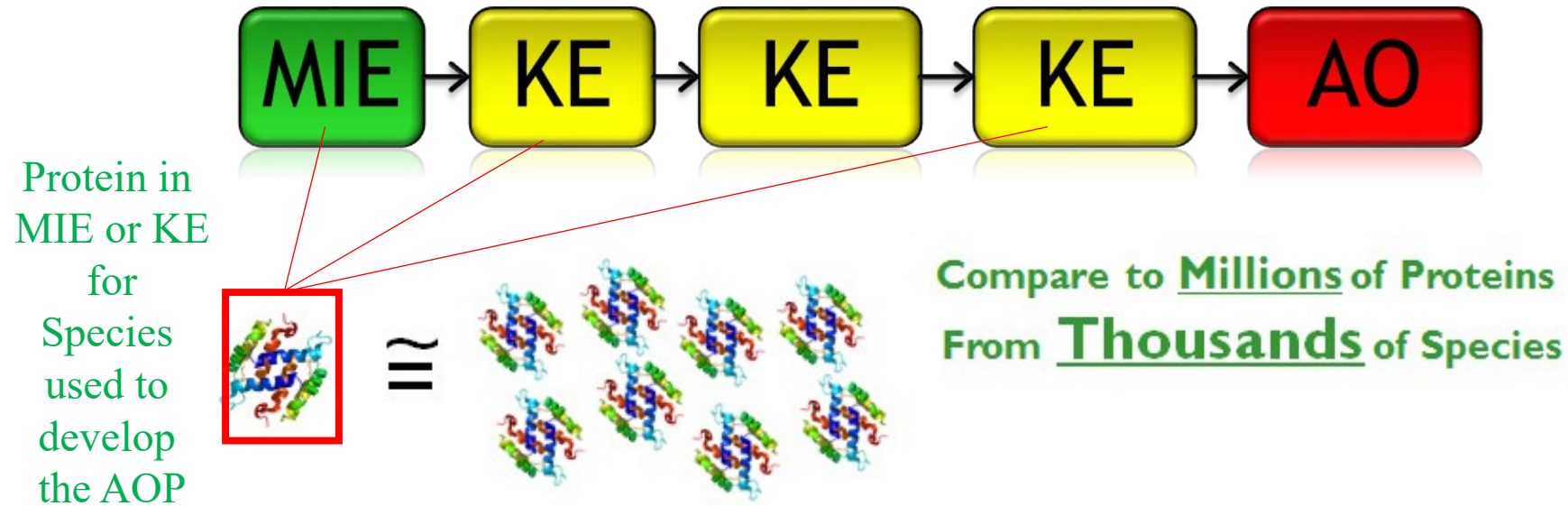
Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

<https://seqapass.epa.gov/seqapass/>



Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool

Evaluation of MIE and KE conservation across species

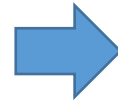


- Greater similarity = Greater likelihood that chemical can act on the protein
- Line of Evidence: Predict Potential Chemical Susceptibility Across Species
 - Receptor/enzyme available for the chemical to act upon
- Conservation of MIE and early KEs: Extrapolate across taxa

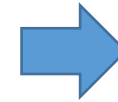
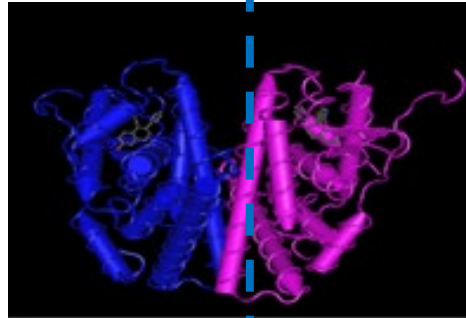


Sequence

```
MTMTLHTKASGMALLHQIQGNELEPLNRPQLKIPLERPLGE  
VYLDSSKPAVYNYPEGAAYEFNAAAAANAQVYGQTGLPYG  
PGSEAAAFGSNGLGGFPPLNSVSPSPLMLLHPPQLSPFLQ  
PHGQQVPYYLENEPSGYTVREAGPPAFYRPNSDNRRQGGR  
ERLASTNDKGSMAKESAKETRYCAVCNDYASGYHYGVWSC  
EGCKAFFKRSIQGHNDYMCNATNQCTIDKNRRKSCQACRLR  
KCYEVGMMKGGIRKDRRGGRMLKHKRQRDDGEGRGEVG  
SAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLA  
EPPILYSEYDPTRPFSEASMMGLLTNLADRELHMINWAKV  
PGFVDLTLDQVHLLCAWLEILMIGLVWRSMHEHPGKLLFA  
PNLLDRNQGKCEGMVEIFDMLLATSSRFMMNLQGEF  
VCLKSILLNSGVYFLSSTLKSLEEKDHIHRVLDKITDTLIHLM
```



Structure



Function

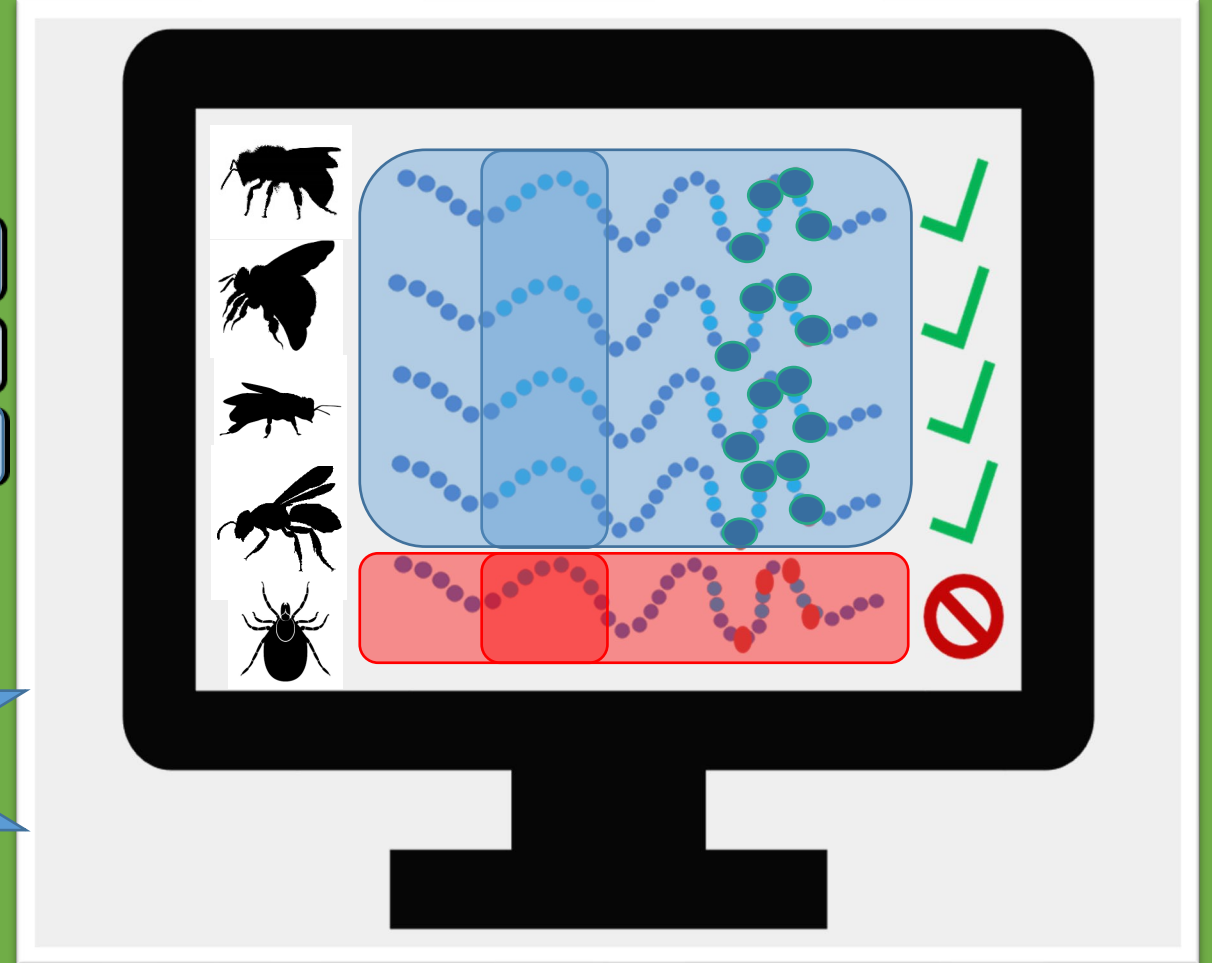


Bioinformatics

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

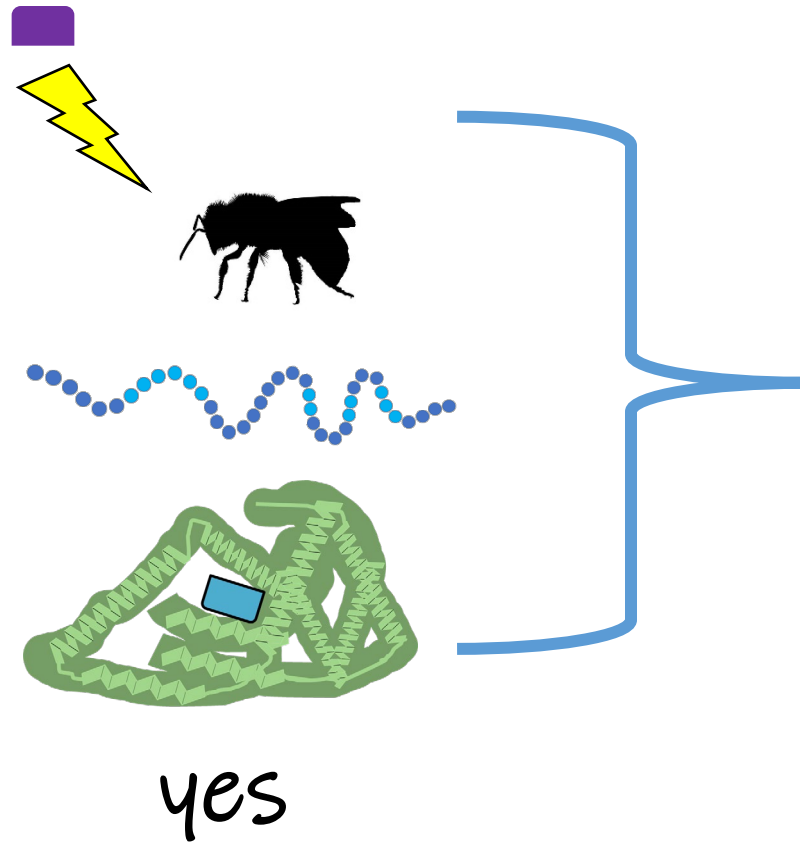
seqapass.epa.gov/seqapass/



Gather Lines of Evidence Toward Protein Conservation



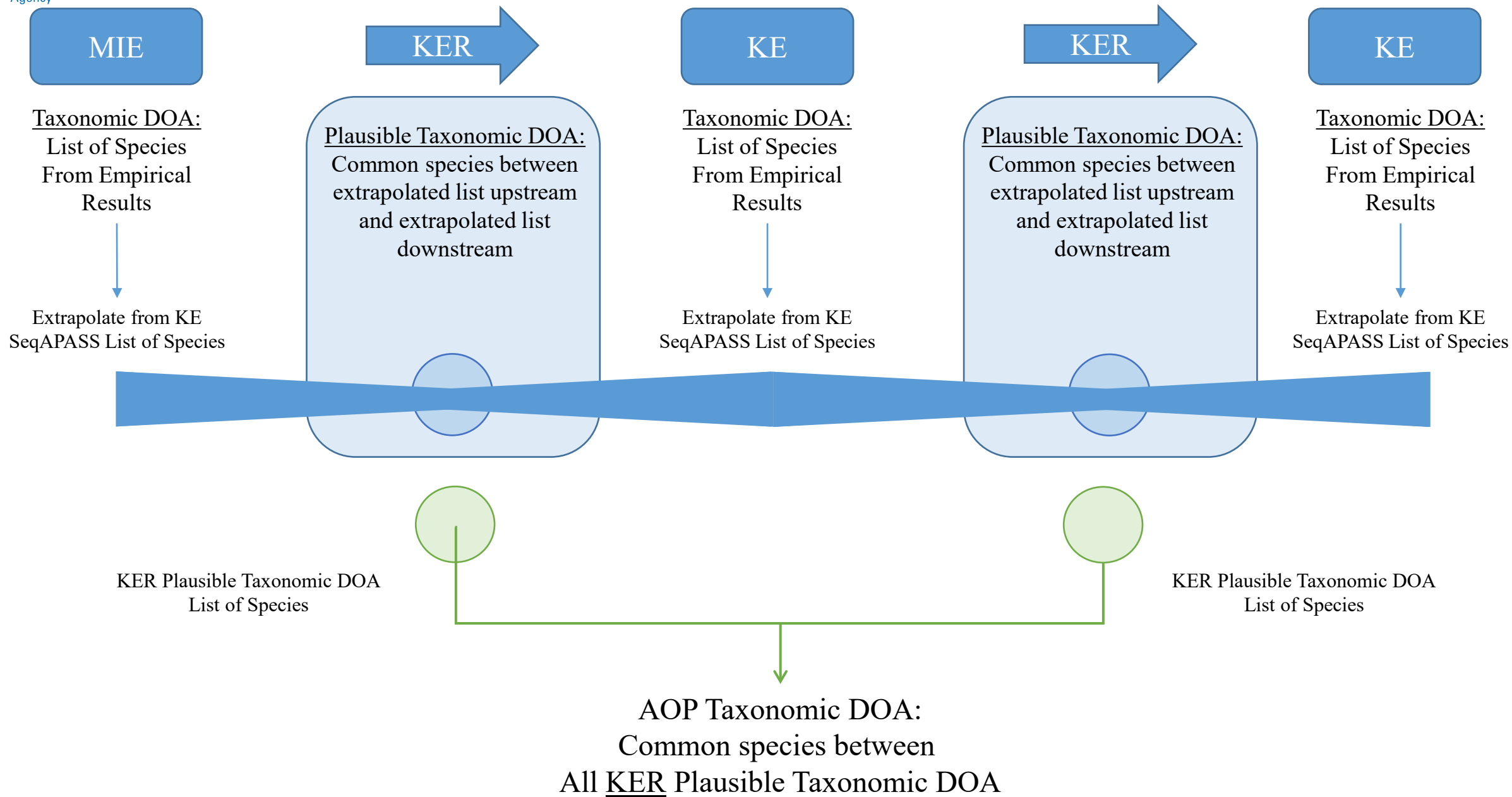
SeqAPASS Predicts Likelihood of Similar Susceptibility based on Sequence Conservation:



	yes
	yes
	yes
	yes
	no

Line(s) of evidence indicate

- The protein is conserved
- The protein is NOT conserved





Recommended Needs

More quality sequence data with better annotation

Better understanding of unique biology of invertebrate species

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

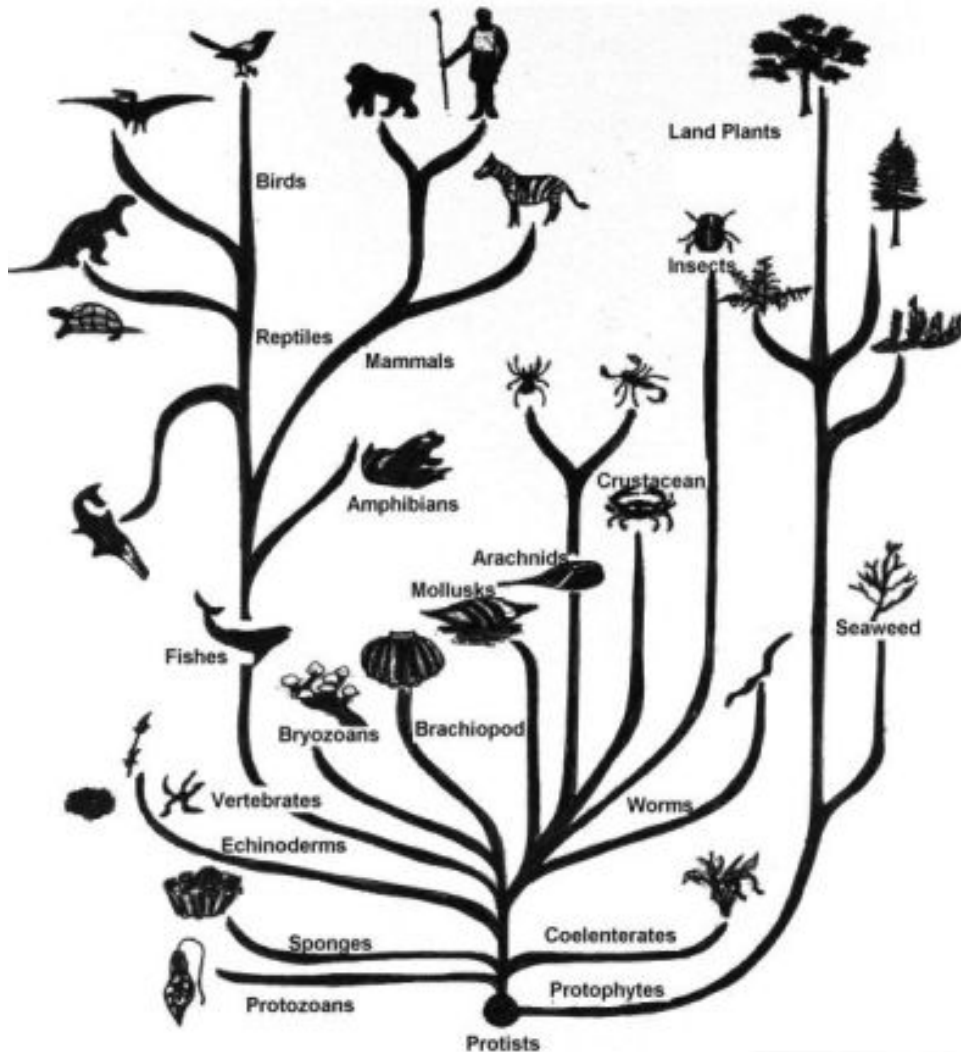
1. *Apis cerana* (Asiatic honeybee)
2. *Apis dorsata* (giant honeybee)
3. *Apis florea* (little honeybee)
4. *Apis mellifera* (honey bee)
5. *Bombus bifarius* (Two Form Bumble Bee)
6. *Bombus impatiens* (common eastern bumble bee)
7. *Bombus terrestris* (buff-tailed bumblebee)
8. *Bombus vancouverensis 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

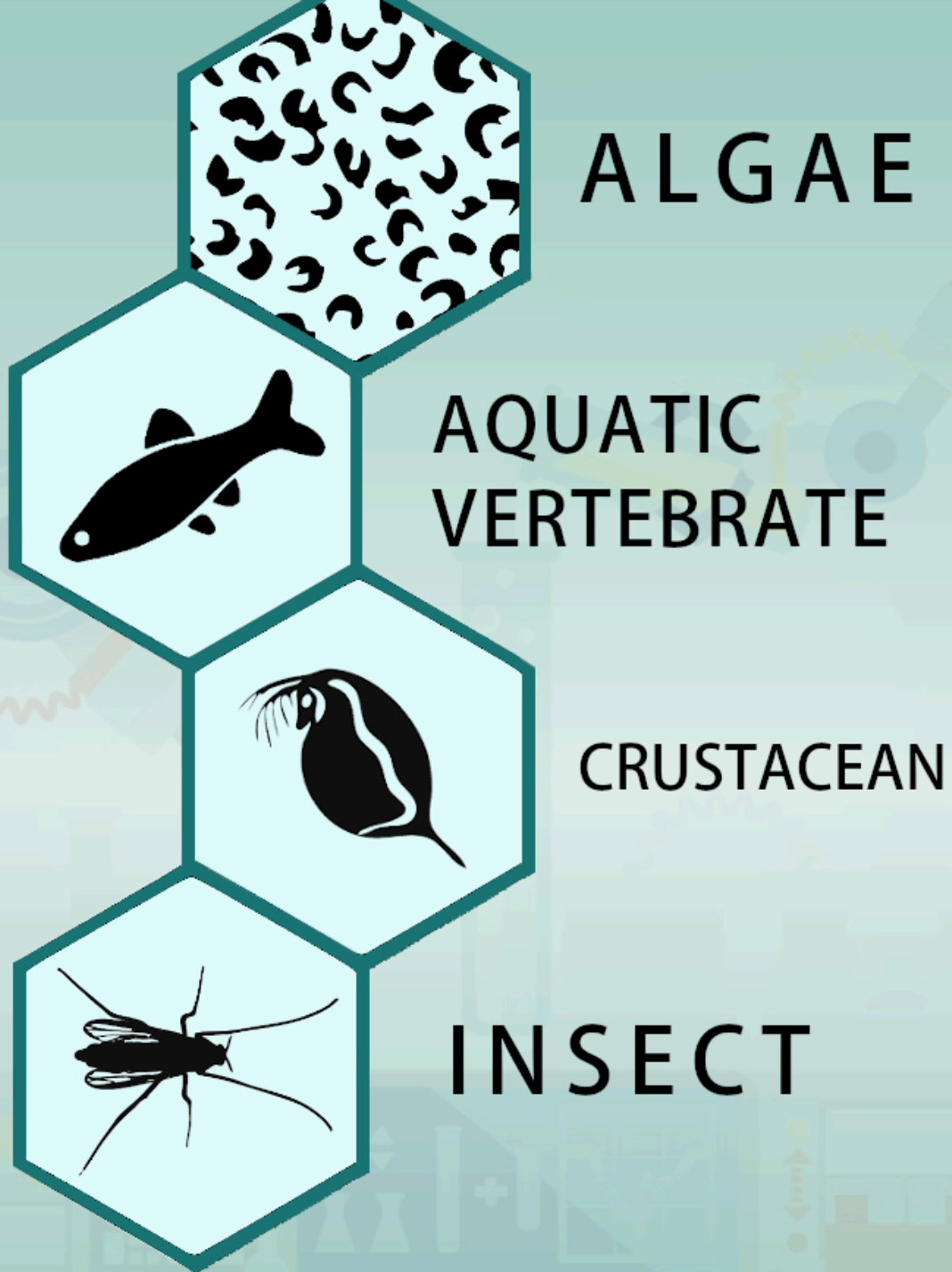
1. *Danaus plexippus plexippus* (monarch butterfly)
2. *Papilio machaon* (common yellow swallowtail)
3. *Papilio polytes* (common Mormon)
4. *Papilio xuthus* (Asian swallowtail)
5. *Pararge aegeria* (specked wood butterfly)
6. *Pieris rapae* (cabbage white)
7. *Vanessa tameamea* (butterflies)
8. *Zerene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



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?



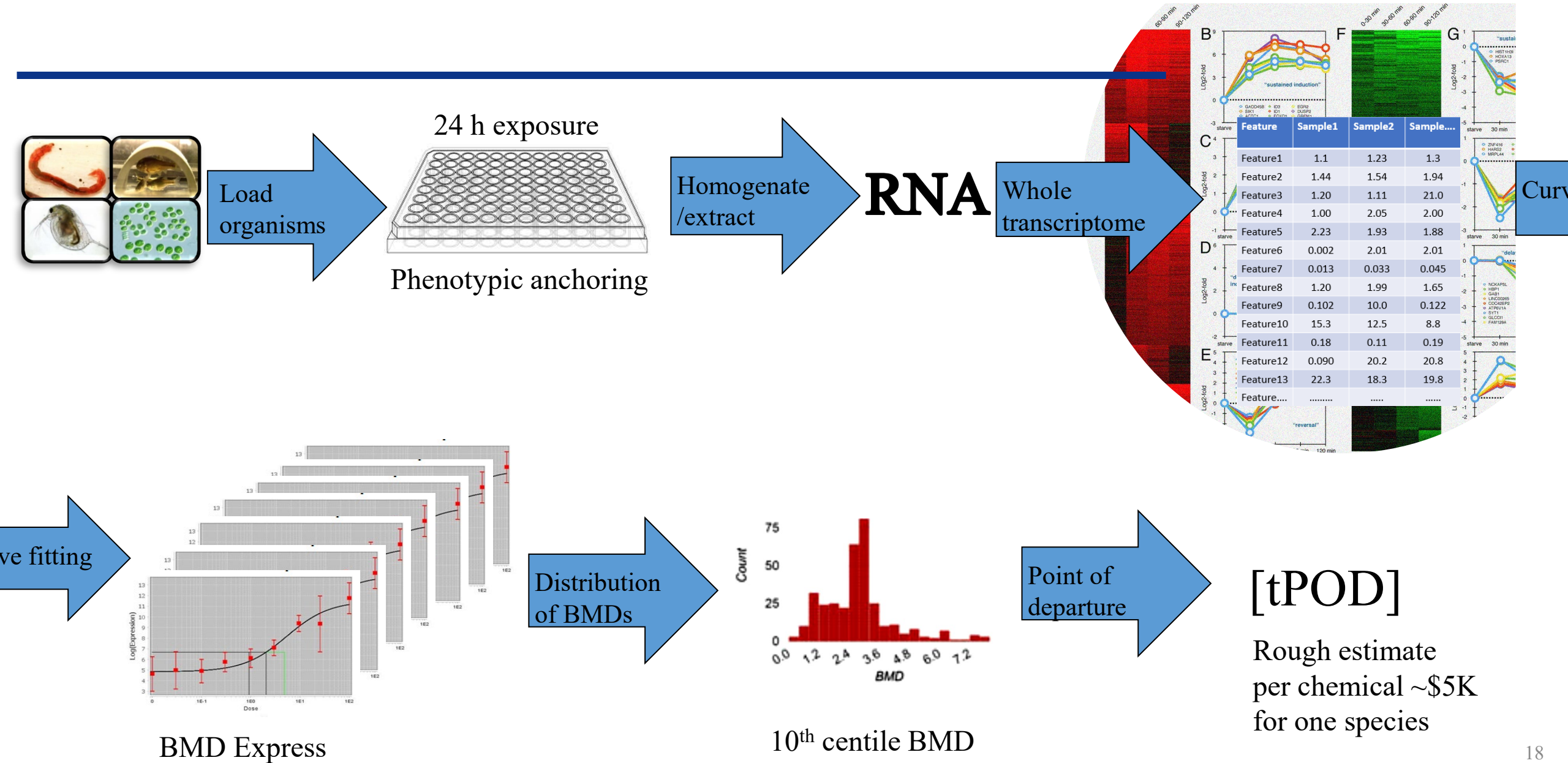
High throughput assays for three major trophic levels of aquatic ecosystems

- 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



Acknowledgements

U.S. EPA, ORD

Marissa Jensen (University of Minnesota Duluth)

Sally Mayasich (University of Wisconsin)

Donovan Blatz (ORISE)

Monique Hazemi (ORISE)

Sara Vliet (US EPA)

Jon Doering (U of Lethbridge)

Colin Finnegan (Iowa State University)

GDIT

Thomas Transue

Cody Simmons

Audrey Wilkinson

Wilson Menendez

SeqAPASS v6.0 (Released Sept. 2021)



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<https://seqapass.epa.gov/seqapass/>