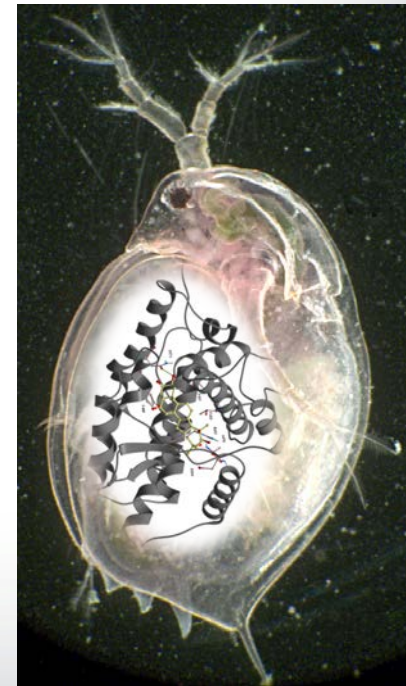# Cross-species Extrapolation of an Adverse Outcome Pathway for Ecdysteroid Receptor Activation

Carlie A. LaLone
University of Minnesota
Water Resources Center
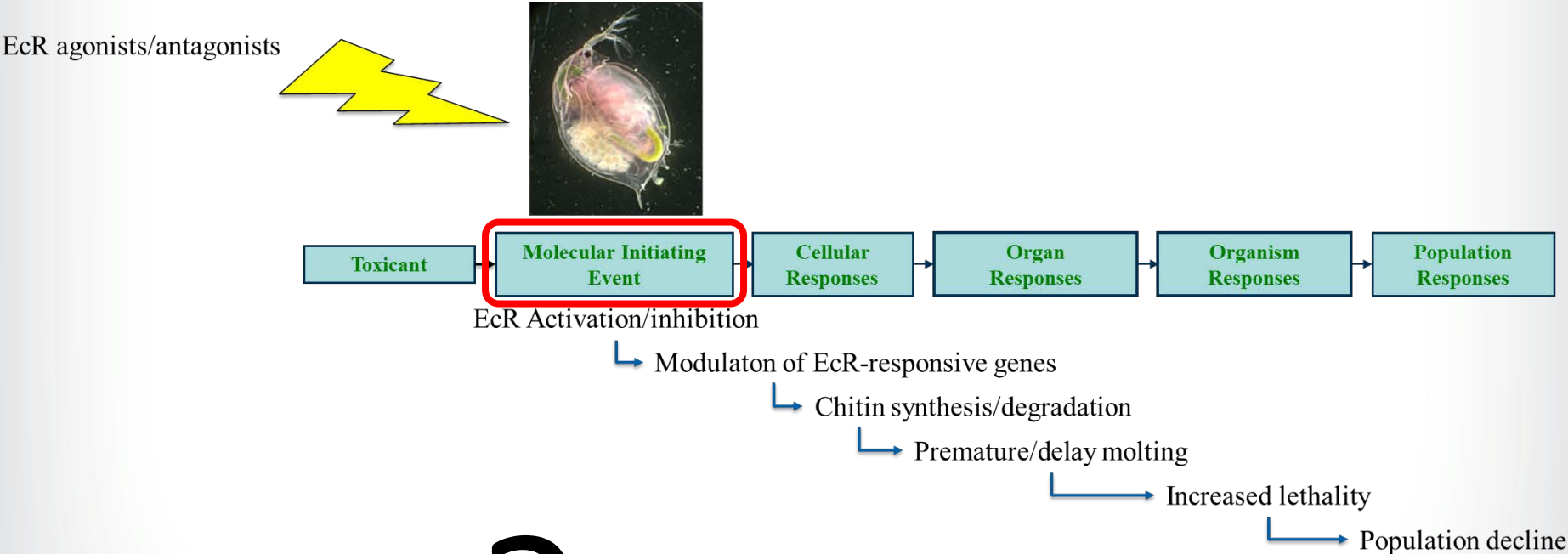
- **Adverse outcome pathway development – Ecdysteroid receptor (EcR) activation leading to mortality**

EcR agonists/antagonists

| Toxicant | Molecular Initiating Event | Cellular Responses | Organ Responses | Organism Responses | Population Responses |

EcR Activation/inhibition
↳ Modulaton of EcR-responsive genes
   ↳ Chitin synthesis/degradation
      ↳ Premature/delay molting
         ↳ Increased lethality
            ↳ Population decline

?

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

Query Species Protein

=

Multiple Hit Species/Proteins with Sequence/Structural Similarity
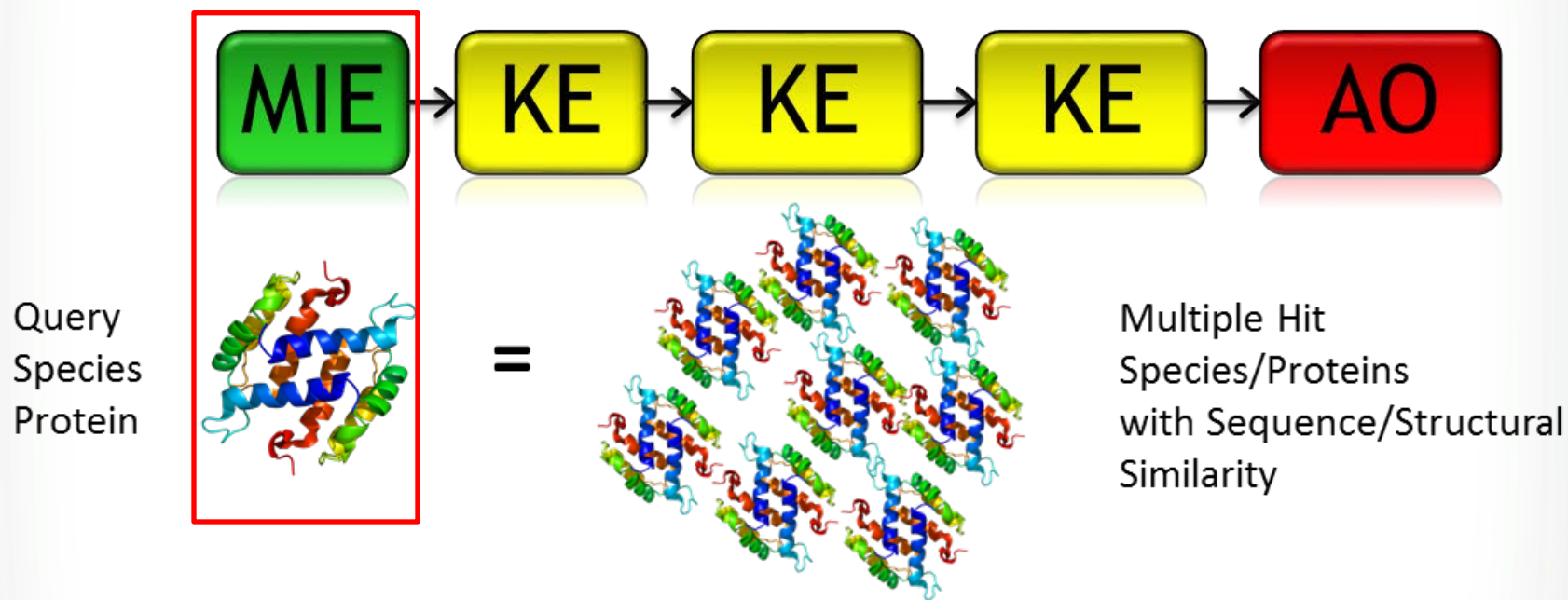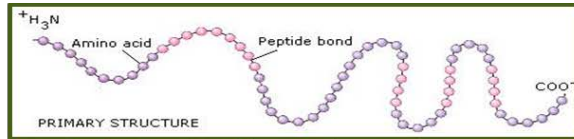
- **Sequence based approach to predict relative intrinsic susceptibility**
  - **Receptor/enzyme available for the chemical to act upon**
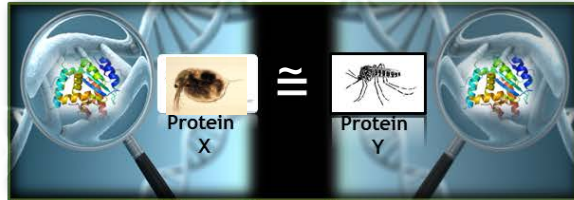- **Conservation of MIE: Extrapolate MIE across taxa**

Facilitates a Strategic Approach for Assessing Protein Similarity Across Species

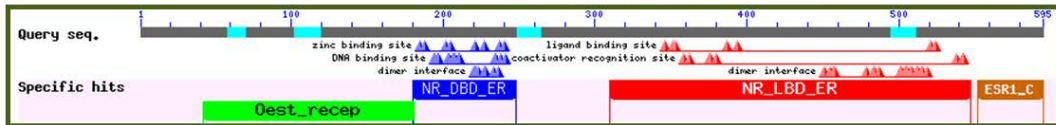**Low Level of Complexity**

High level of Complexity

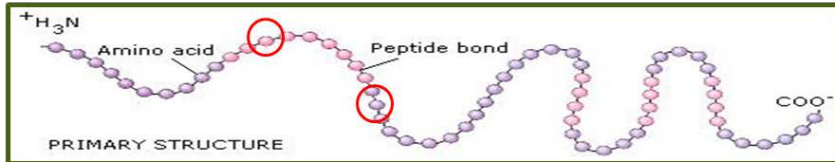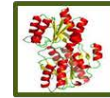- Primary Amino Acid Sequence Alignments
- Ortholog Candidate Identification (RBH)
- Conserved Functional Domain Alignments
- Individual Amino Acid Residue Queries
- Tertiary Protein Structure Considerations

Conservation of molecular target: Line-of-evidence that MIE may be relevant

SeqAPASS Query

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

Logo

| Home | Request SeqAPASS Run | View SeqAPASS Reports | Susceptibility Cutoff Formulas |

Request SeqAPASS Run

Choose Search Type — By Species ▾

Query Species Search — *Daphnia magna*

[Add Query Species]

Selected Query Species

Query Protein Search

[Filter Protein]

http://www.ncbi.nlm.nih.gov/protein

Query Proteins — Ecdysteroid Receptor

Final Query Protein(s) for SeqAPASS Run

[Request Run]   [Clear]

All Protein Sequences in the NCBI protein database

- **Query *Daphnia magna* ecdysteroid receptor**
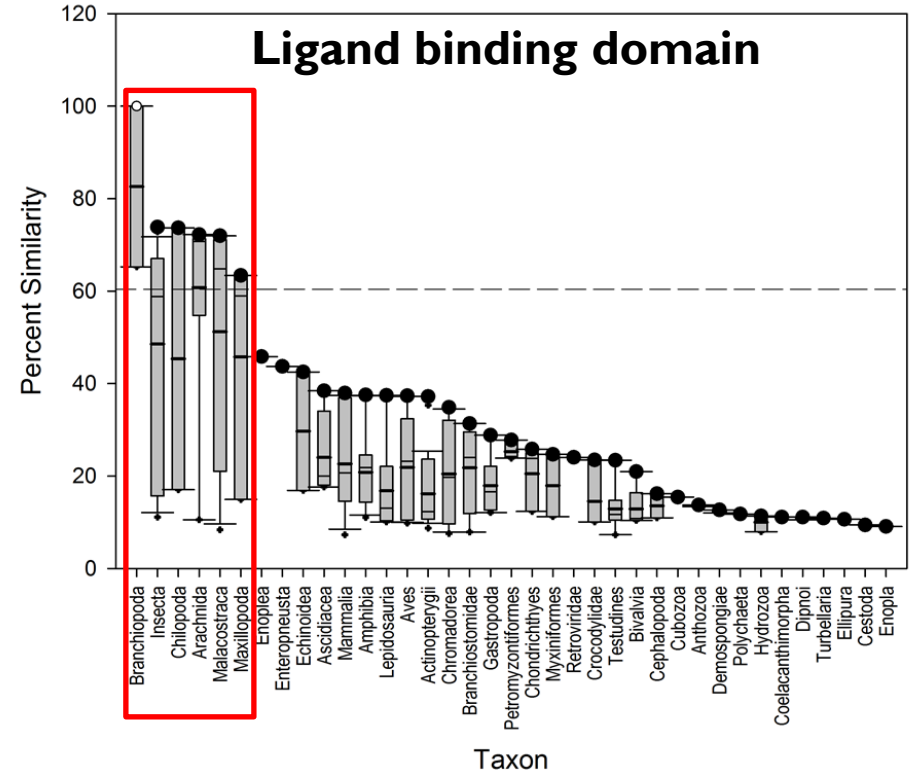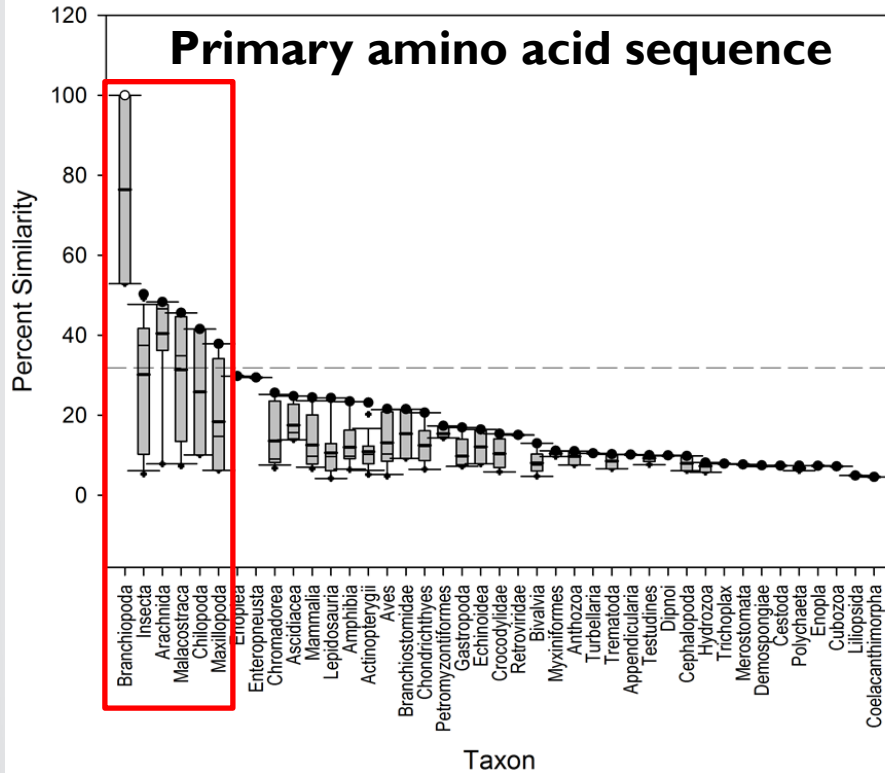
Key amino acid residues

- **Collaborators developed homology model to identify key residues**

Scientific Name	Common Name	Class Name	Protein Name	D384	T415	T418	R455	A470	Y480	N573
Daphnia magna	crustaceans	Branchiopoda	ecdysteroid receptor	D	T	T	R	A	Y	N
Daphnia pulex	common water flea	Branchiopoda	hypothetical protein DAPPUDRAFT_319648	D	T	T	R	A	Y	-
Monochamus alternatus	Japanese pine sawyer	Insecta	ecdysone receptor isoform A	E	T	T	R	V	Y	N
Pediculus humanus corporis	human body louse	Insecta	Ecdysone receptor, putative	E	T	T	R	A	Y	N
Locusta migratoria	migratory locust	Insecta	ecdysone receptor	E	T	T	R	A	Y	N
Lithobius peregrinus	centipedes	Chilopoda	ecdysone receptor, isoform S	E	T	T	R	A	Y	N
Liocheles australasiae	scorpions	Arachnida	ecdysone receptor	E	T	T	R	A	Y	N
Amblyomma americanum	Lone Star tick	Arachnida	AamEcRA1	E	T	T	R	A	Y	N
Ornithodoros moubata	mites & ticks	Arachnida	nuclear receptor	E	T	T	R	A	Y	N
Ixodes scapularis	black-legged tick	Arachnida	AamEcRA1, putative	E	T	T	R	A	Y	N
Agelena silvatica	spiders	Arachnida	ecdysone receptor	E	T	T	R	A	Y	N
Crangon crangon	Atlantic sand fiddler crab	Malacostraca	ecdysteroid receptor isoform 1	E	T	T	R	G	Y	N
Celuca pugilator	American lobster	Malacostraca	ecdysteroid receptor	E	T	T	R	G	Y	N
Homarus americanus	crustaceans	Malacostraca	ecdysteroid receptor splice variant	E	T	T	R	G	Y	N
Marsupenaeus japonicus	green crab	Malacostraca	ecdysone receptor	E	T	T	R	G	Y	N
Carcinus maenas	blackback land crab	Malacostraca	ecdysteroid receptor	E	T	T	R	G	Y	N
Tigriopus japonicus	crustaceans	Maxillopoda	ecdysone receptor	E	T	T	R	A	Y	N
Amphiascus tenuiremis	crustaceans	Maxillopoda	ecdysone receptor isoform 2	E	T	T	R	A	Y	N
Trichinella spiralis	nematodes	Enoplea	ecdysone receptor	D	T	N	K	G	Y	H
Saccoglossus kowalevskii	hemichordates	Enteropneusta	liver X-like nuclear receptor	E	T	T	R	G	L	H
Strongylocentrotus purpuratus	purple sea urchin	Echinoidea	Ecr/Fxr protein	E	T	T	R	G	L	H
Ciona intestinalis	tunicates	Ascidiacea	nuclear receptor	Q	A	I	R	L	F	H
Macaca mulatta	Rhesus monkey	Mammalia	hypothetical protein EGK_06297	-	A	S	E	L	-	H
Ailuropoda melanoleuca	giant panda	Mammalia	hypothetical protein PANDA_010593	-	A	S	E	L	-	H
Cricetulus griseus	Chinese hamster	Mammalia	Oxysterols receptor LXR-alpha	-	A	S	E	L	-	H
Loxodonta africana	African savanna elephant	Mammalia	oxysterols receptor LXR-alpha-like isoform 1	-	A	S	E	L	-	H

- **EcR sequence and structural conservation: MIE likely relevant**

  - **Branchiopoda**
  - **Insecta**
  - **Chilopoda**
  - **Arachnida**
  - **Malacostraca**
  - **Maxillopoda**

MIE → KE → KE → KE → AO

Taxonomic domain of applicability

- **U.S. Environmental Protection Agency**
  - Daniel Villeneuve
  - Gerald Ankley
- **Computer Sciences Corporation**
  - Henry Helgen
  - Steve Walata
  - Derek Lane
- **Norwegian Institute for Water Research (NIVA)**
  - Knut Erik Tollefsen
  - You Song
  - Tore Høgåsen
- **Universitetet I Tromsø, The Arctic University of Norway**
  - Ingebrigt Sylte
  - Kurt Kristiansen

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