Integrating Informatics to Broaden the Taxonomic Domain of Applicability for Adverse Outcome Pathways

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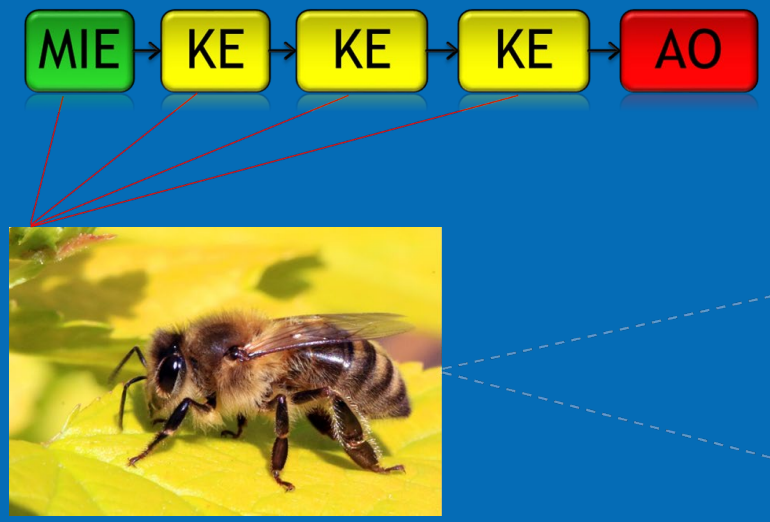
Research Bioinformaticist



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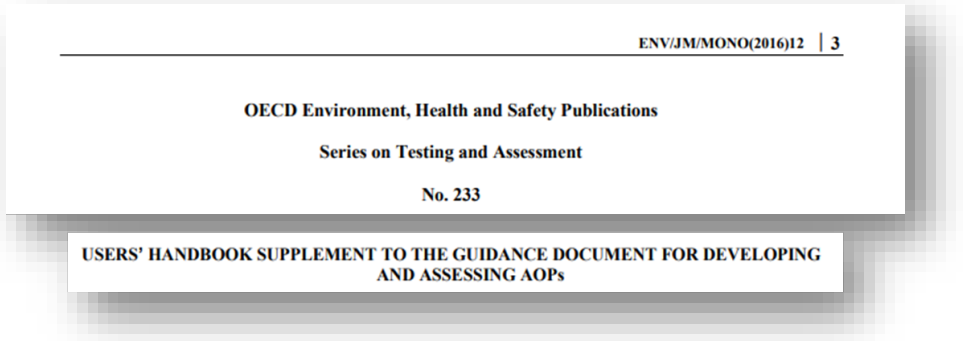
Office of Research and Development
Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division

May 19th, 2022

Current Status for Defining the Taxonomic Domain of Applicability

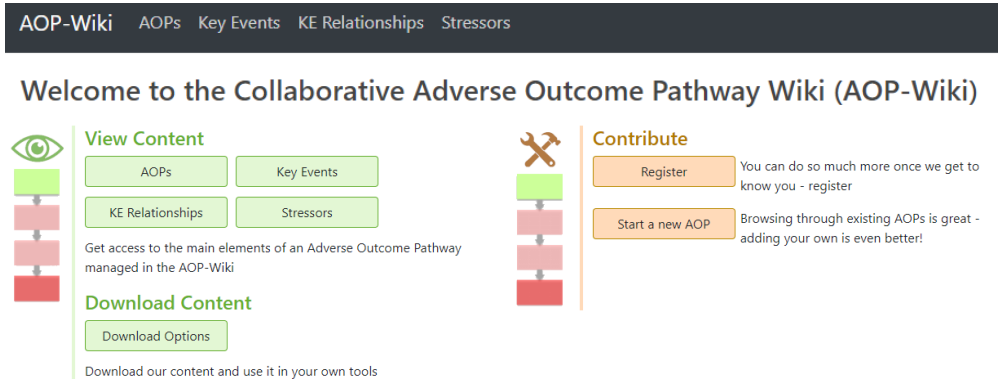


OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 233

USERS' HANDBOOK SUPPLEMENT TO THE GUIDANCE DOCUMENT FOR DEVELOPING AND ASSESSING AOPs



Biological Domain of Applicability

1. Structure: Is the biological object being measured/observed present/conserved in the taxa/sex/life-stage of interest? Here biological object may refer to a protein, a cell type, an organ, etc.

2. Function: Is the function of that biological object and the process being measured via the KE conserved and relevant in the taxa/sex/life-stage of interest. Does it play the same role?

Any specification of tDOA

KEs: 26%

KERs: 22%

AOPs: 25%

Evidence Supporting this KER

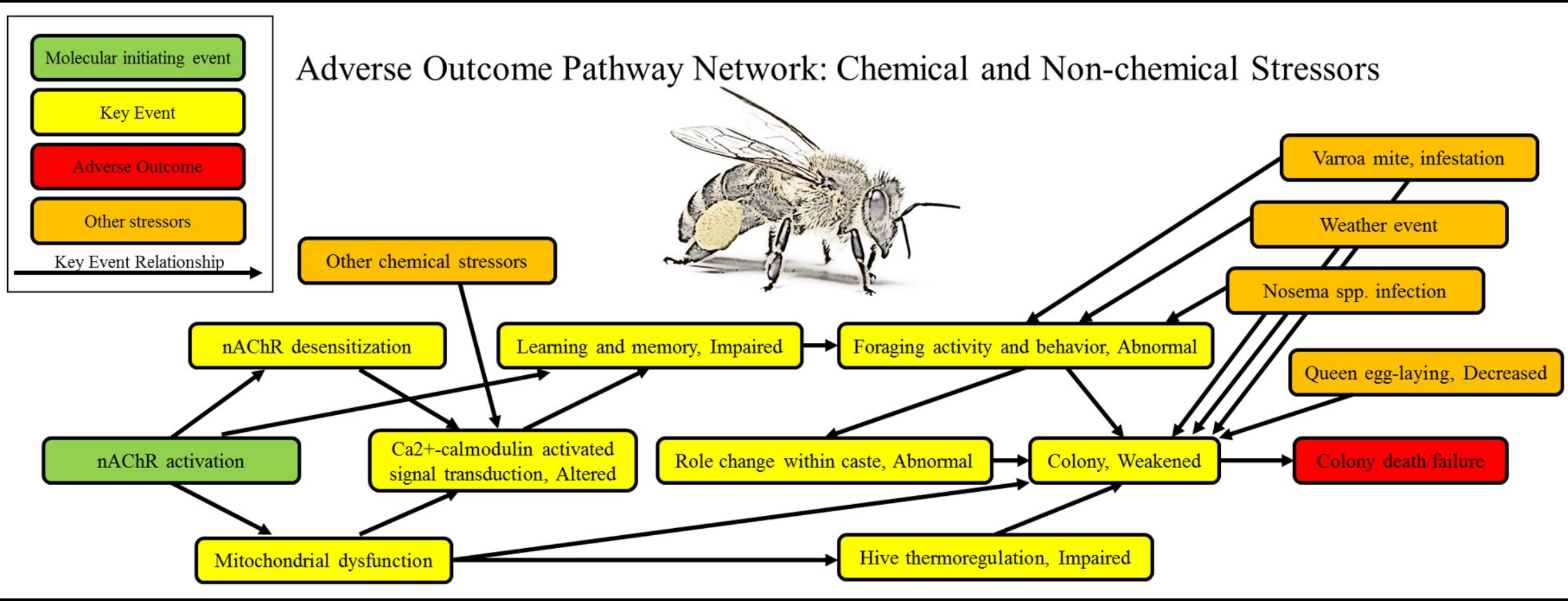
Empirical Evidence

In this section authors are encouraged to cite specific evidence that supports the idea that a change in the upstream KE (KEupstream) will lead to, or is associated with, a subsequent change in the downstream KE (KEdownstream), assuming the perturbation of KEupstream is sufficient.

Biological Plausibility

Define, in free text, the biological rationale for a connection between KEupstream and KEdownstream. What are the structural or functional relationships between the KEs? For example, there is a functional relationship between an enzyme's activity and the product of a reaction it catalyses.

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 an AOP?

How to define the taxonomic relevance of an AOP?

Empirical Evidence tDOA

Biological Plausible tDOA: Decision Tree

MIE

Is there evidence that the nAChR is conserved in the organism?

No

Yes

KE1

Is there evidence that the nAChR is conserved in the organism?

No

Yes

KE2

Is there evidence that the calcium-calmodulin signal transduction pathway is conserved in the organism?

No

Yes

KE3

Does the organism rely on learning and memory for survival?

No

Yes

KE4

Does species rely on foraging food sources to support a colony? Does species use signals to relay information on food source location to other colony members?

No

Yes

KE5

Do inappropriate or untimely role changes of the organism lead to adverse impacts on the colony?

No

Yes

KE6

Does the organism depend on a colony structure for survival?

No

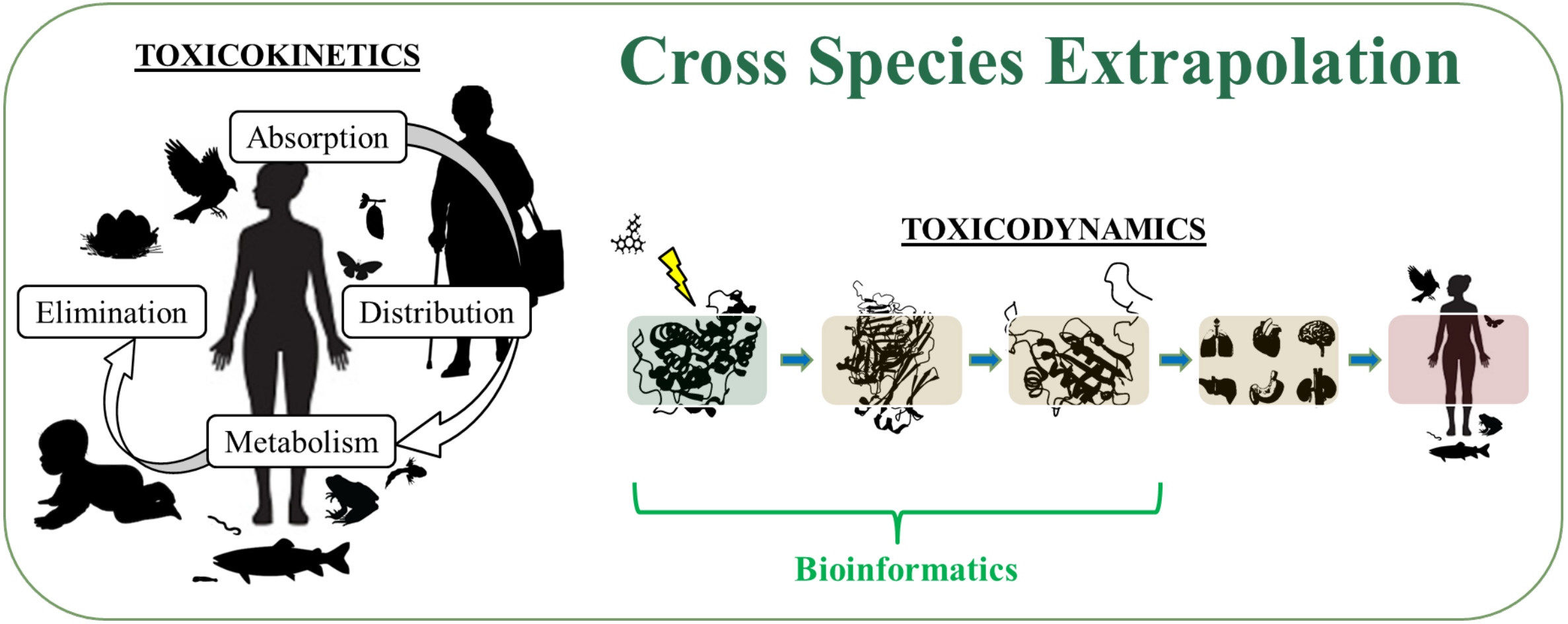
Yes

AO

Does the species live in a colony structure?

No

Sensitivity to Chemical Perturbation



Simplify Complexity

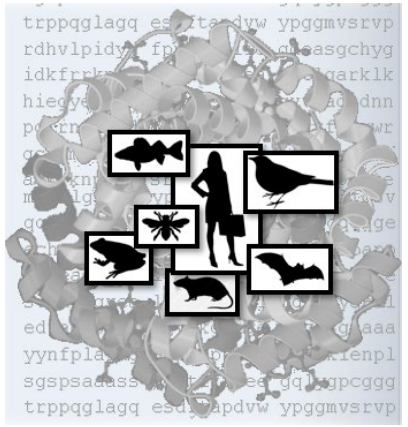
Bioinformatics

- 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

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*

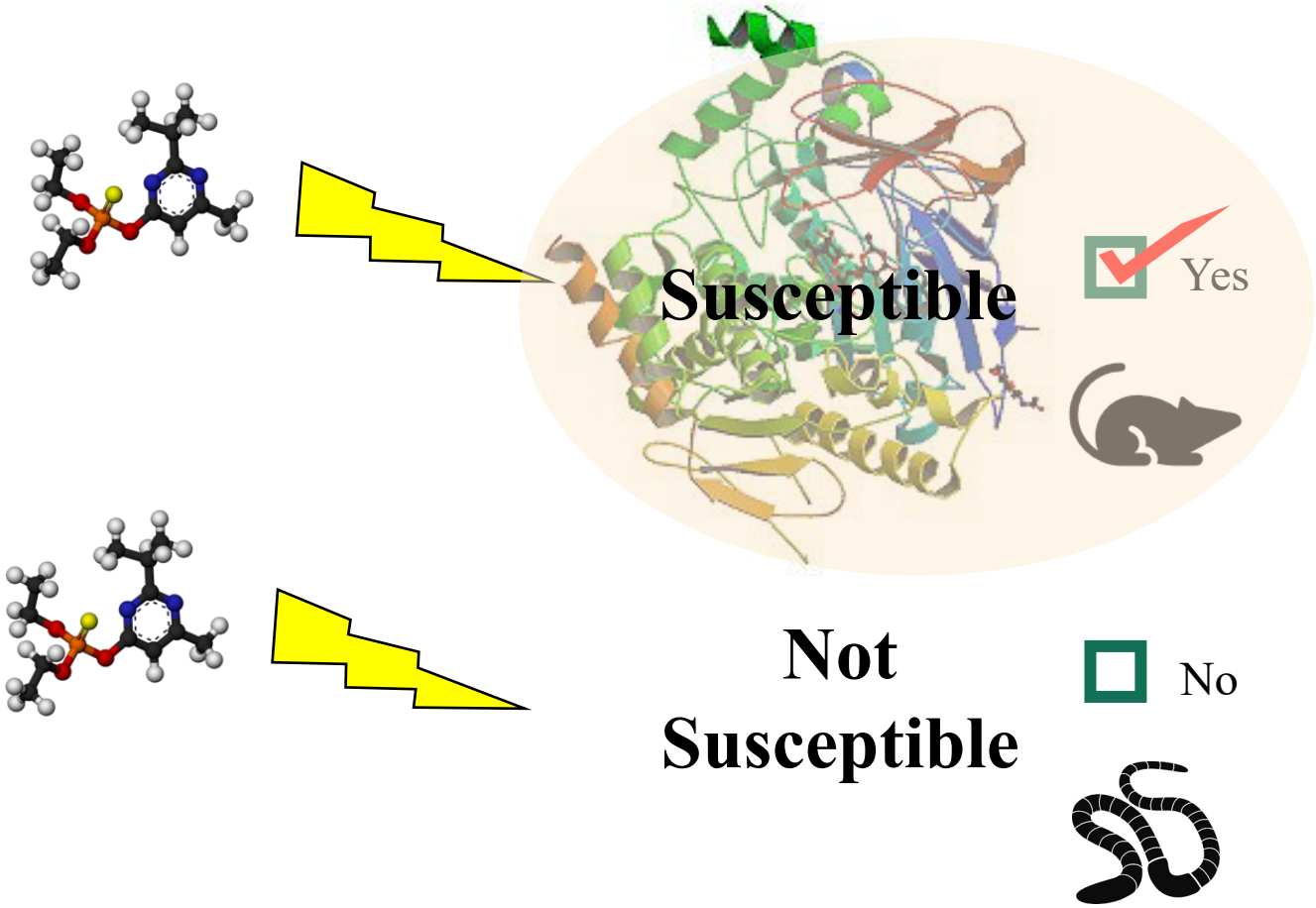
https://seqapass.epa.gov/seqapass/

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

Considering chemical sensitivity?

Factors that make a species sensitive

- Exposure
- Dose
- ADME
- Target receptor availability
- Life stage
- Life history
- etc.
- etc.



Simple question to address:

Is the known chemical target available in a species for a chemical to act upon?

Yes or No

Likely susceptible or Not likely susceptible (at least through the known mechanism)

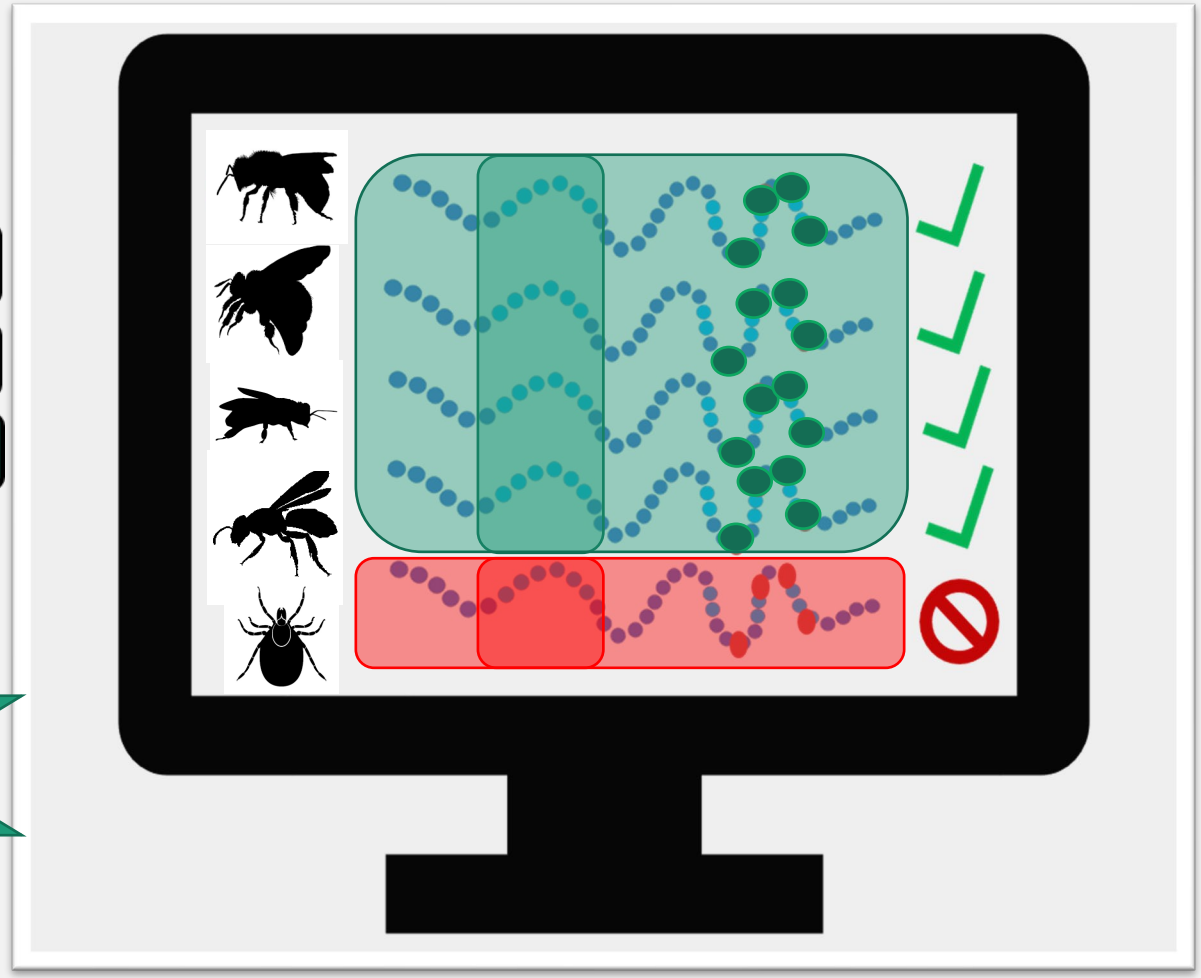
Flexible Analysis Based On Available Data

Level 1 Primary Amino Acid Sequence Alignments

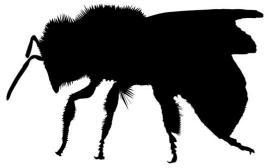
Level 2 Conserved Functional Domain Alignments

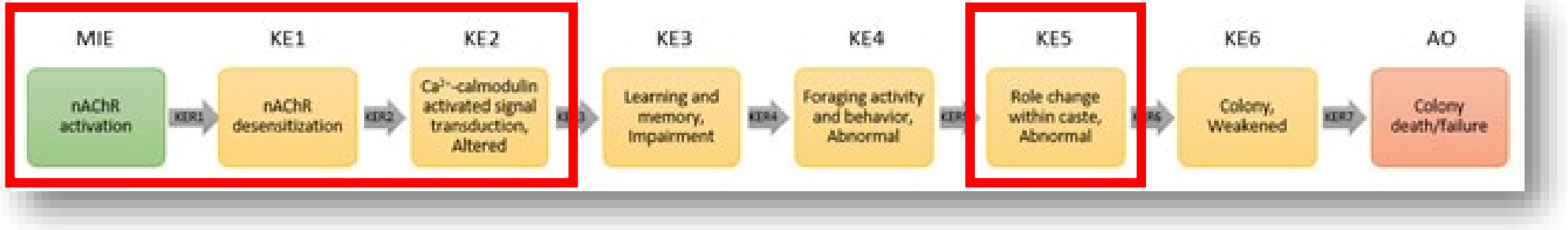
Level 3 Critical (Close Contact) Amino Acid Conservation

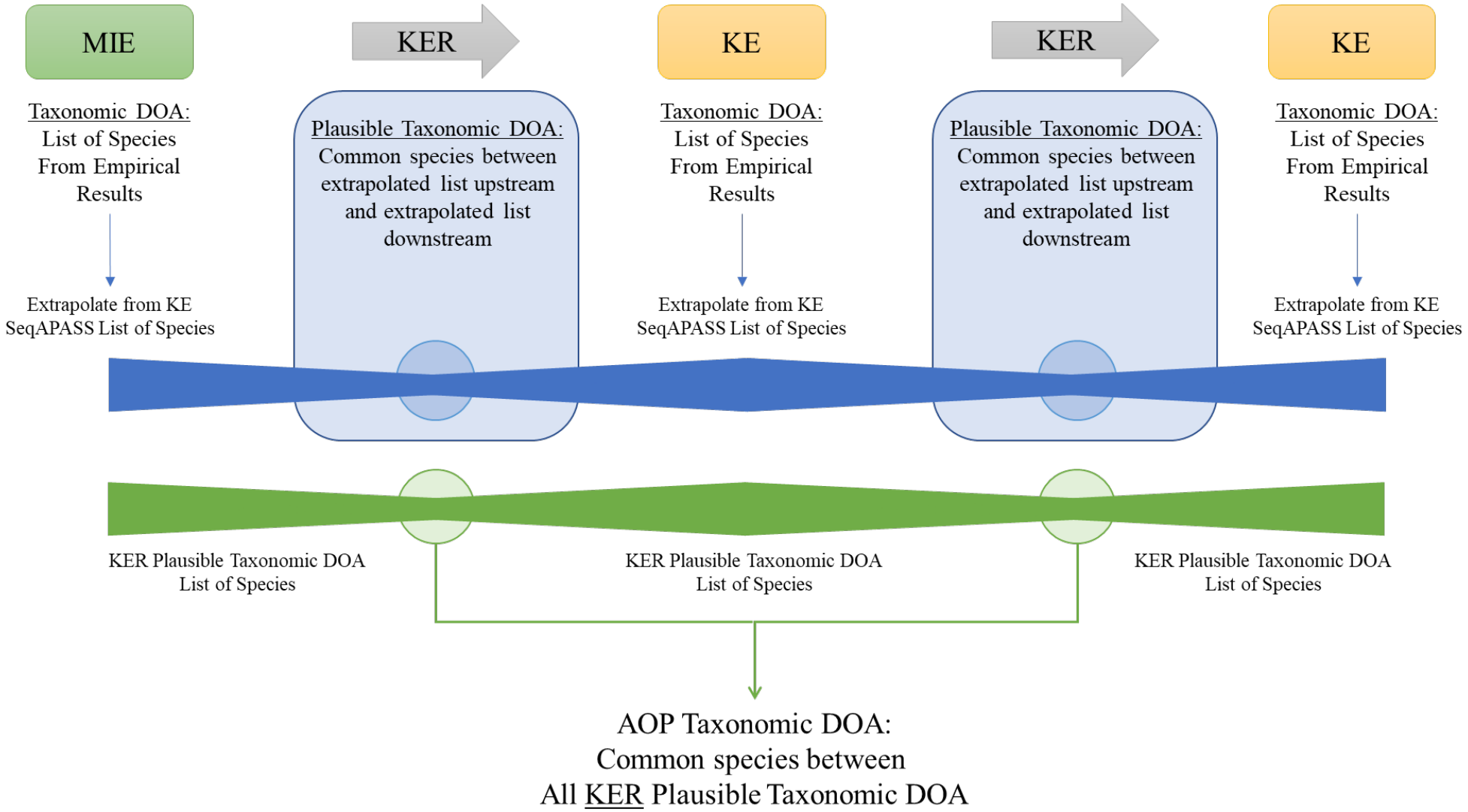
seqapass.epa.gov/seqapass/



Gather Lines of Evidence Toward Protein Conservation

Apis  ≈  *Non-Apis*


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


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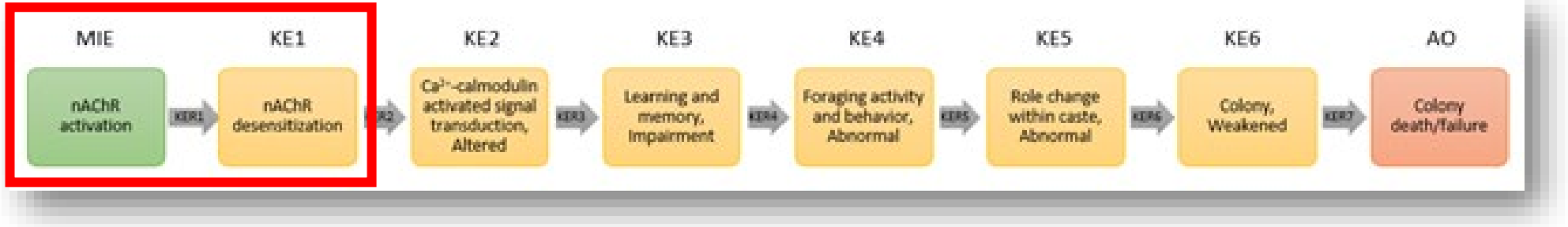
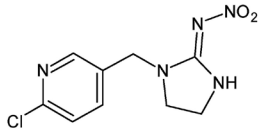
Plausible Taxonomic DOA: Common species between extrapolated list upstream and extrapolated list downstream
Taxonomic DOA: List of Species From Empirical Results
Extrapolate from KE SeqAPASS List of Species
Extrapolate from KE SeqAPASS List of Species
Extrapolate from KE SeqAPASS List of Species
KER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

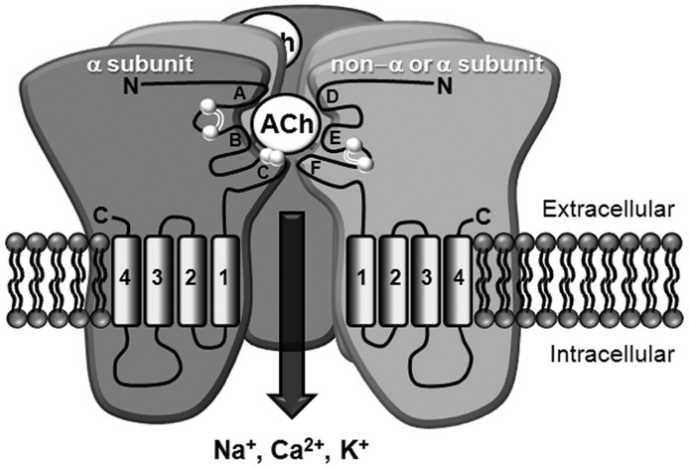


As an example: nAChR protein involved in MIE and KE1

Nicotinic acetylcholine receptor (nAChR)



Molecular Initiating Event

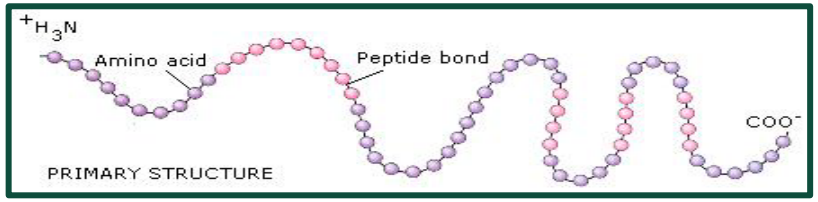


Adverse Outcome

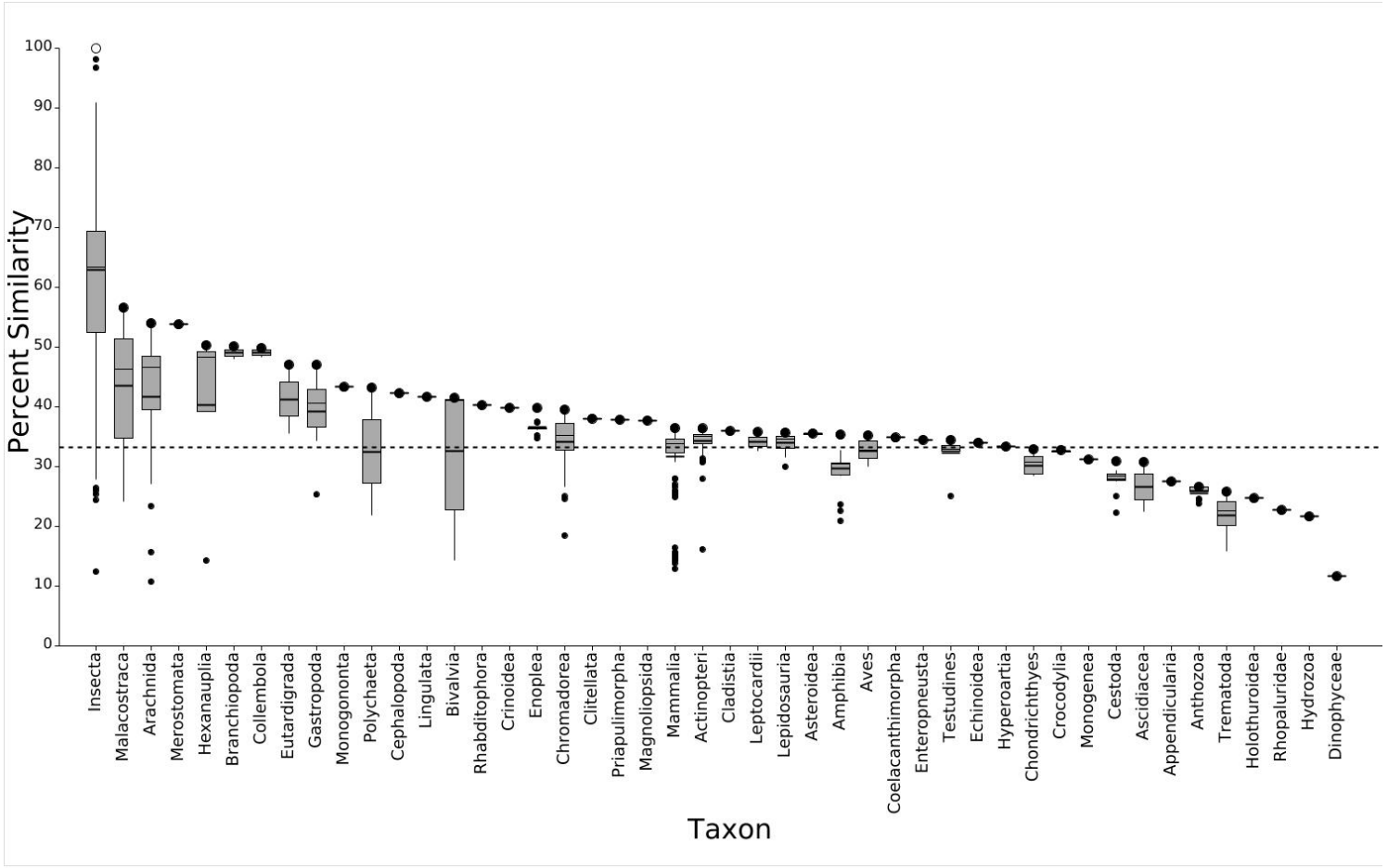
Taken from: Jones and Sattelle, 2010

Level 1 SeqAPASS Results: MIE and KEI Protein Conserved

Primary Amino Acid Sequence Alignment

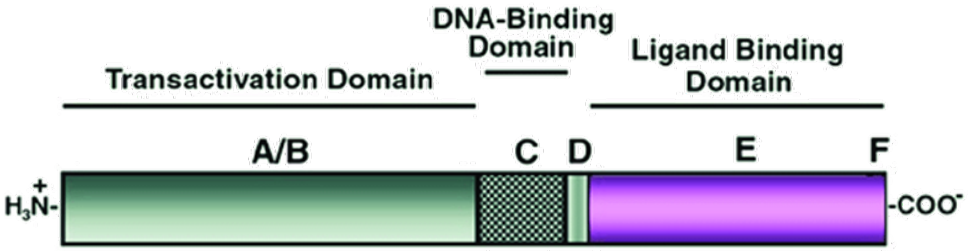


Nicotinic Acetylcholine Receptor α1 Subunit



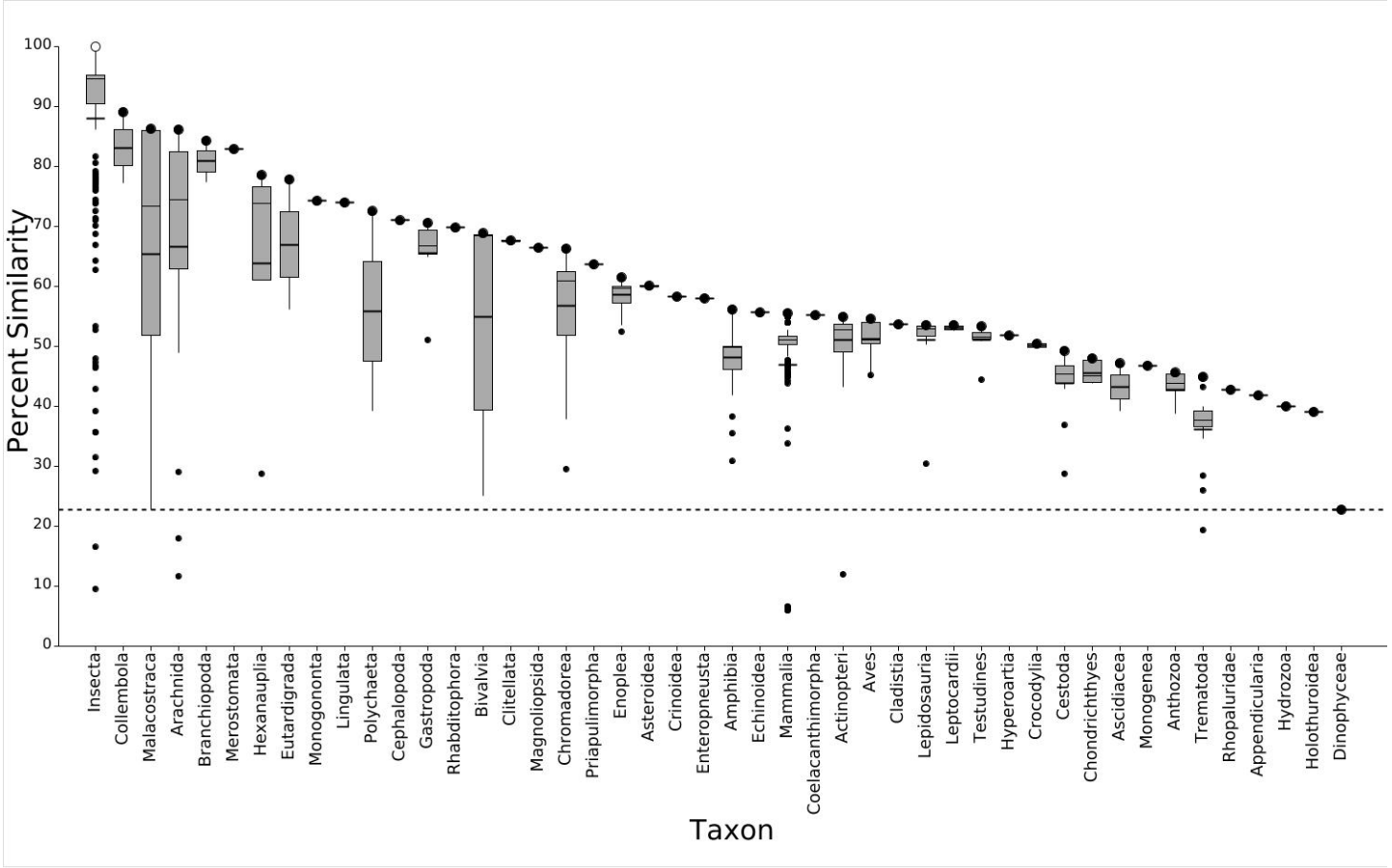
Level 2 SeqAPASS Results: MIE and KEI Protein Conserved

Conserved Functional Domain Alignment ✓

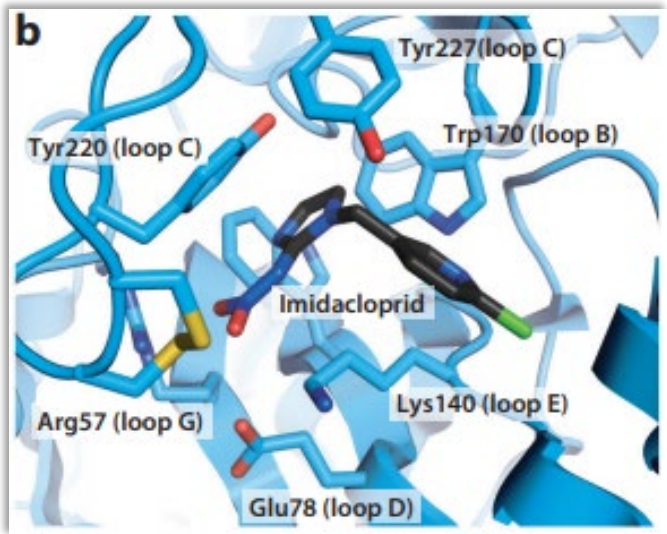


Nicotinic Acetylcholine Receptor α1 Subunit

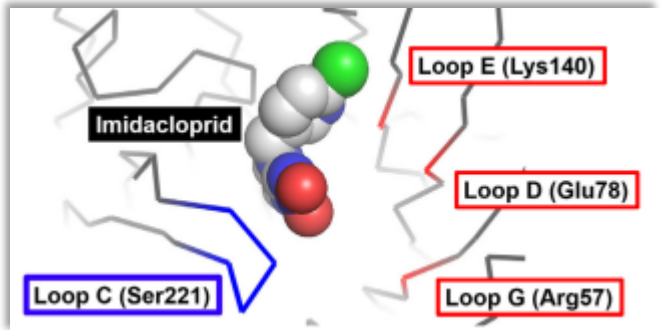
Neurotransmitter-gated ion-channel ligand binding domain



Level 3 SeqAPASS Results: MIE and KEI Protein Conserved



Matsuda, Kazuhiko, et al. "Neonicotinoid Insecticides: Molecular Targets, Resistance, and Toxicity." *Annual Review of Pharmacology and Toxicology*, vol. 60, no. 1, 2020, pp. 241–255., doi:10.1146/annurev-pharmtox-010818-021747.



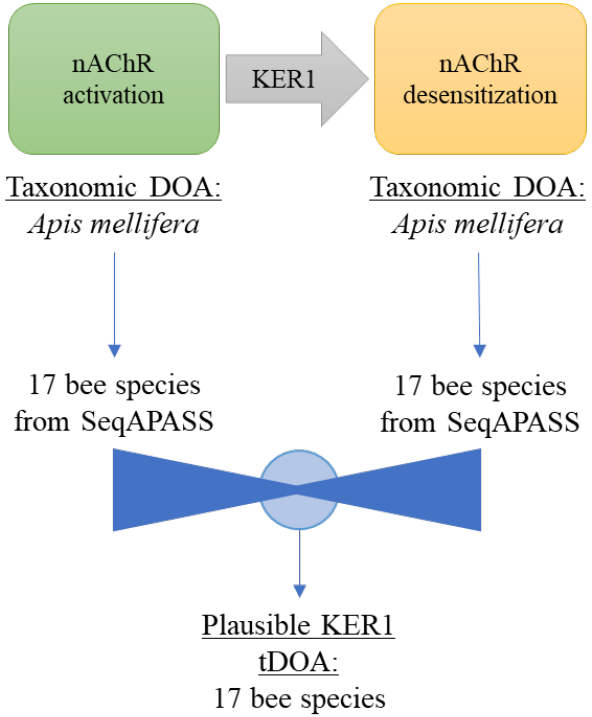
Matsuda, Kazuhiko. "Robust Functional Expression of Insect Nicotinic Acetylcholine Receptors Provides New Insights into Neonicotinoid Actions and New Opportunities for Pest and Vector Control." *Pest Management Science*, 2020, doi:10.1002/ps.6182.

Total Match
Partial Match
Not a Match
Susceptible Yes
Susceptible No

Nicotinic Acetylcholine Receptor α1 Subunit

Scientific Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7
Drosophila melanogaster	Y	57R	78E	140K	170W	220Y	221S	227Y
Apis mellifera	Y	53R	74E	136K	166W	216Y	217I	223Y
Apis cerana	Y	79R	100E	162K	192W	242Y	243I	249Y
Apis florea	Y	79R	100E	162K	192W	242Y	243I	249Y
Habropoda laboriosa	Y	53R	74E	136K	166W	216Y	217I	223Y
Osmia bicornis bicornis	Y	79R	100E	162K	192W	242Y	243I	249Y
Osmia lignaria	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus bifarius	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vancouverensis nearcticus	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vosnesenskii	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus terrestris	Y	79R	100E	162K	192W	242Y	243I	249Y
Megachile rotundata	Y	79R	100E	162K	192W	242Y	243I	249Y
Dufourea novaeangliae	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus impatiens	Y	79R	100E	162K	192W	242Y	243I	249Y
Nomia melanderi	Y	79R	100E	162K	192W	242Y	243I	249F
Eufriesea mexicana	Y	79R	100E	162K	192W	242Y	243I	249Y
Megalopta genalis	N	--	8E	70K	100W	150Y	151I	157F
Apis dorsata	Y	57K	78E	140K	170W	220Y	221T	227Y
Ceratina calcarata	Y	61K	82E	144K	174W	224Y	225T	231Y

Level 3 SeqAPASS Results: MIE and KEI Protein Conserved



MIE and KE1 Conserved Among:

Apis cerana
Apis dorsata
Apis florea
Apis mellifera
Bombus biffarius
Bombus impatiens
Bombus terrestris
Bombus vancouverensis nearcticus
Bombus vosnesenskii
Ceratina calcarata
Dufourea novaeangliae
Eurfriesea mexicana
Habropoda laboriosa
Megachile rotundata
Nomia melanderi
Osmia bicornis bicornis
Osmia lignaria

Total Match
Partial Match
Not a Match
Susceptible Yes
Susceptible No

Nicotinic Acetylcholine Receptor α1 Subunit

Scientific Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7
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Apis mellifera	Y	53R	74E	136K	166W	216Y	217I	223Y
Apis cerana	Y	79R	100E	162K	192W	242Y	243I	249Y
Apis florea	Y	79R	100E	162K	192W	242Y	243I	249Y
Habropoda laboriosa	Y	53R	74E	136K	166W	216Y	217I	223Y
Osmia bicornis bicornis	Y	79R	100E	162K	192W	242Y	243I	249Y
Osmia lignaria	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus bifarius	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vancouverensis nearcticus	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vosnesenskii	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus terrestris	Y	79R	100E	162K	192W	242Y	243I	249Y
Megachile rotundata	Y	79R	100E	162K	192W	242Y	243I	249Y
Dufourea novaeangliae	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus impatiens	Y	79R	100E	162K	192W	242Y	243I	249Y
Nomia melanderi	Y	79R	100E	162K	192W	242Y	243I	249F
Eufriesea mexicana	Y	79R	100E	162K	192W	242Y	243I	249Y
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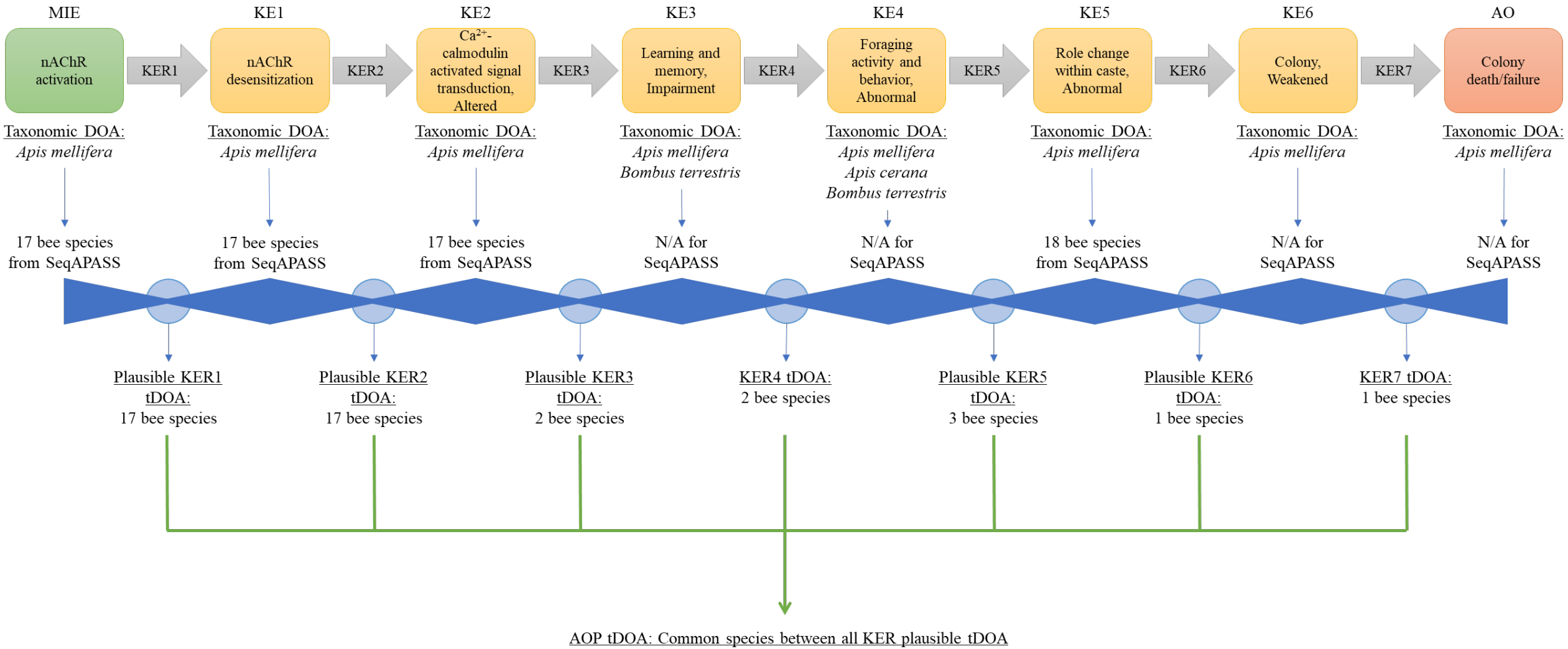
MIE
nAChR activation
KER1
KE1
nAChR desensitization
KER2
KE2
Ca2+-calmodulin activated signal transduction, Altered
KER3
KE3
Learning and memory, Impairment
KER4
KE4
Foraging activity and behavior, Abnormal
KER5
KE5
Role change within caste, Abnormal
KER6
KE6
Colony, Weakened
KER7
AO
Colony death/failure
Taxonomic DOA: Apis mellifera
Taxonomic DOA: Apis mellifera
Taxonomic DOA: Apis mellifera
Taxonomic DOA: Apis mellifera Bombus terrestris
Taxonomic DOA: Apis mellifera Apis cerana Bombus terrestris
Taxonomic DOA: Apis mellifera
Taxonomic DOA: Apis mellifera
Taxonomic DOA: Apis mellifera
17 bee species from SeqAPASS
17 bee species from SeqAPASS
17 bee species from SeqAPASS
N/A for SeqAPASS
N/A for SeqAPASS
18 bee species from SeqAPASS
N/A for SeqAPASS
N/A for SeqAPASS
Plausible KER1 tDOA: 17 bee species
Plausible KER2 tDOA: 17 bee species
Plausible KER3 tDOA: 2 bee species
KER4 tDOA: 2 bee species
Plausible KER5 tDOA: 3 bee species
Plausible KER6 tDOA: 1 bee species
KER7 tDOA: 1 bee species
AOP tDOA: Common species between all KER plausible tDOA

MIE

Is there evidence that the nAChR is conserved in the organism?

No

Yes

KE1

Is there evidence that the nAChR is conserved in the organism?

No

Yes

KE2

Is there evidence that the calcium-calmodulin signal transduction pathway is conserved in the organism?

No

Yes

KE3

Does the organism rely on learning and memory for survival?

No

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KE4

Does species rely on foraging food sources to support a colony?
Does species use signals to relay information on food source location to other colony members?

No

Yes

KE5

Do inappropriate or untimely role changes of the organism lead to adverse impacts on the colony?

No

Yes

KE6

Does the organism depend on a colony structure for survival?

No

Yes

AO

Does the species live in a colony structure?

No

Empirical Evidence tDOA

SeqAPASS Expands tDOA

Apis cerana	*Bombus terrestris*	*Habropoda laboriosa*
Apis dorsata	*Bombus vancouverensis nearcticus*	*Megachile rotundata*
Apis florea	*Bombus vosnesenskii*	*Nomia melanderi*
Apis mellifera	*Ceratina calcarata*	*Osmia bicornis bicornis*
Bombus biffarius	*Dufourea novaeangliae*	*Osmia lignaria*
Bombus impatiens	*Eurfriesea mexicana*	

Note: This is a rather focused example – for

Government

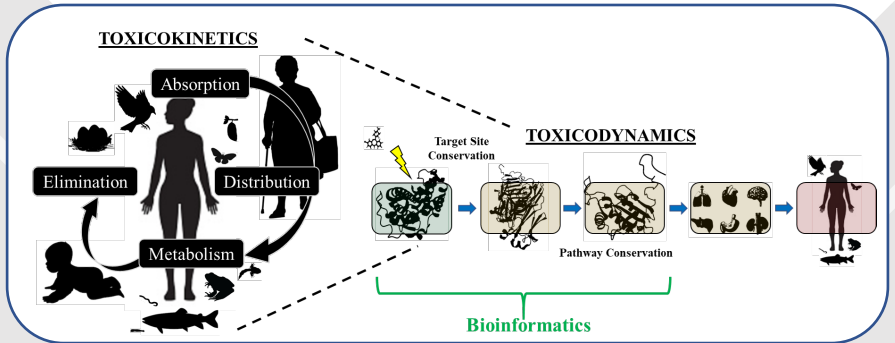
Industry

Consortium to Advance Cross Species Extrapolation in Regulation

Steering Committee:
Carlie LaLone (US EPA)
Geoff Hodges (Unilever)
Nil Basu (McGill U)
Steve Edwards (RTI)
Fiona Sewell (NC3Rs)
Michelle Embry (HESI)
Patience Browne (OECD)

1. Define the taxonomic domain of applicability
2. Define the global regulatory landscape/need
3. Develop a bioinformatics toolbox
4. Communicate a shared scientific vision

Interested in Learning more or Joining: Contact LaLone.Carlie@epa.gov or Geoff.Hodges@unilever.com



Academia

NGO

Acknowledgements

U.S. EPA, ORD

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Sally Mayasich (University of Wisconsin)
Monique Hazemi (ORISE)
Sara Vliet (past ORISE 2021)
Jon Doering (past NRC 2018)
Colin Finnegan (past ORISE 2018)
Donovan Blatz (past ORISE 2021)

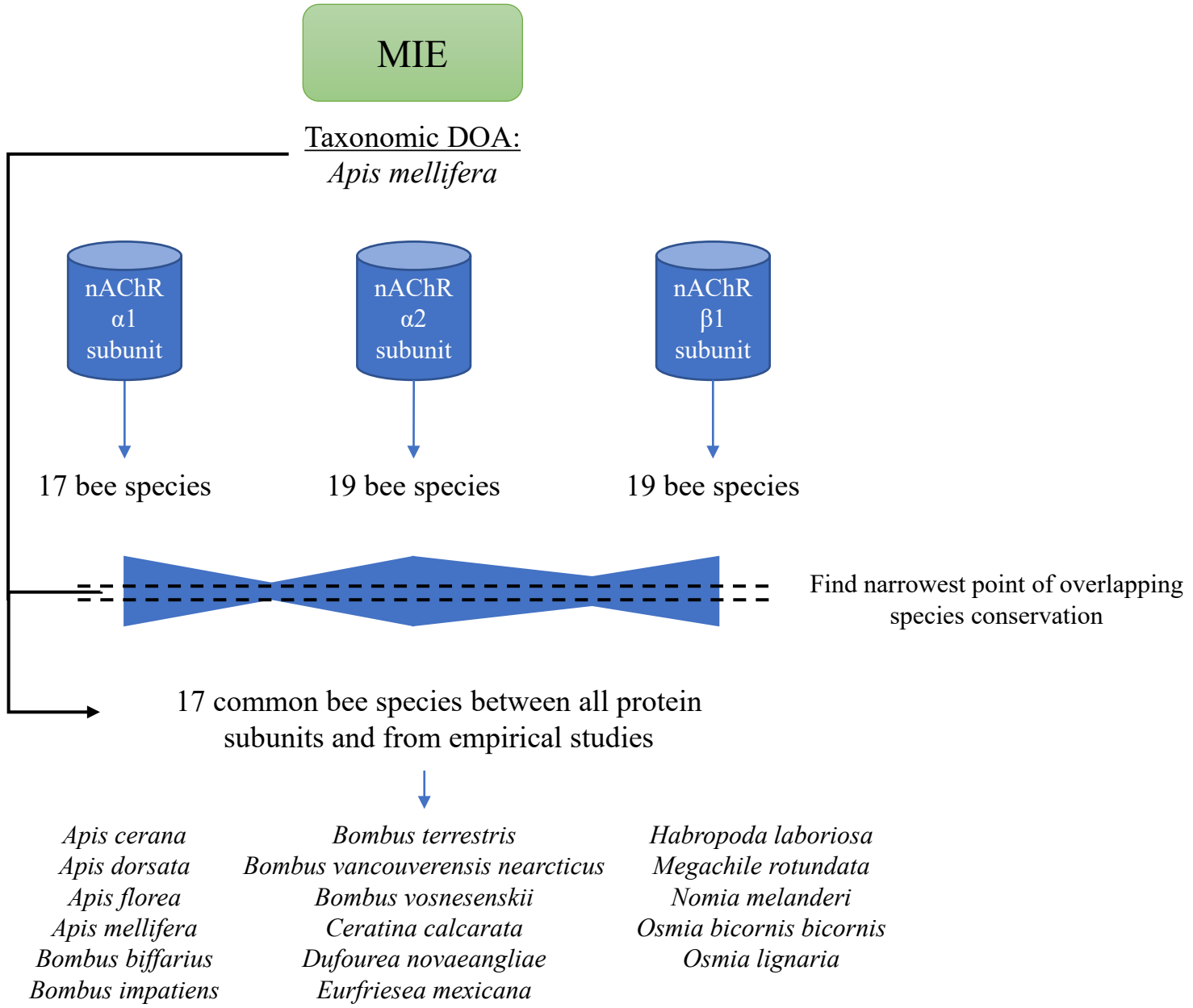
GDIT

Cody Simmons
Audrey Wilkinson
Wilson Menendez
Thomas Transue (past GDIT 2022)

SeqAPASS v6.0 (Released Sept. 2021)

LaLone.Carlie@epa.gov

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


MIE
Taxonomic DOA:
Apis mellifera
nAChR α1 subunit
nAChR α2 subunit
nAChR β1 subunit
17 bee species
19 bee species
19 bee species
Find narrowest point of overlapping species conservation
17 common bee species between all protein subunits and from empirical studies
Apis cerana
Apis dorsata
Apis florea
Apis mellifera
Bombus biffarius
Bombus impatiens
Bombus terrestris
Bombus vancouverensis nearcticus
Bombus vosnesenskii
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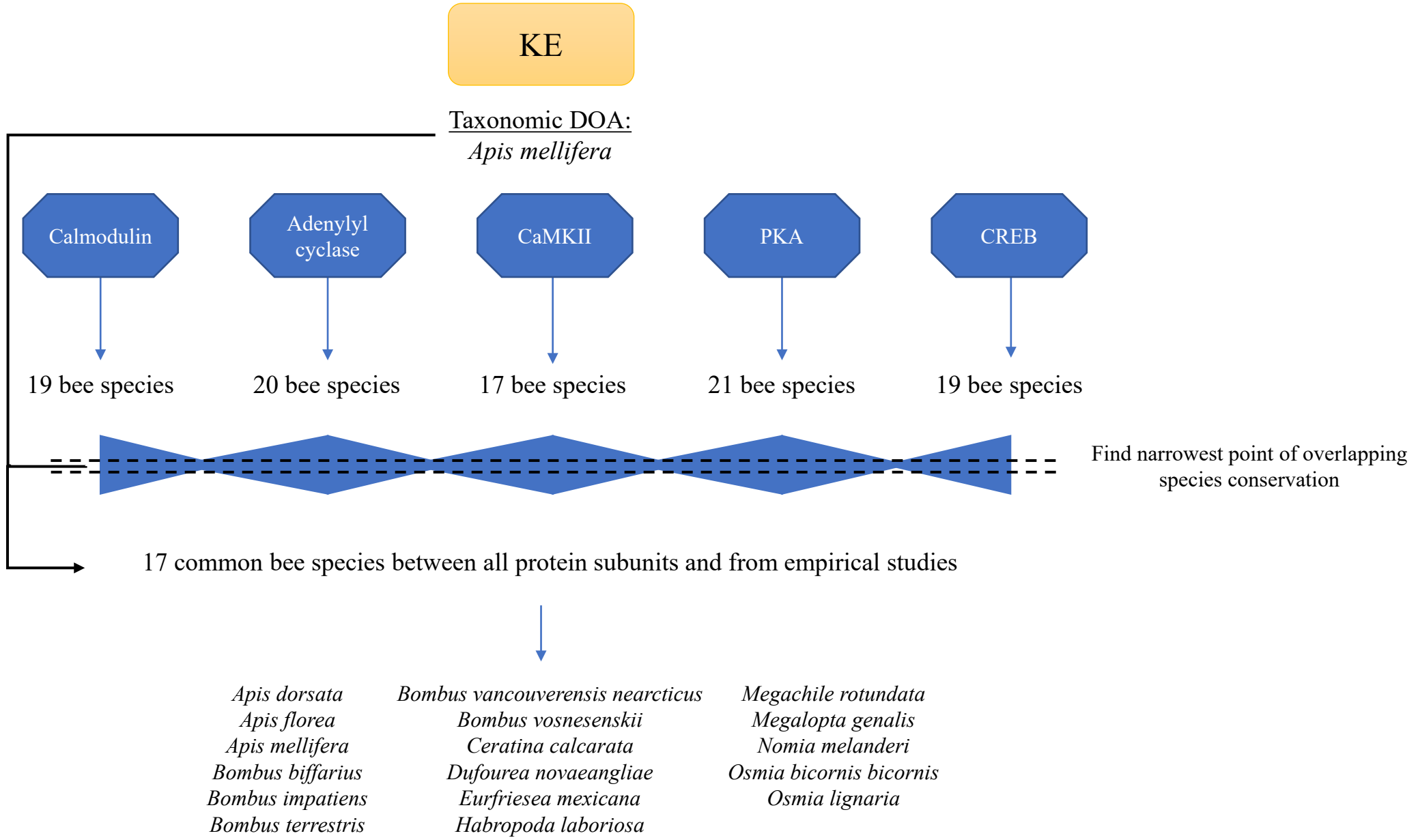
KE
Taxonomic DOA:
Apis mellifera
Calmodulin
Adenylyl cyclase
CaMKII
PKA
CREB
19 bee species
20 bee species
17 bee species
21 bee species
19 bee species
Find narrowest point of overlapping species conservation
17 common bee species between all protein subunits and from empirical studies
Apis dorsata
Apis florea
Apis mellifera
Bombus biffarius
Bombus impatiens
Bombus terrestris
Bombus vancouverensis nearcticus
Bombus vosnesenskii
Ceratina calcarata
Dufourea novaeangliae
Eurfriesea mexicana
Habropoda laboriosa
Megachile rotundata
Megalopta genalis
Nomia melanderi
Osmia bicornis bicornis
Osmia lignaria