Evaluation of Cross-Species Conservation of the Androgen Receptor and the basis for Identifying Androgenic Chemicals in Nonmammalian Taxa Using Mammalian Test Systems

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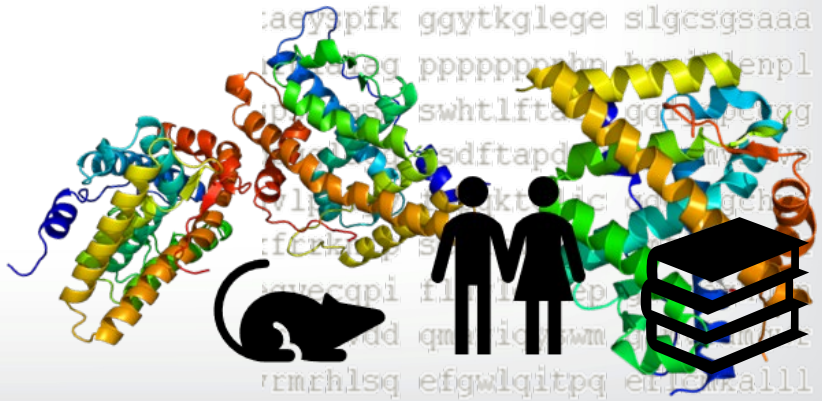

U.S. Environmental Protection Agency – Japan Ministry of the Environment
14th Bilateral Meeting on Endocrine Disruption Test Methods Development
June 9-10, 2021

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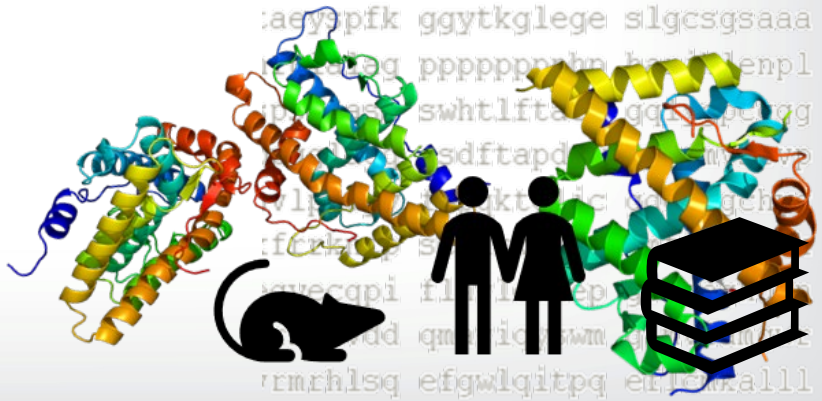
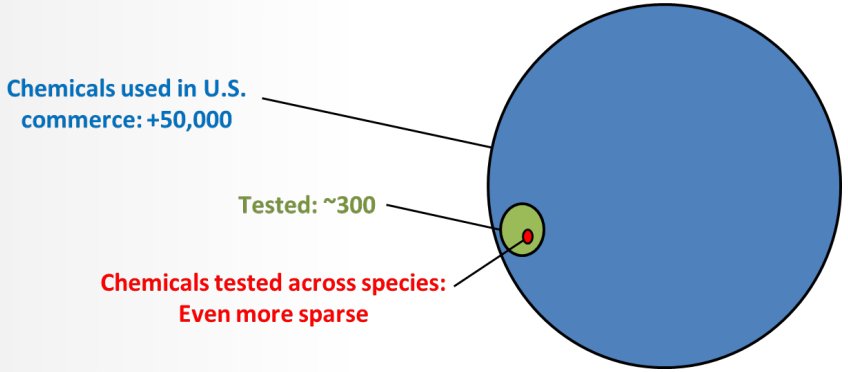
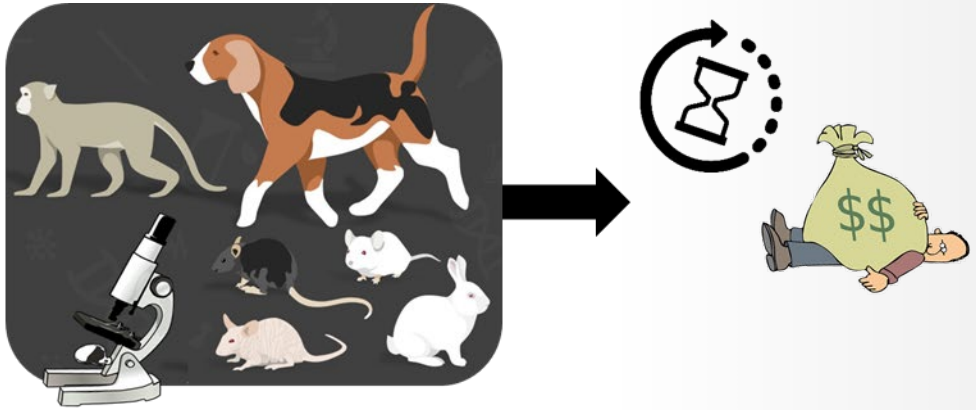


The Need for New Approach Methods (NAMs)

- EPA & the Endocrine Disruptor Screening Program (EDSP) are tasked with evaluating thousands of chemicals for their potential to adversely impact human health and the environment through perturbation of endocrine pathway targets.
- Large numbers of chemicals lacking bioactivity data requires the use of new methods to rapidly screen compounds for the prioritization of chemicals for further evaluation

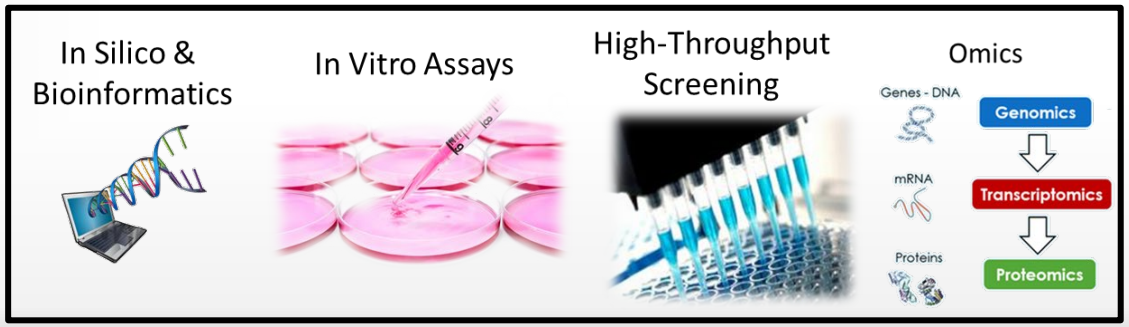


Whole Animal Testing Methods



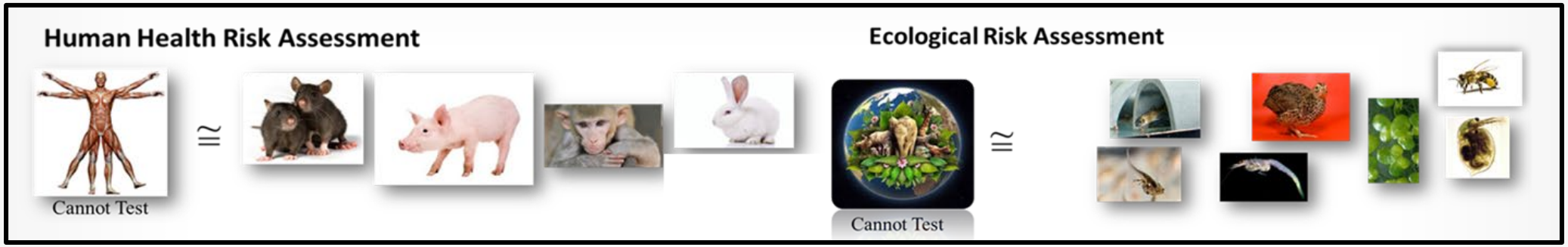
- Limited data for many compounds, limited resources for traditional toxicity testing, and international efforts to reduce animal use all necessitate the development of **new approach methods (NAMs)**

New Approach Methods (NAMs)



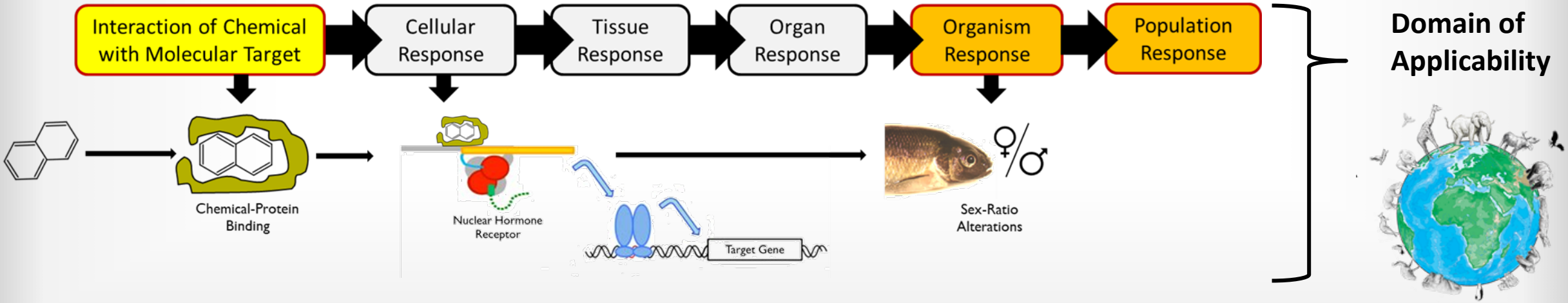
Surrogate Species in Toxicity Testing

- In whole animal testing, it is assumed that the sensitivity of species to a chemical is a function of their relatedness



- High throughput screening assays (US EPA ToxCast) rapidly test chemicals, identify those most likely to be endocrine disruptors, and help inform putative **molecular targets** for chemicals using **<u>mammalian cells</u>**

- Knowledge of the **molecular target** can be linked, though biochemical events, to an **adverse outcome** of regulatory concern



U.S. EPA Toxicity Forecaster (ToxCast)

U.S. EPA ToxCast Program:

- Screens thousands of chemicals **in mammalian-based** high throughput assays for potential bioactivity
- Predicts chemical toxicity and prioritizes chemicals for further testing
- Identifies putative **molecular targets**

Assay Name	Assay Target	Model organism
ATG_TRANS	Androgen receptor, AR	Human (*Homo sapiens*)
NVS_NR_hAR	Androgen receptor, AR	Human (*Homo sapiens*)
OT_AR_ARELUC_AG_1440	Androgen receptor, AR	Human (*Homo sapiens*)
OT_AR_ARSRC1_0480	Androgen receptor, AR	Human (*Homo sapiens*)
OT_AR_ARSRC1_0960	Androgen receptor, AR	Human (*Homo sapiens*)
TOX21_AR_BLA_Agonist	Androgen receptor, AR	Human (*Homo sapiens*)
TOX21_AR_BLA_Antagonist	Androgen receptor, AR	Human (*Homo sapiens*)
TOX21_AR_LUC_MDAKB2_Agonist	Androgen receptor, AR	Human (*Homo sapiens*)
TOX21_AR_LUC_MDAKB2_Antagonist	Androgen receptor, AR	Human (*Homo sapiens*)
NVS_NR_cAR	Androgen receptor, AR	Chimpanzee (*Pan troglodytes*)
NVS_NR_rAR	Androgen receptor, AR	Norway rat (*Rattus norvegicus*)

- Do mammalian-based screening approaches reasonably reflect potential impacts to other species?
  - **Can we expect compounds that interact with the mammalian androgen receptor (AR) to also interact with the AR in other species?**

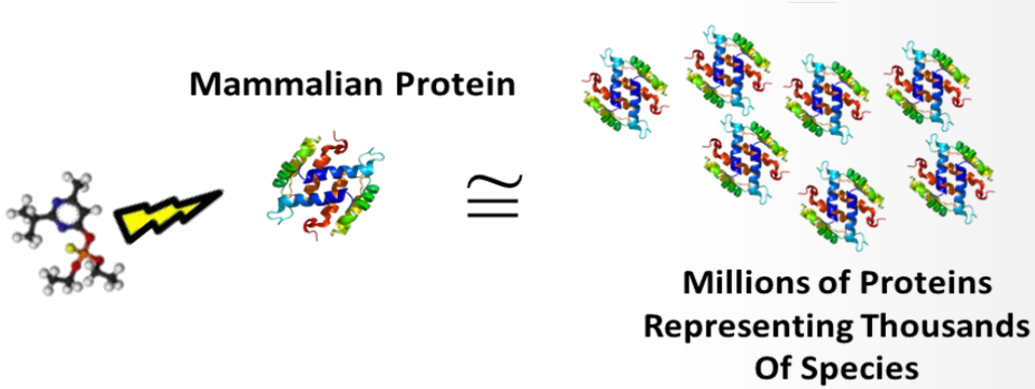
SeqAPASS: Sequence Alignment to Predict Across Species Susceptibility

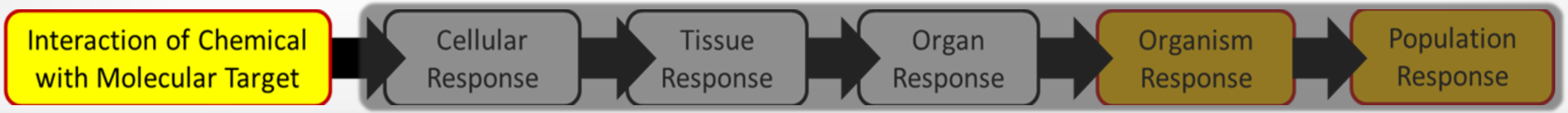
- Online, publicly available tool for understanding **target conservation** across thousands of diverse species

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

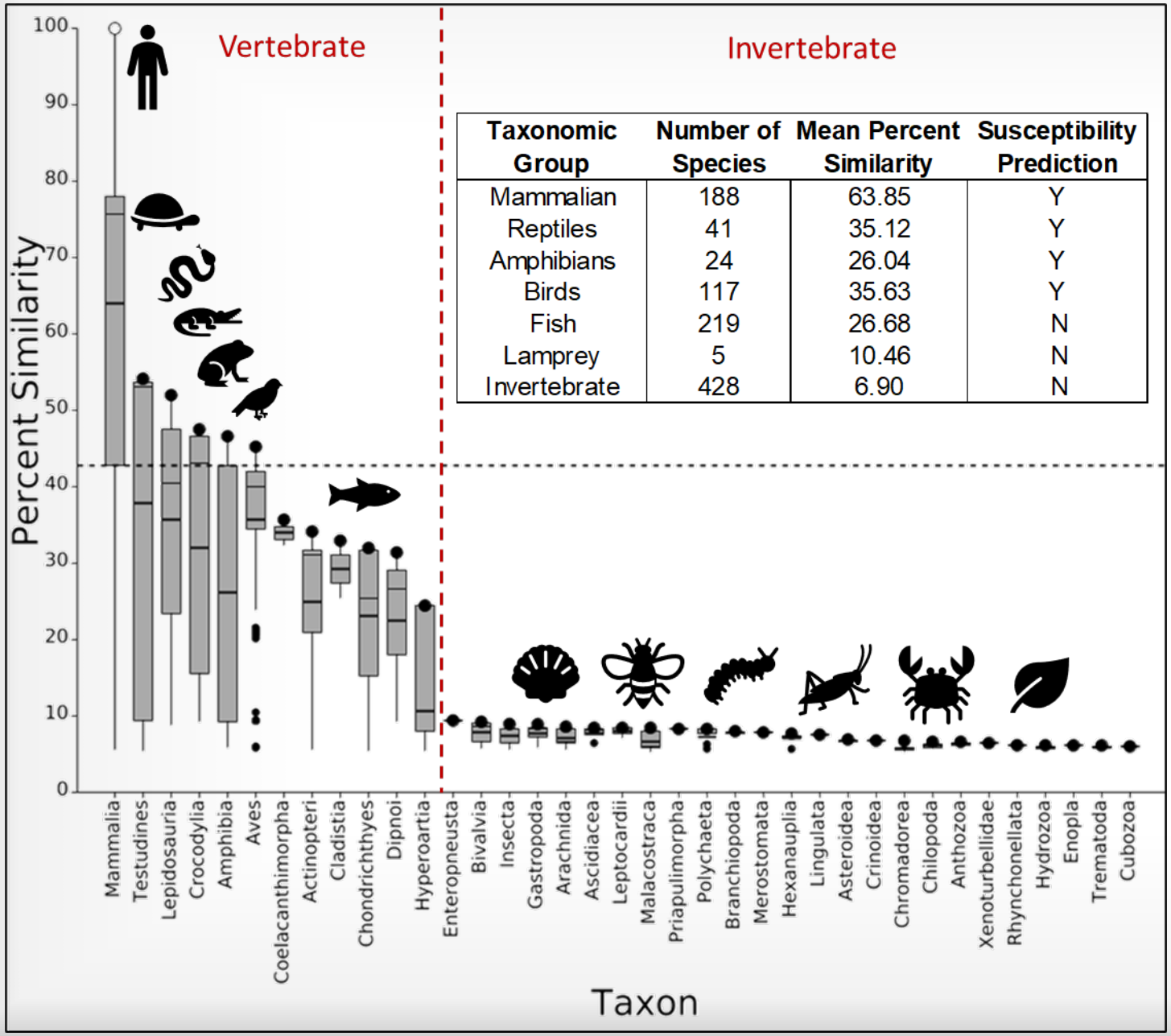
SeqAPASS Applications

- **Extrapolate high throughput screening data**
- **Extrapolate biological pathway knowledge across species**
- Predict relative intrinsic susceptibility
- Generate research hypotheses
- Prioritize testing efforts

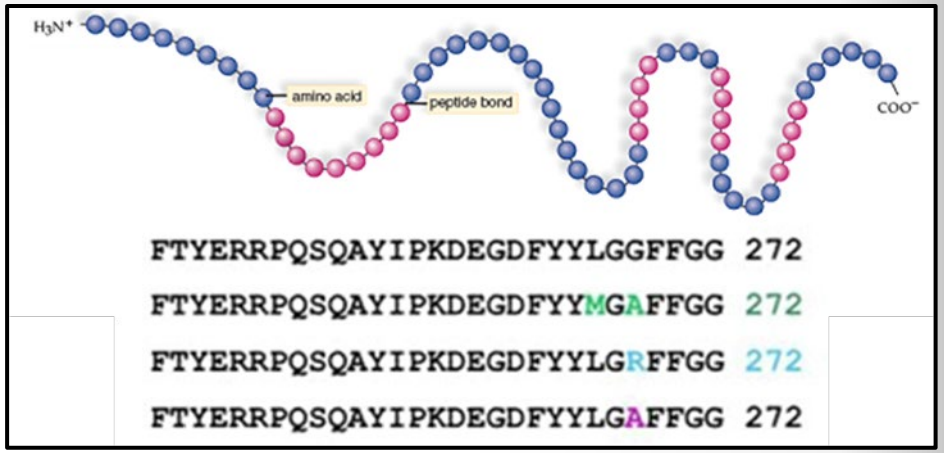




Assessing AR Conservation Across Species Using the SeqAPASS Tool

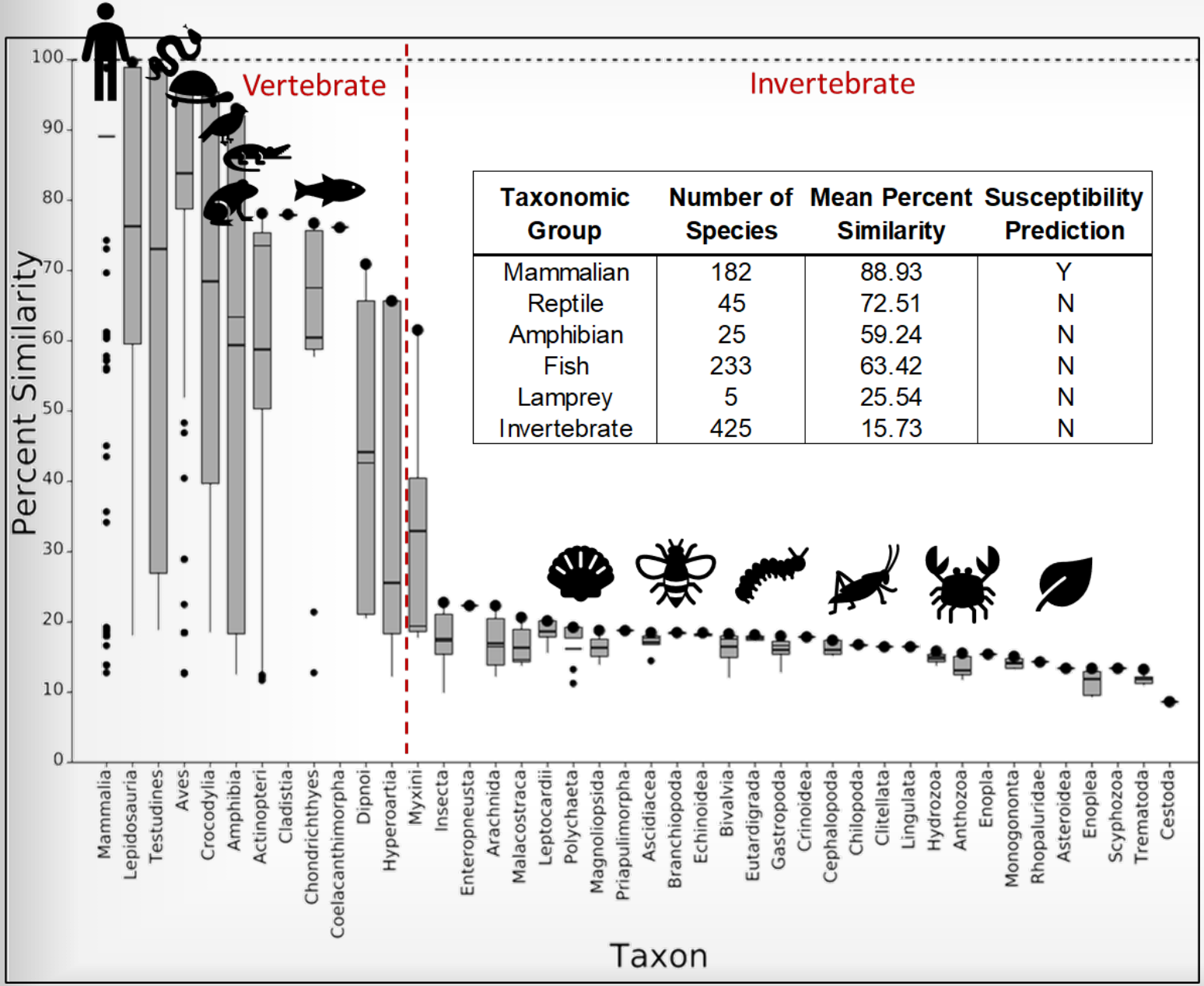


Taxonomic Group	Number of Species	Mean Percent Similarity	Susceptibility Prediction
Mammalian	188	63.85	Y
Reptiles	41	35.12	Y
Amphibians	24	26.04	Y
Birds	117	35.63	Y
Fish	219	26.68	N
Lamprey	5	10.46	N
Invertebrate	428	6.90	N

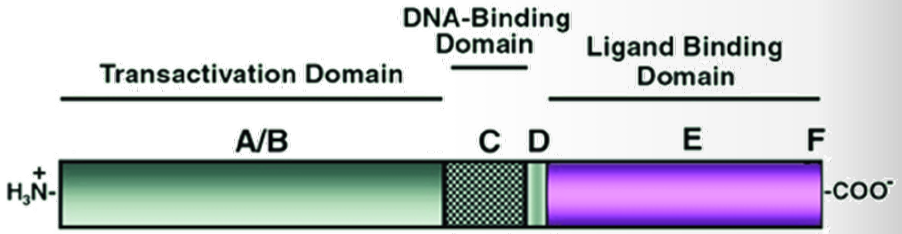


- Level 1 SeqAPASS Analysis
- Primary Amino Acid Sequence Alignments
- Large difference in AR amino acid sequence seen between vertebrates and invertebrates
- Results suggest fish species have lower percent similarity relative to other vertebrate species

Assessing AR Conservation Across Species Using the SeqAPASS Tool



Taxonomic Group	Number of Species	Mean Percent Similarity	Susceptibility Prediction
Mammalian	182	88.93	Y
Reptile	45	72.51	N
Amphibian	25	59.24	N
Fish	233	63.42	N
Lamprey	5	25.54	N
Invertebrate	425	15.73	N

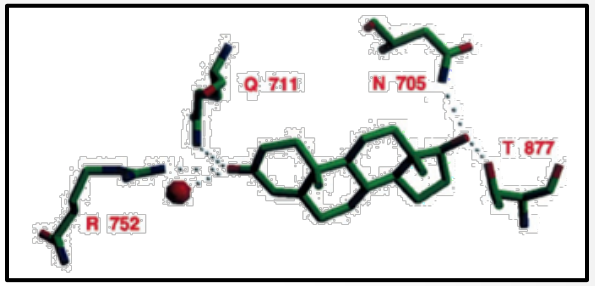


- Level 2 SeqAPASS Analysis
- Primary Amino Acid Sequence Alignments
- Large difference in AR-LBD amino acid sequence seen between vertebrates and invertebrates
- In contrast to Level 1, the AR LBD of fish species show higher similarity to other vertebrates

Assessing AR Conservation Across Species Using the SeqAPASS Tool

Total Match
Partial Match
Susceptible Yes
Not a Match
Susceptible No

Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4
Human	Y	706N	712Q	753R	878T
Rhesus monkey	Y	681N	687Q	728R	853T
Rabbit	Y	687N	693Q	734R	859T
Pig	Y	682N	688Q	729R	854T
Black rat	Y	686N	692Q	733R	858T
Mice	Y	685N	691Q	732R	857T
Mainland tiger snake	Y	575N	581H	622R	747T
Western terrestrial garter snake	Y	575N	581H	622R	747T
Western painted turtle	Y	576N	582Q	623R	748T
Japanese quail	Y	489N	495Q	536R	661T
Chicken	Y	489N	495Q	536R	661T
Zebra finch	Y	470N	476Q	517R	642T
Chinese alligator	Y	508N	514Q	555R	680T
Tropical clawed frog	Y	574N	580Q	621R	746T
African clawed frog	Y	576N	582Q	623R	748T
Reedfish	Y	578N	584Q	625R	750T
Gray bichir	Y	109N	115Q	156R	281T
Rainbow trout	Y	640N	646Q	687R	810T
Fathead minnow	Y	626N	632Q	673R	796T
Zebrafish	Y	656N	662Q	703R	826T
Japanese medaka	Y	531N	537Q	578R	701T
Little skate	Y	525N	531Q	572R	697S
West African lungfish	Y	665N	671Q	712R	833T

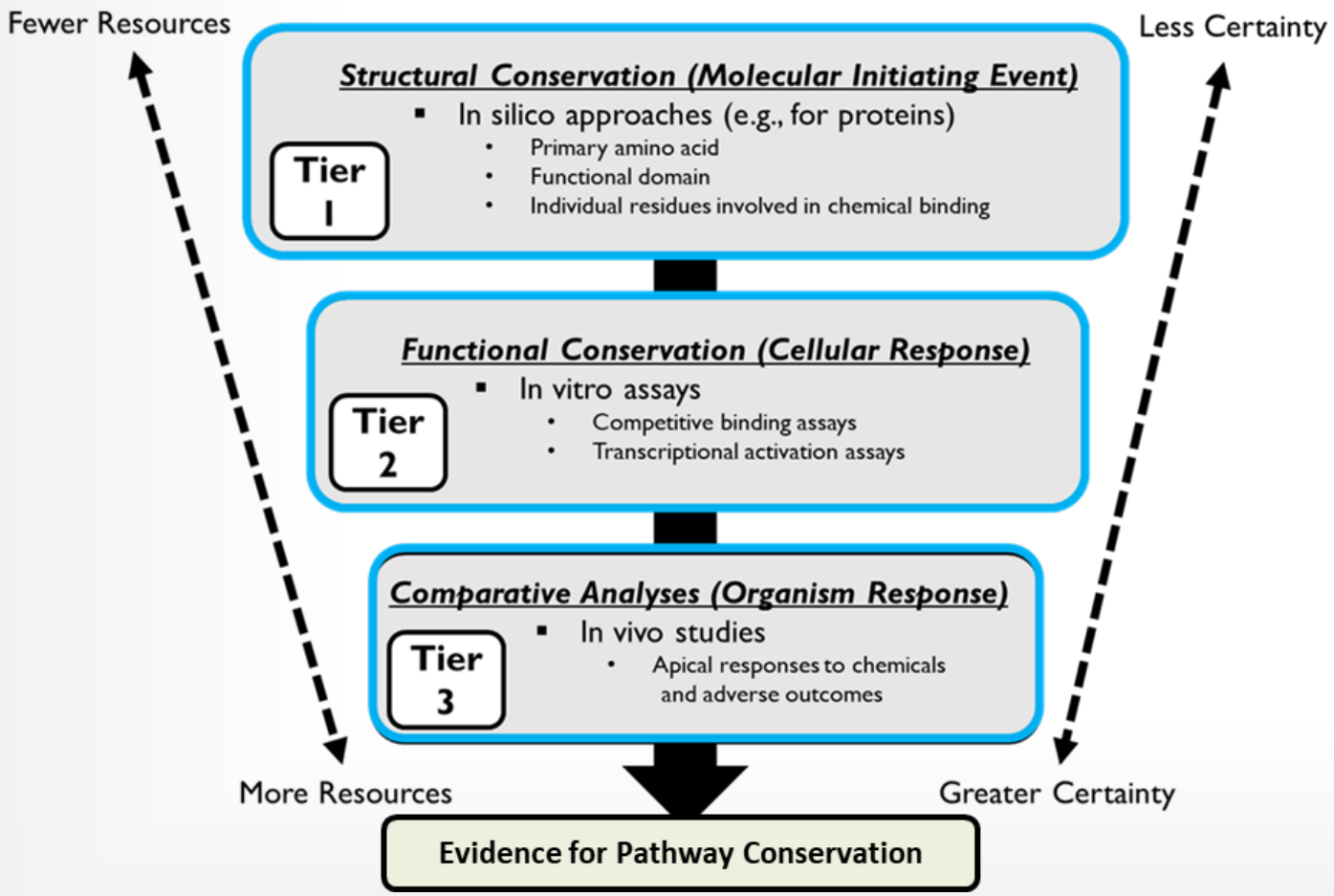


- Level 3 SeqAPASS Analysis
- Close-contact amino acids
- Vertebrate species with available data are predicted to share similar suseptibility

Overall, SeqAPASS analysis suggests chemicals may interact similarly with all mammalian androgen receptors

Evaluating Existing Data to Extrapolate High-Throughput Androgen Receptor Screening Data Across Species

For In Silico tools to be used in a regulatory context it is **essential** to understand how computational predictions relate to empirical data across species



In the lab

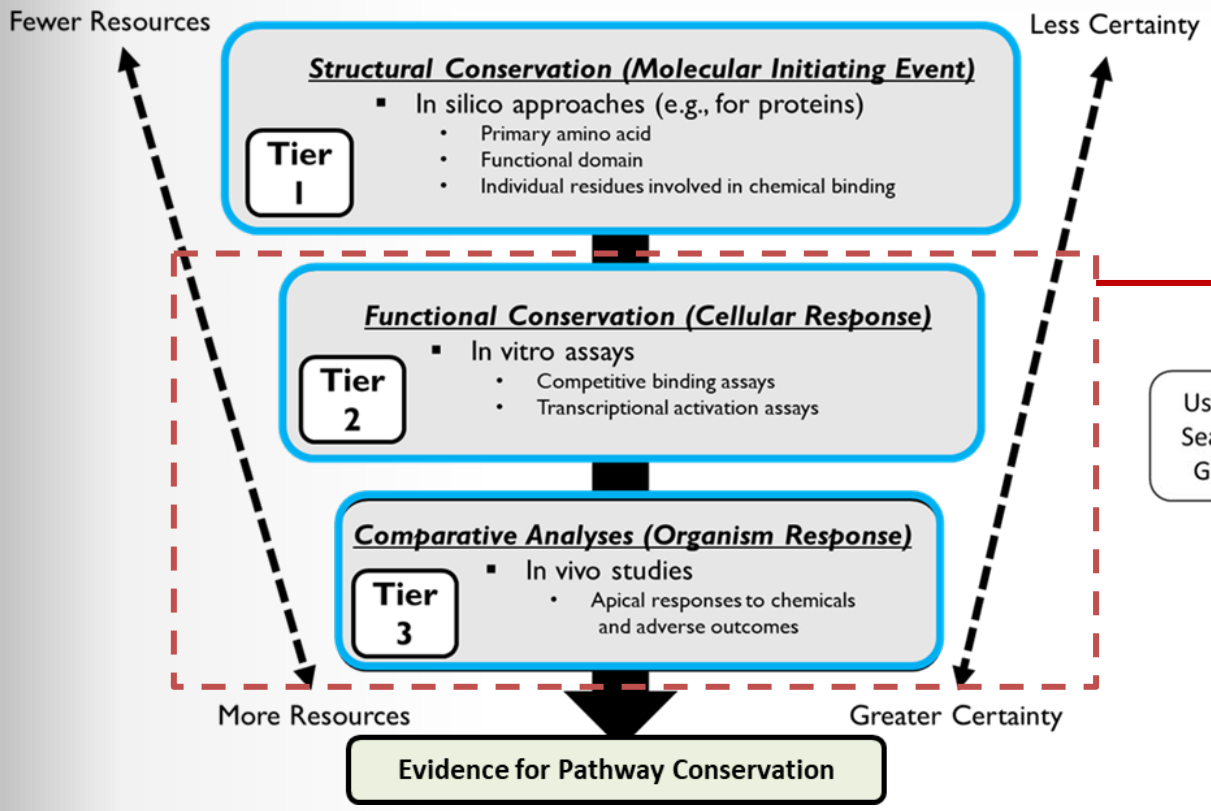
- Cross species In vitro studies
- Cross species In vivo studies
- Chemical proteomics
- Etc.

Out of the lab

- Molecular modeling & docking
- Review of existing evidence
- Etc.

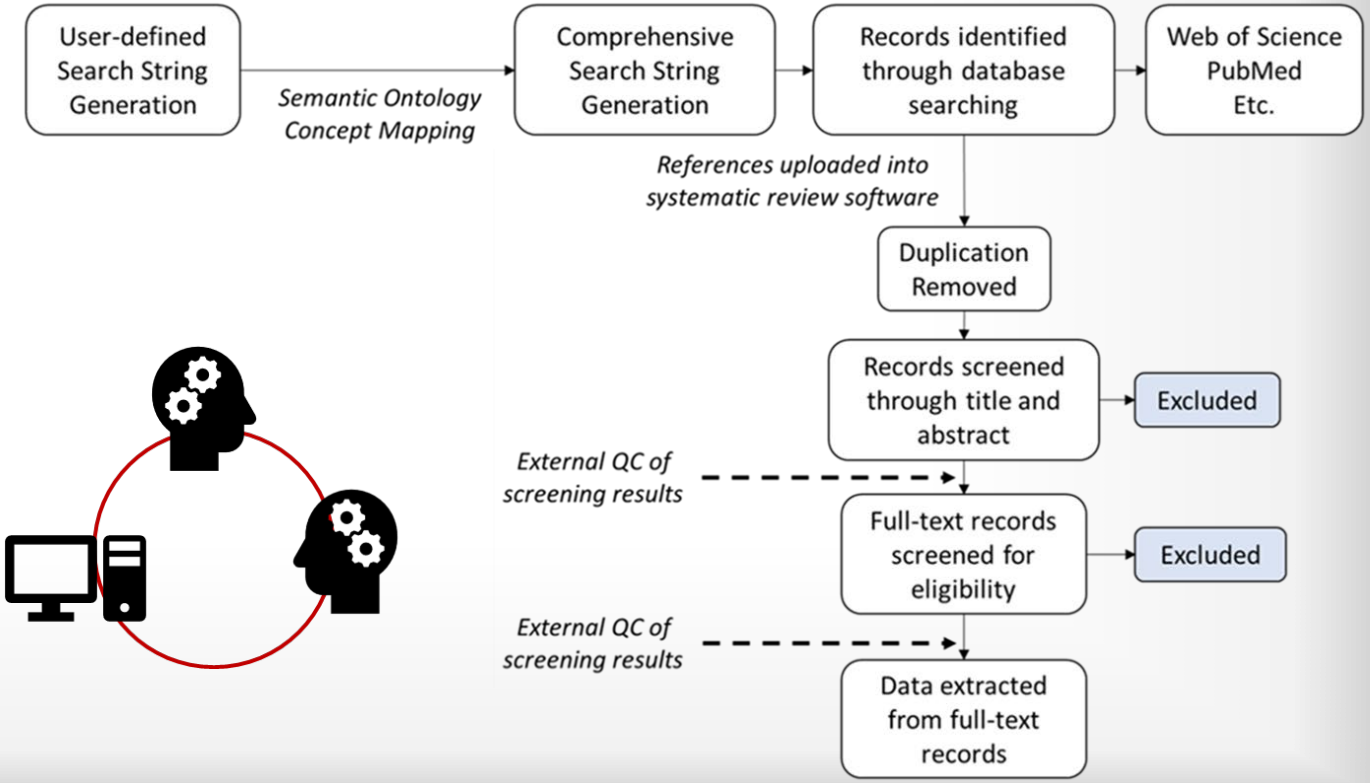
We are currently working across these areas to evaluate and refine computational predictions of cross-species sensitivity

Evaluating Existing Data to Extrapolate High-Throughput Androgen Receptor Screening Data Across Species

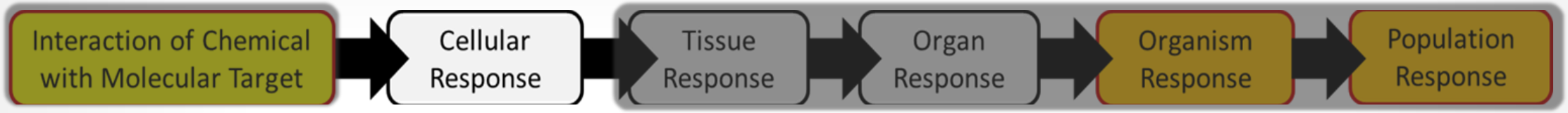


Uses **available tools** and **existing data** to assemble evidence for pathway conservation that can be used to define risk assessment applications

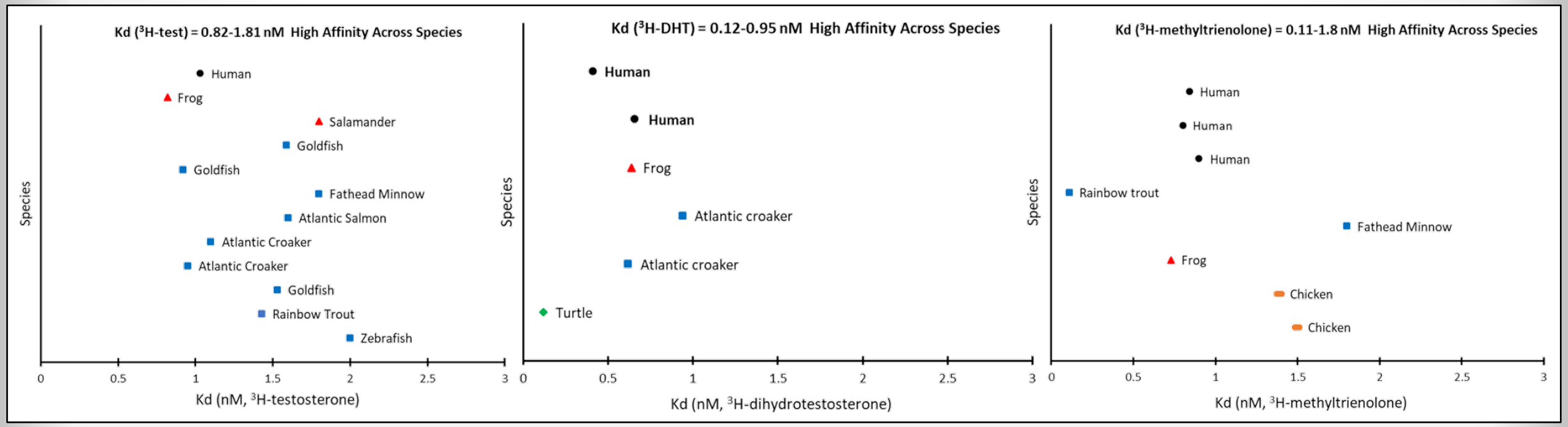
Systematic Literature Review



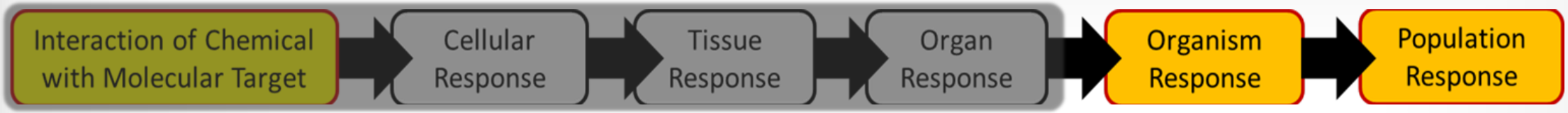
Cross-Species In Vitro Androgen Receptor Responses



- Preliminary results (small sample of initial papers) suggest functional conservation of AR across vertebrate species for three high-affinity AR ligands



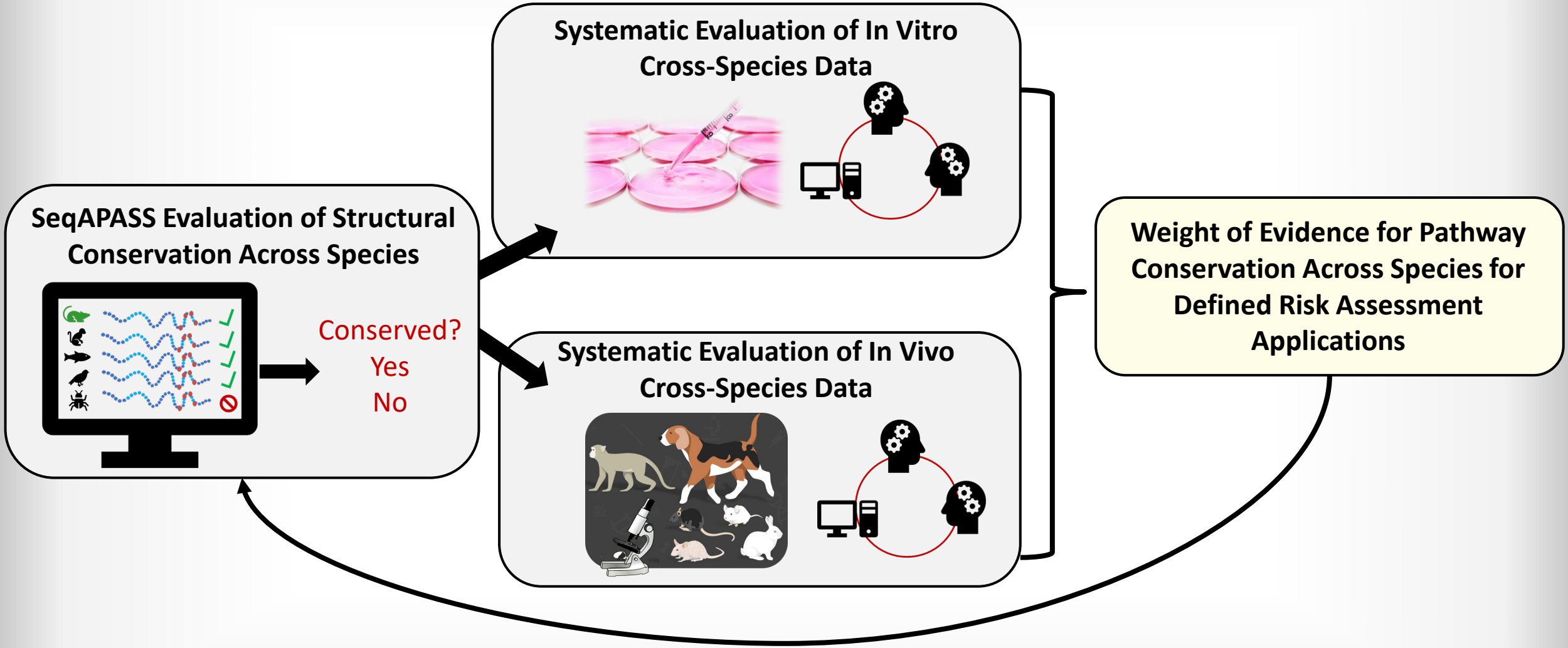
Cross-Species In Vivo Androgen Receptor Responses



- Preliminary results (small sample of initial papers) suggest comparative AR across vertebrate species for three high-affinity AR ligands

	Vertebrate Class			
Chemical	**Fish**	**Reptile**	**Amphibian**	**Bird**
Testosterone (endogenous androgen)	◦Female sex reversal	◦Female development of male sex characteristics ◦Masculinized gonad tissue ◦Altered population sex-rations towards male-based populations	◦Altered population sex-rations towards male-based populations	◦Cloacal gland induction ◦Increase in crowing behavior
Methyltestosterone (synthetic androgen)	◦Reduced gonadosomatic index	◦Female development of male sex characteristics ◦Masculinized gonad tissue ◦Altered population sex-rations towards male-based populations	◦Altered population sex-rations towards male-based populations	◦Reduced egg laying in females
17ß-trenbolone (environmental androgen)	◦Female development of male secondary sex characteristics ◦Reduced circulating E2 Levels ◦Masculinized gonad tissue ◦Reduced vitellogenin levels	◦Female development of male secondary sex characteristics ◦Masculinized gonad tissue ◦Altered population sex-rations towards male-based populations	◦Altered population sex-rations towards male-based populations ◦Female development of male secondary sex characteristics ◦Masculinized gonad tissue	◦Cloacal gland induction ◦Altered population sex-rations towards male-based populations

Evaluating Existing Data to Extrapolate High-Throughput Androgen Receptor Screening Data Across Species



- Apply pathway to other targets of interest
- Repeat process to account for the emergence of new information

Mentor: Carlie LaLone (ORD)

Collaboration

Scott Lynn (OSCP)

Kristan Markey (OSCP)

Thanks!

Any questions?

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

Anyone can use SeqAPASS to help inform their own research questions!
If you are interested in using SeqAPASS we are happy to help!

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