



# Gathering Evidence of Endocrine Pathway Conservation for Cross-Species Extrapolation Using New Approach Methods

**Sara M. Vliet**

ORISE Postdoctoral Fellow  
Great Lakes Toxicology & Ecology Division  
Office of Research and Development  
U.S Environmental Protection Agency



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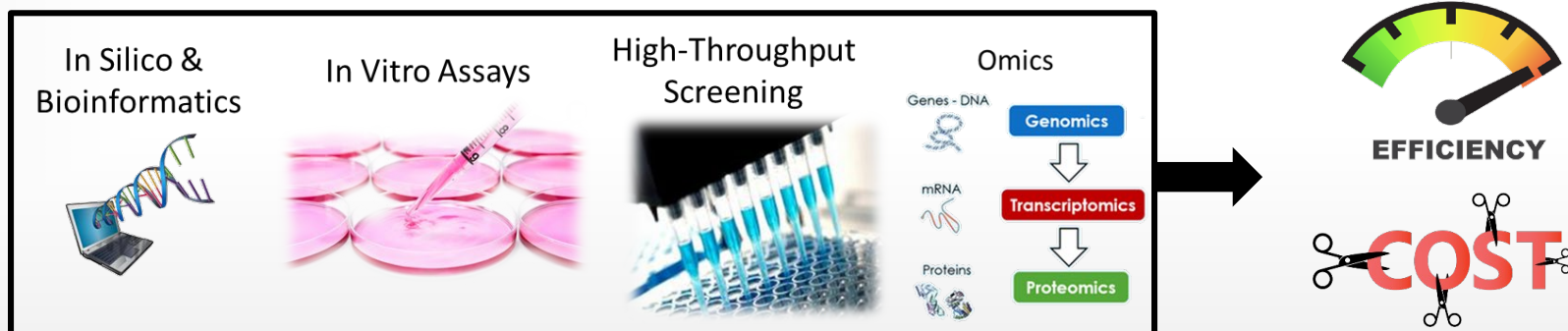
# The Need for New Approach Methods (NAMs)

- The US Environmental Protection Agency's Endocrine Disruptor Screening Program (EDSP) is required to screen pesticidal chemicals for their potential to adversely impact human health and the environment through perturbation of endocrine pathway targets.



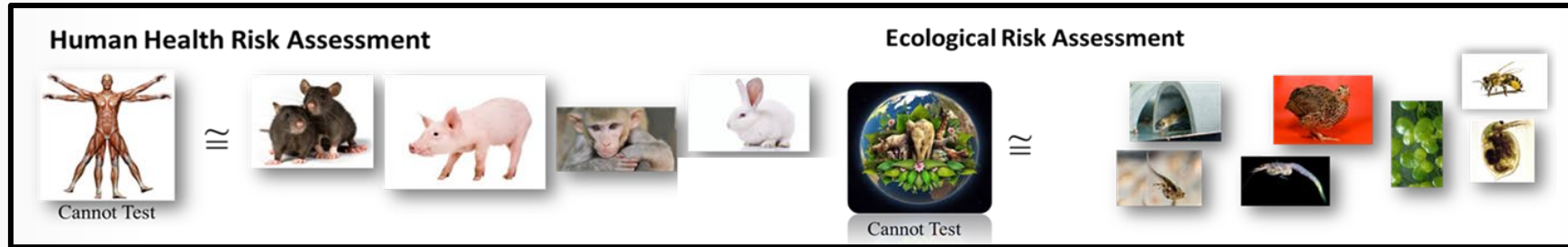
- Limited data for many compounds, limited resources for traditional toxicity testing, and international efforts to reduce animal use all necessitate the development of **new approach methods (NAMs)**

## New Approach Methods (NAMs)

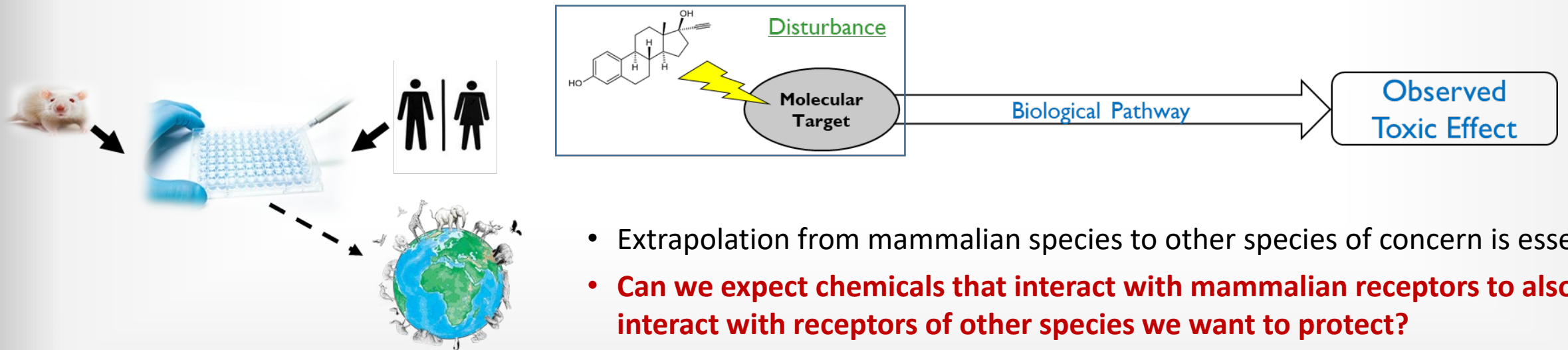


# Surrogate Species in Toxicity Testing

- 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 mammalian cells
- Knowledge of the molecular target be linked to an adverse outcome of regulatory concern

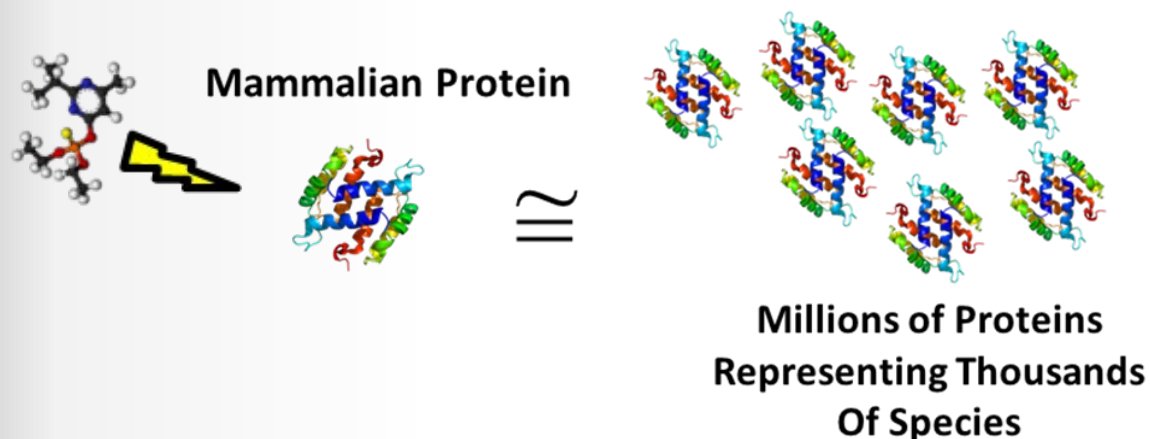


- Extrapolation from mammalian species to other species of concern is essential
- Can we expect chemicals that interact with mammalian receptors to also interact with receptors of other species we want to protect?**



# SeqAPASS: Sequence Alignment to Predict Across Species Susceptibility

- Online, publicly available tool for understanding **target conservation** across thousands of diverse species
- Facilitates rapid and quantitative assessment of protein similarity and provides a foundation for predicting the taxonomic domain of applicability
- Developed with both researchers and risk assessors in mind



## SeqAPASS Applications

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

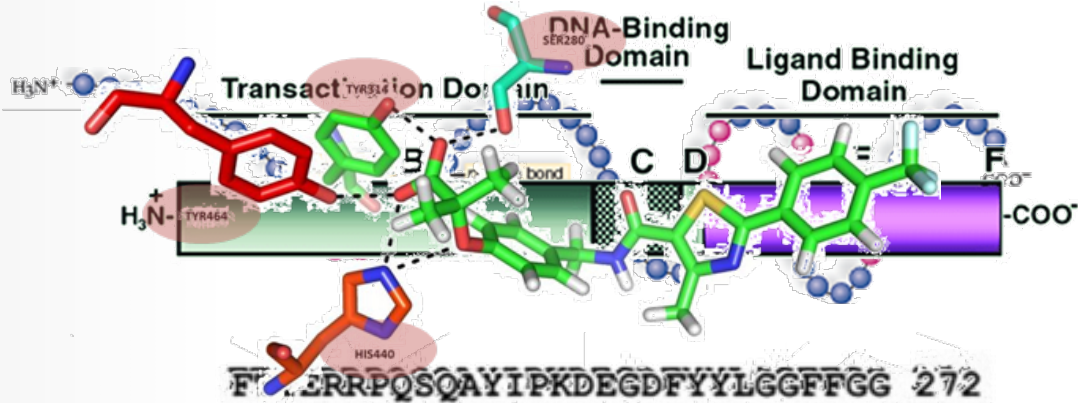
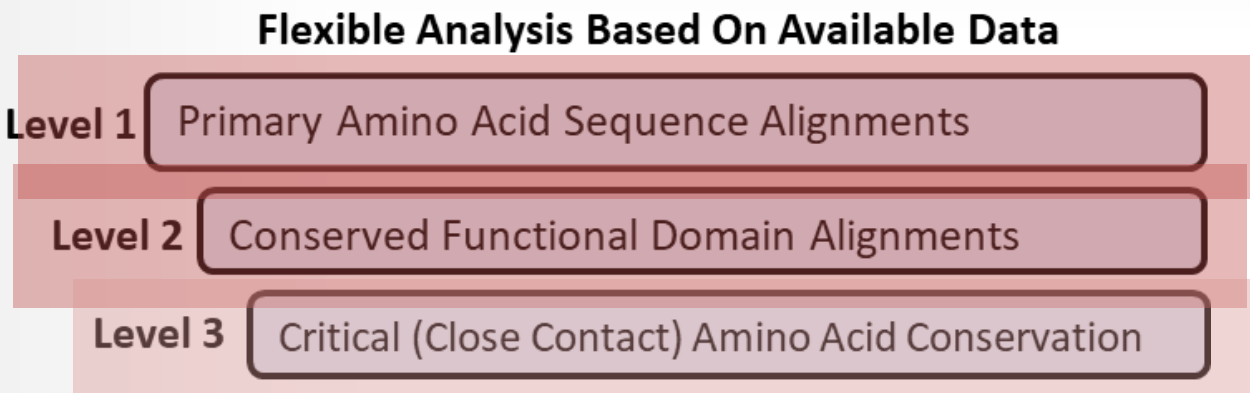


Sequence Alignment to Predict Across Species  
Susceptibility (SeqAPASS): A Web-Based Tool for  
Addressing the Challenges of Cross-Species  
Extrapolation of Chemical Toxicity

Carlie A. LaLone,<sup>\*,1</sup> Daniel L. Villeneuve,<sup>\*</sup> David Lyons,<sup>†</sup> Henry W. Helgen,<sup>‡</sup>  
Serina L. Robinson,<sup>§,2</sup> Joseph A. Swintek,<sup>¶</sup> Travis W. Saari,<sup>\*</sup> and  
Gerald T. Ankley<sup>\*</sup>

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

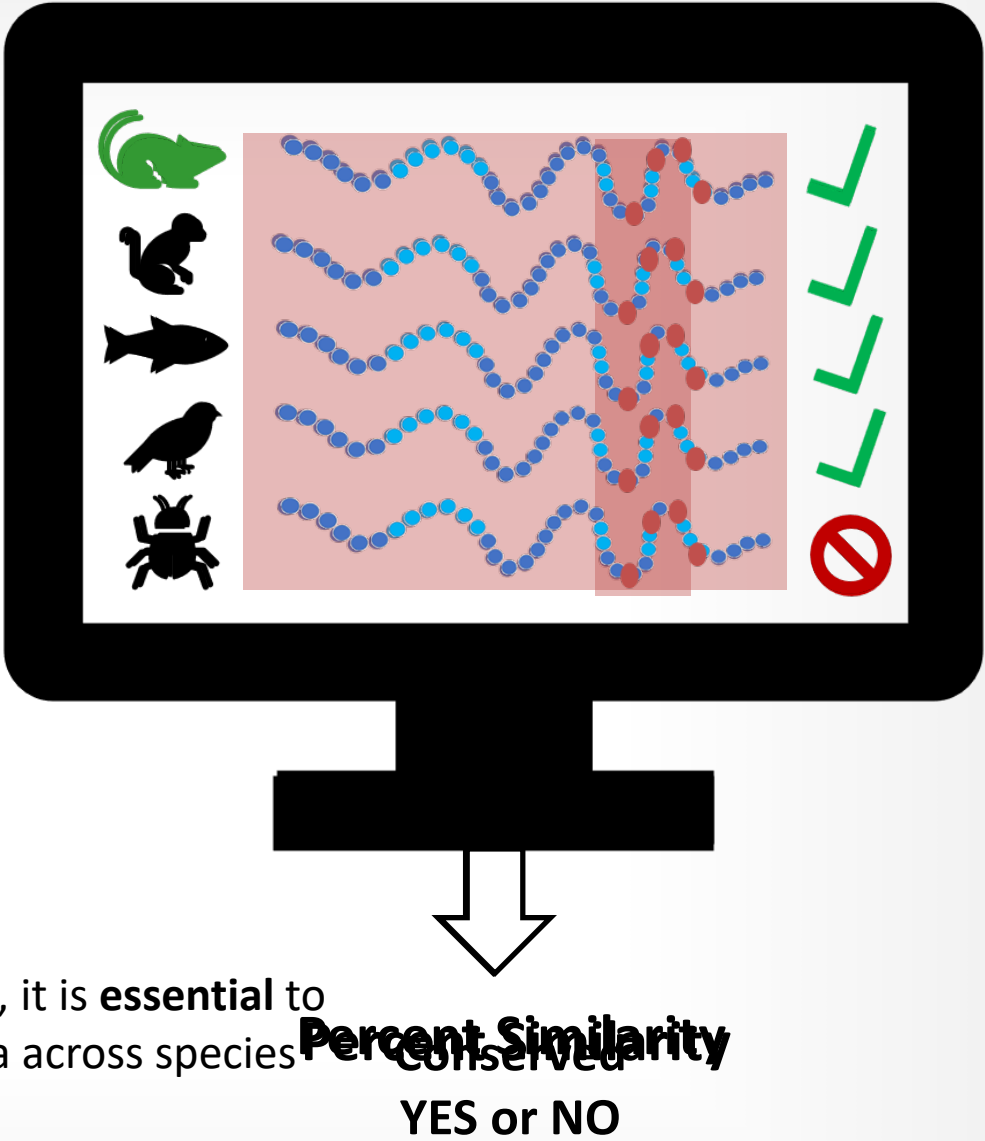
# SeqAPASS: The Basics



For SeqAPASS to be used in a regulatory context and the EDSP pipeline, it is **essential** to understand how our computational predictions relate to empirical data across species

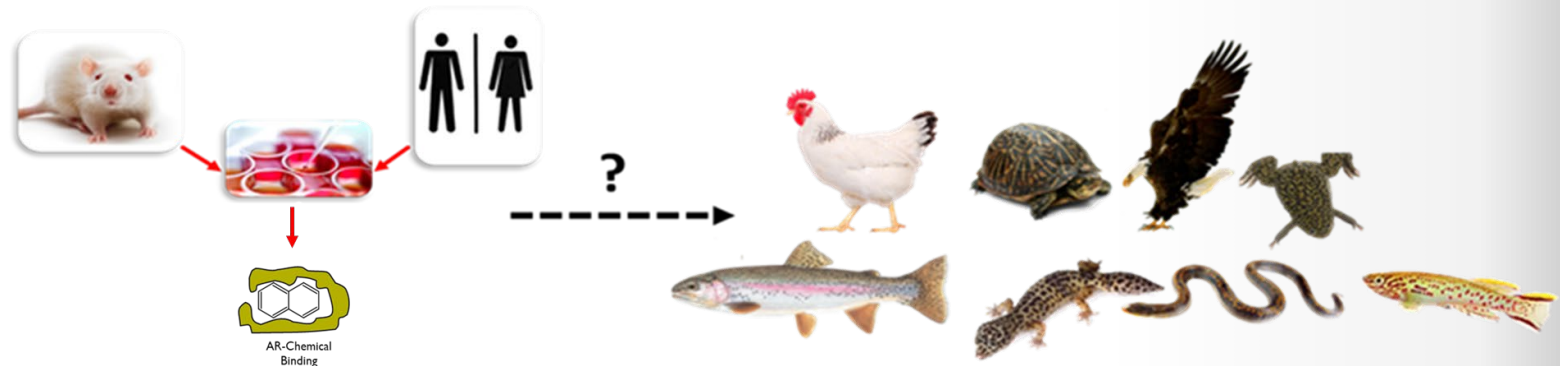
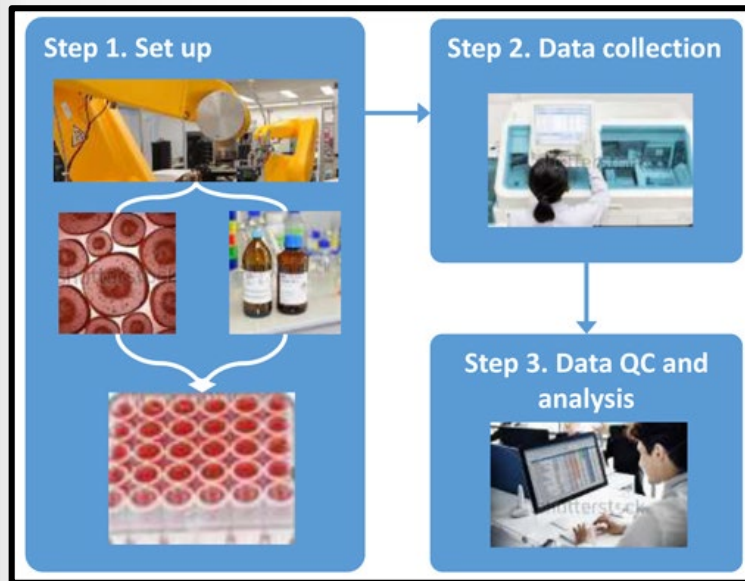
1. Evaluating **existing data and literature**

- 2. Conducting **in vitro** molecular biology studies
- 3. Conducting further **in silico** work (E.g. molecular docking, etc.)



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

- The androgen receptor (AR) is an important endocrine target for many environmental chemicals
- Exposure to AR-binding compounds can result in impaired endocrine physiology and reproductive behaviors in exposed animals.

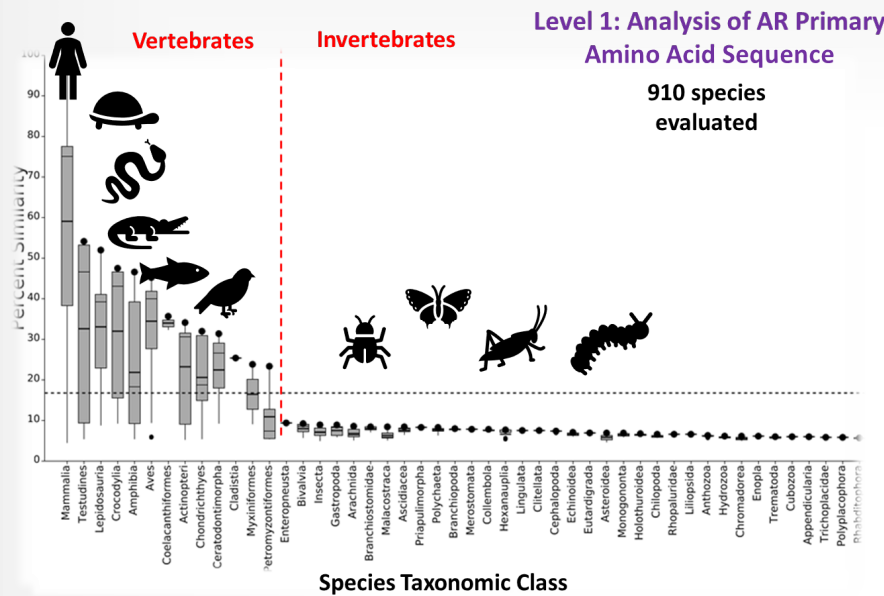


## Guiding Question:

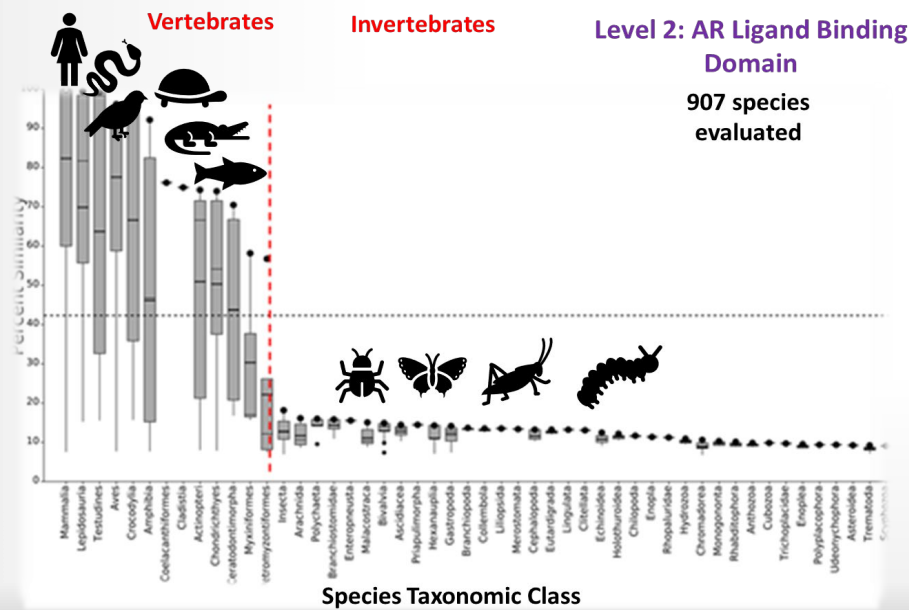
Can we expect chemicals that interact with AR in mammalian screening models to reflect potential toxicity across ecologically-relevant species?

# Assessing AR Conservation Across Species Using the SeqAPASS Tool

1.



2.



3.

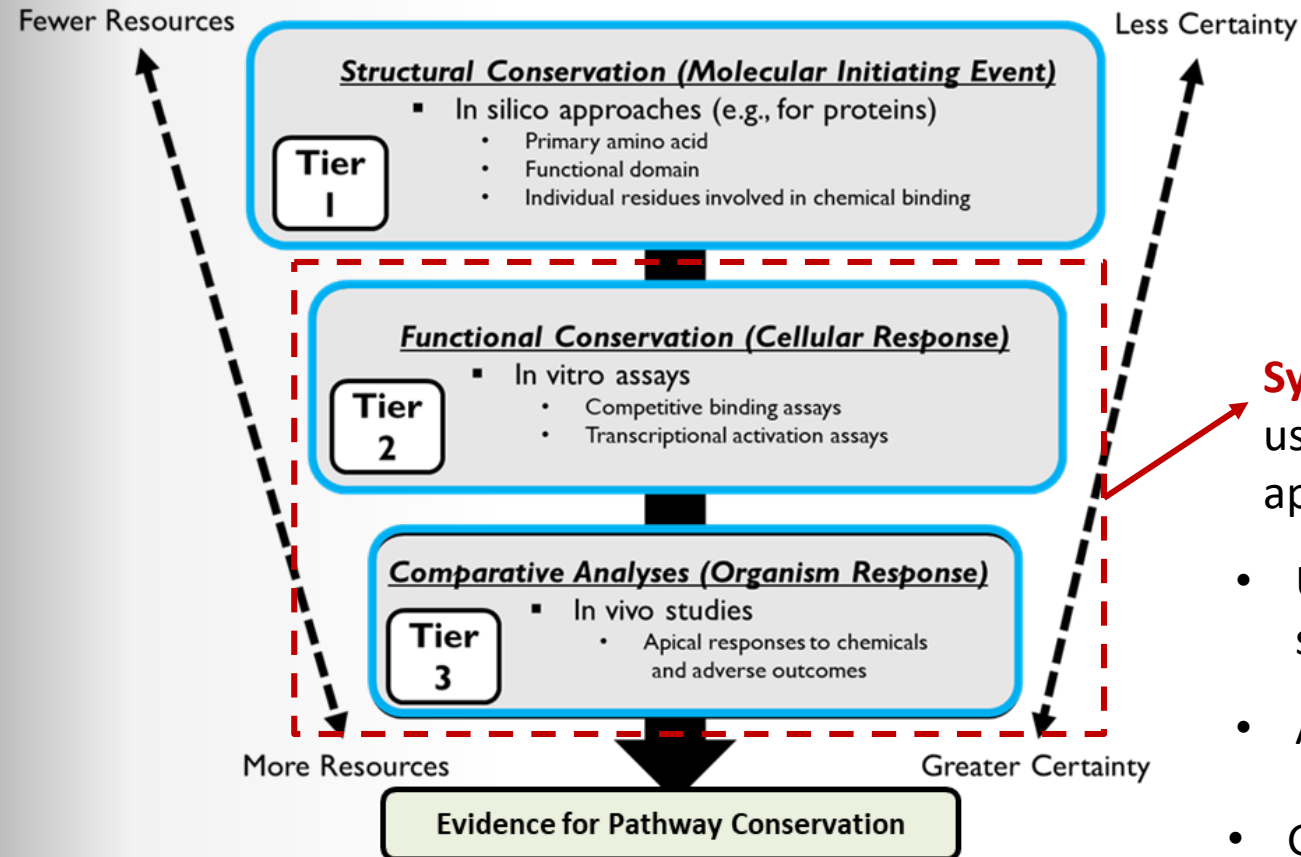
**Level 3: Analysis of Conservation of Individual Amino Acid Residues**  
250 species evaluated

Taxonomic Group	# of Spp.	Shared Susceptibility
Mammals	117/1	Yes/No
Lizards, Snakes	11	Yes
Turtles	3	Yes
Birds	58	Yes
Crocodiles, Alligators	4	Yes
Amphibians	13	Yes
Coelacanths	2	Yes
Eel-shaped	1	Yes
Bony Fish	87/1	Yes/No
Sharks, Rays	4	Yes
Lungfish	2	Yes

- Across all three levels, SeqAPASS results suggest conservation of AR across vertebrate species
- Overall, these predictions suggest that chemicals that bind and activate AR in mammalian-based assays, are likely to interfere with AR in other vertebrate species
- Line of evidence for pathway conservation



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



> *Environ Toxicol Chem.* 2016 Nov;35(11):2806-2816. doi: 10.1002/etc.3456. Epub 2016 Jun 28.

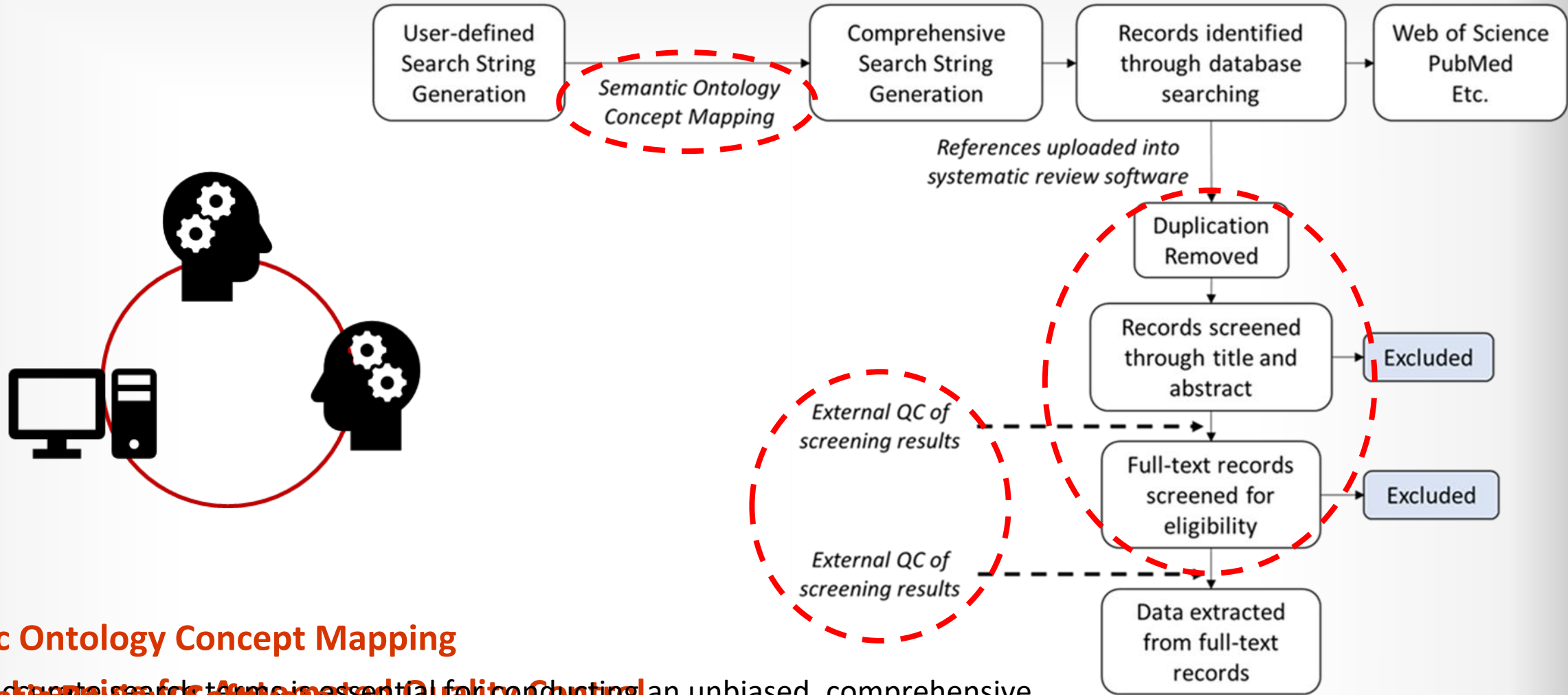
Evaluation of the scientific underpinnings for identifying estrogenic chemicals in nonmammalian taxa using mammalian test systems

Gerald T Ankley<sup>1</sup>, Carlie A LaLone<sup>2</sup>, L Earl Gray<sup>3</sup>, Daniel L Villeneuve<sup>2</sup>, Michael W Hornung<sup>2</sup>

**Systematic Literature Review:** A type of literature review that uses systematic methods to collect secondary data, critically appraise research studies, and synthesize findings

- Using existing evidence (literature), we can evaluate the scientific basis of our cross-species predictions
- Advances in data science can improve this workflow
- Gathering in vivo and in vitro data from vertebrate species exposed to known androgenic compounds provides **additional lines of evidence** for the conservation of the biological pathway across species

# Incorporation of Technical Advances and Tools for Improved Systematic Review

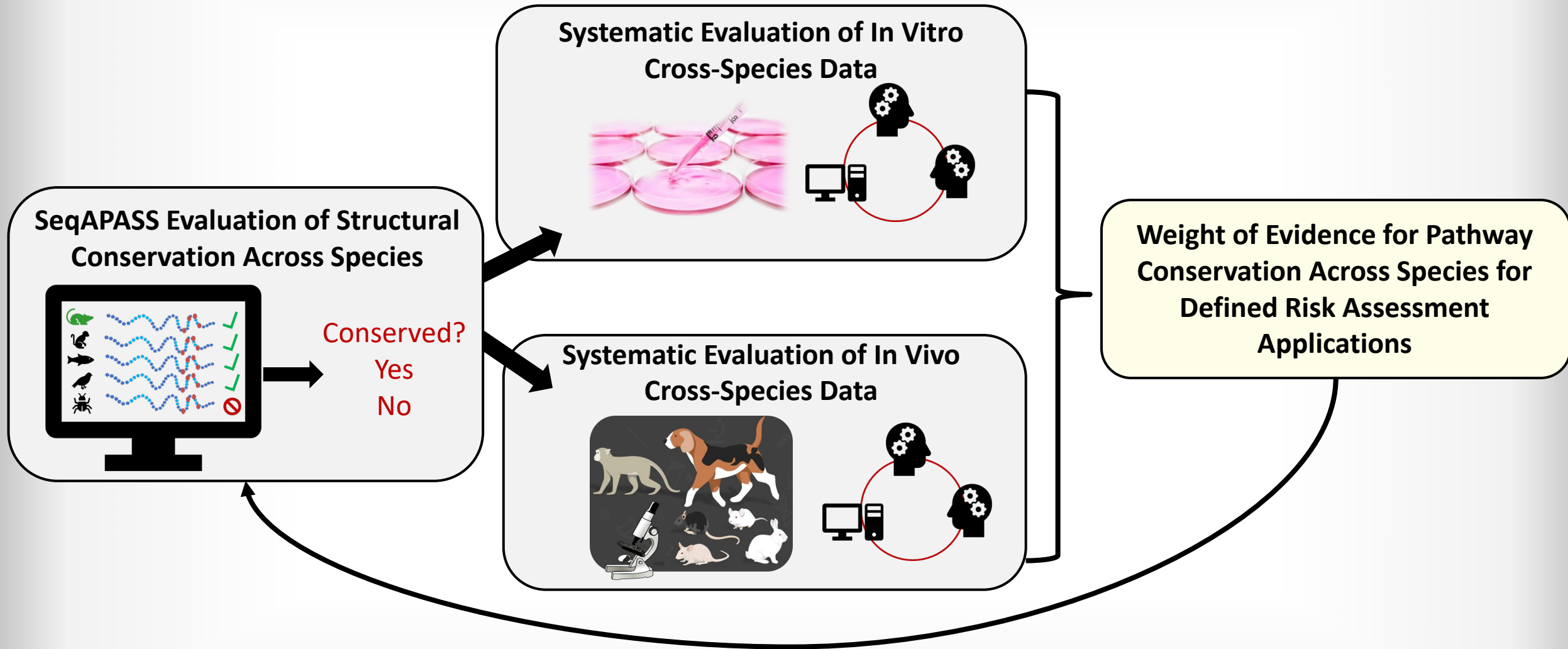


## Semantic Ontology Concept Mapping

### Systematic Review Software and Quality Control

- Using accurate search terms is essential for conducting an unbiased, comprehensive survey of the literature landscape
- Collaborative web-based systematic literature review software provides a platform for unbiased article evaluation and data collection
- Scientific language is often non-standard, redundant, and mischaracterized
- Semantic ontology mapping approaches can develop comprehensive literature search strings by expanding vocabulary based on knowledge of related concepts

# 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



# Gathering Evidence of Endocrine Pathway Conservation for Cross-Species Extrapolation Using New Approach Methods

## Wrapping it up

1. The US EPA SeqAPASS tool is a New Approach Method that can be used to computationally examine biological pathway conservation across taxa and predict chemical susceptibility across diverse species
2. Using systematic literature review techniques and technological advances in data curation science, we demonstrated a framework for the evaluation of existing data.
3. This pipeline provides a means of evaluating computational predictions.
4. Overall, we provide a framework for addressing the conservation of molecular targets across species and understanding the degree to which mammalian-based methods can accurately reflect chemical interactions with non-mammalian targets.







# Thanks!

## Any questions?



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

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

[Vliet.Sara@epa.gov](mailto:Vliet.Sara@epa.gov)  
[LaLone.Carlie@epa.gov](mailto:LaLone.Carlie@epa.gov)  
[Blatz.Donovan@epa.gov](mailto:Blatz.Donovan@epa.gov)