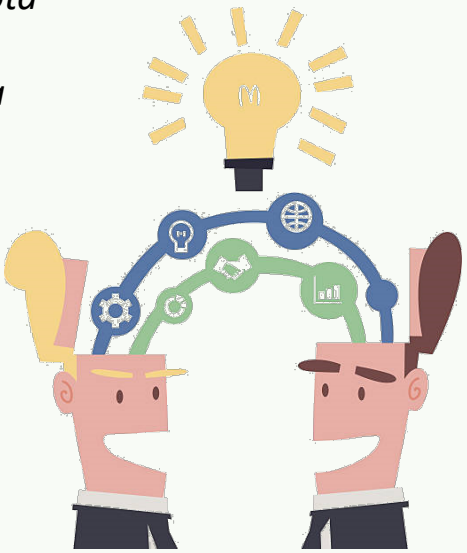
Bridging the Gap: Integrating Systematic Review Strategies and New Approach Methodologies for the Cross-Species Extrapolation of Endocrine Targets

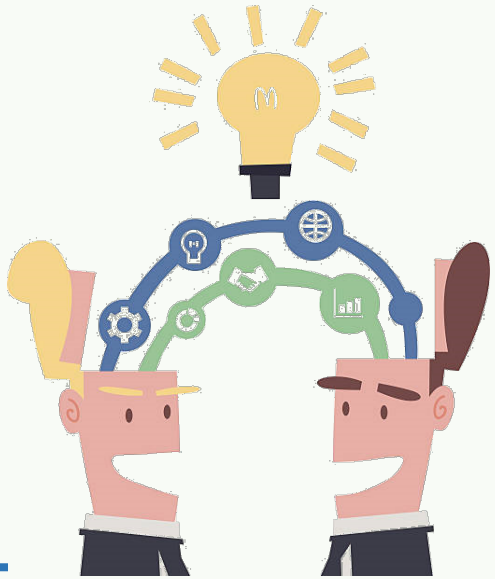
Sara M. Vliet[a], Scott G. Lynn[b], Kristan Markey[b], Carlie A. LaLone[c]

[a] *ORISE Research Participation Program, Great Lakes Toxicology and Ecology Division, Duluth, Minnesota*
[b] *Office of Pesticide Programs, US Environmental Protection Agency, Washington DC*
[c] *Great Lakes Toxicology and Ecology Division, US Environmental Protection Agency, Duluth, Minnesota*



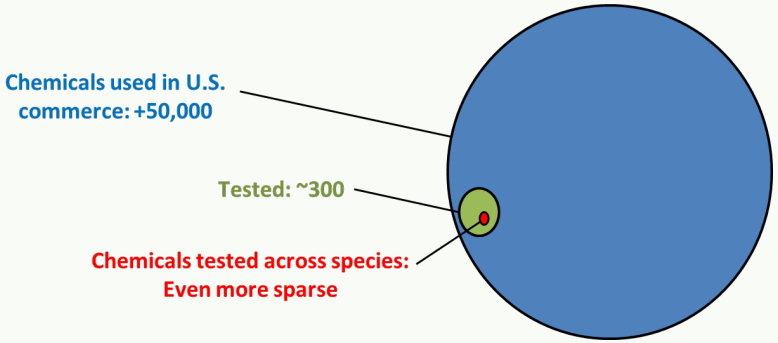
Society for Environmental Toxicology and Chemistry (SETAC)
North America 42nd Annual Meeting
November 14-18, 2021, Virtual

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the US EPA.

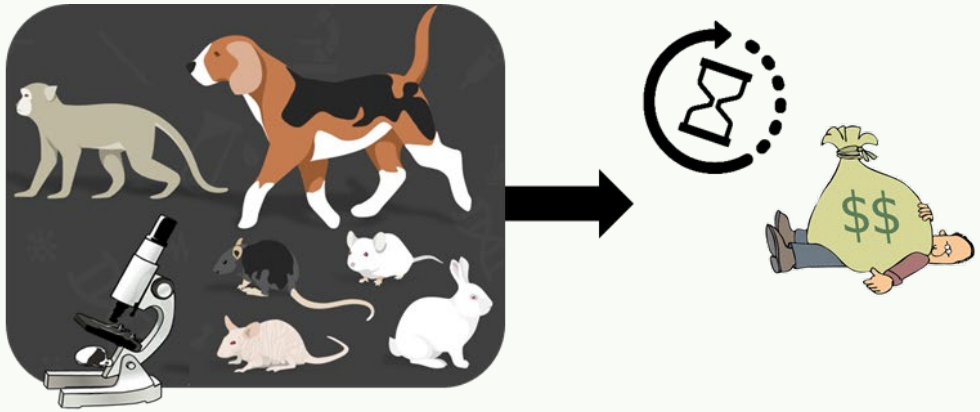


US EPA Endocrine Disruptor Screening Program (EDSP)

- The U.S. EPA's Endocrine Disruptor Screening Program (EDSP) is tasked with evaluating thousands of chemicals for their potential to adversely impact human health and the environment through perturbation of endocrine pathways
- 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

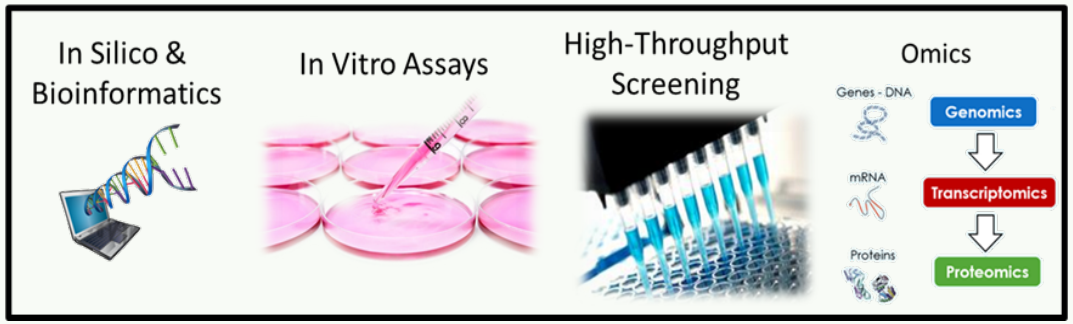


Whole Animal Testing Methods



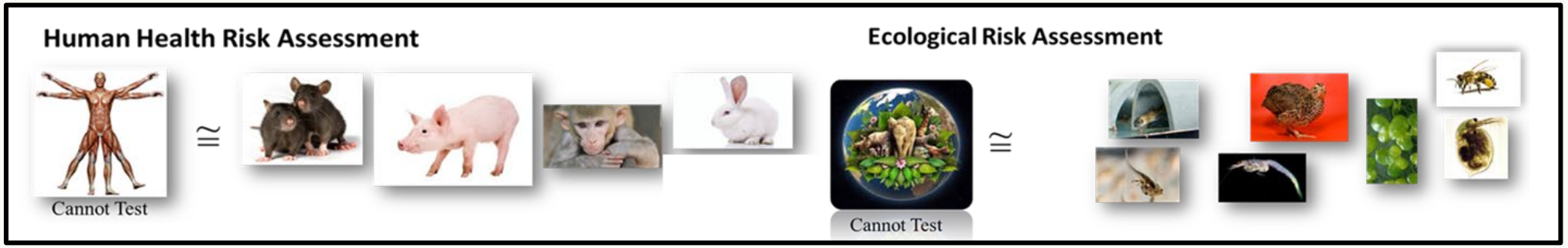
- Limited data for many compounds, limited resources for traditional toxicity testing, and international efforts to reduce animal use all necessitate the development of **new approach methods (NAMs)**

New Approach Methods (NAMs)



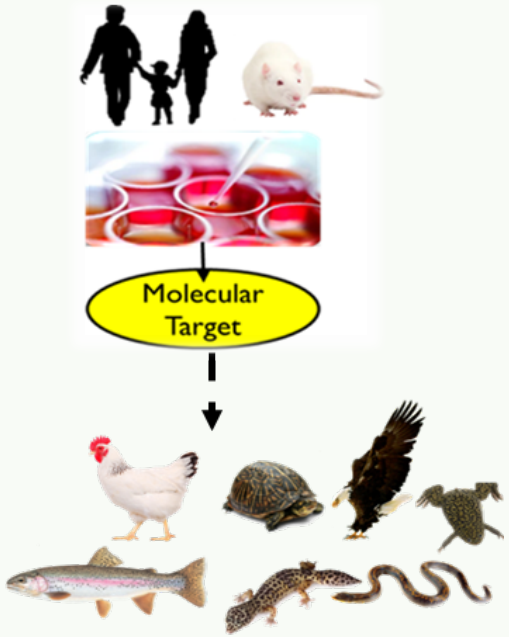
The Challenge of Cross-Species Extrapolation

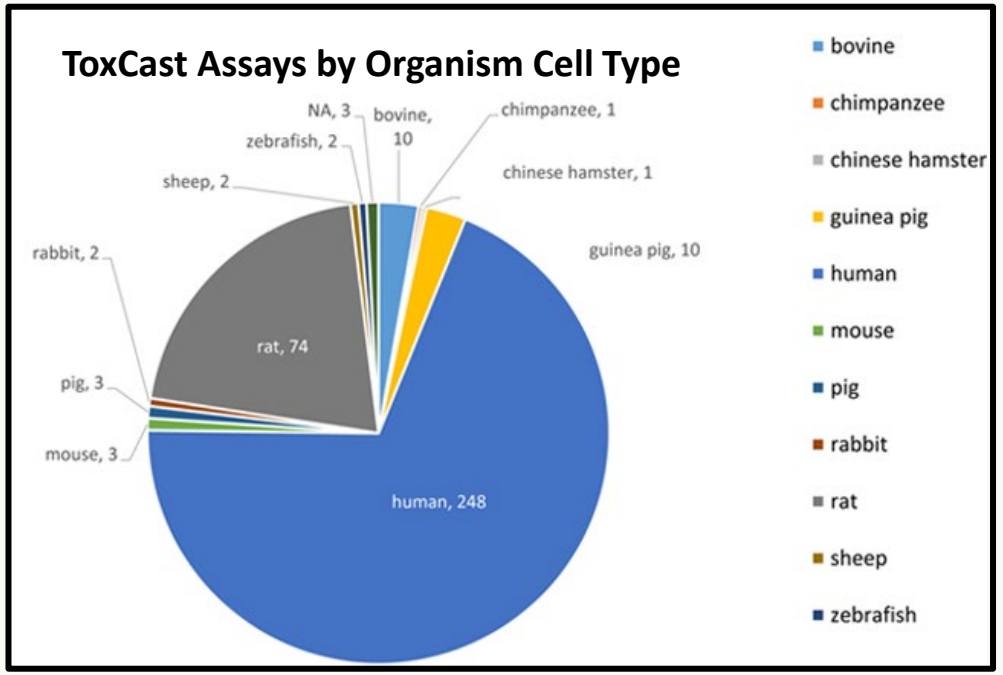
- In whole animal testing, it is assumed that the sensitivity of species to a chemical is a function of their relatedness



- Many NAMs also rely on select model species which may not be sufficient to evaluate the broad diversity of species potentially impacted by chemical exposures

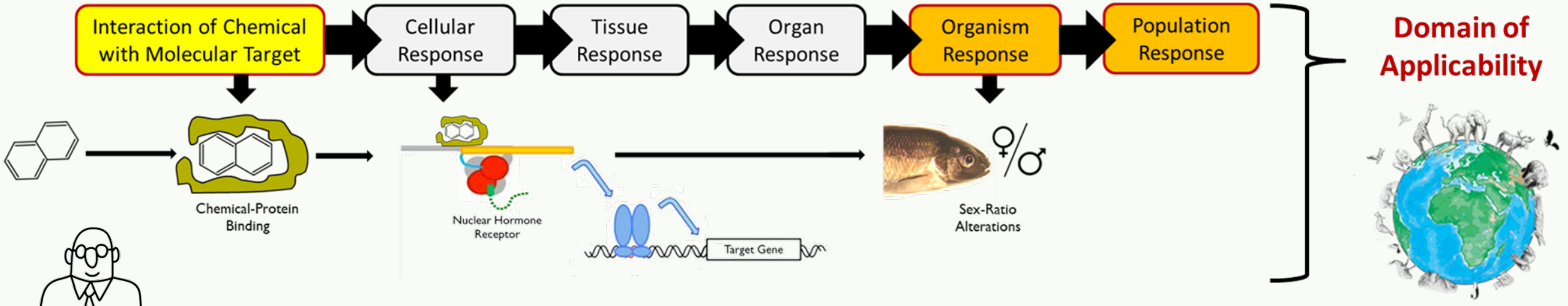
- For example, the US EPA ToxCast program rapidly screens chemicals, identifies potential bioactivity, and helps inform putative **molecular targets** for chemicals different cell types.

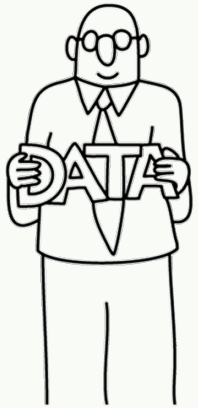




Adverse Outcome Pathways: Linking the Molecular to the Organism

- For NAMs such as SeqAPASS and high-throughput screening to play useful roles in decision-making, we need to understand how changes at the molecular level in cells and tissues are related to apical adverse outcomes
- This can be done through the Adverse Outcome Pathway (AOP) framework, which anchors molecular and cell-level responses, such as those obtained through many NAMs, to in vivo ecological endpoints of regulatory concern
- AOPs also provide a framework for understanding **domain of applicability** (i.e., for what species is this pathway applicable?)





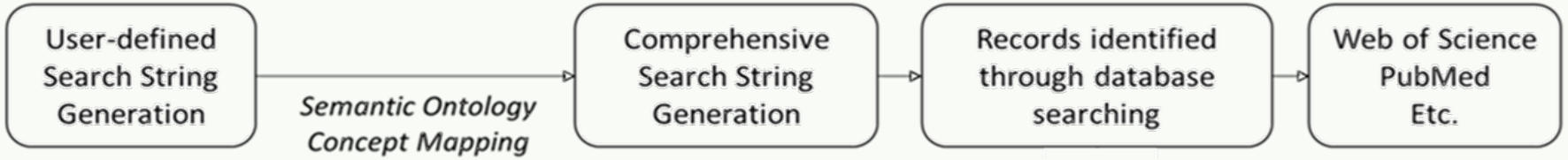
However, the development of a complete and useful AOP requires high-quality chemical toxicity data across different levels of biological organization, different taxa, and different life-stages

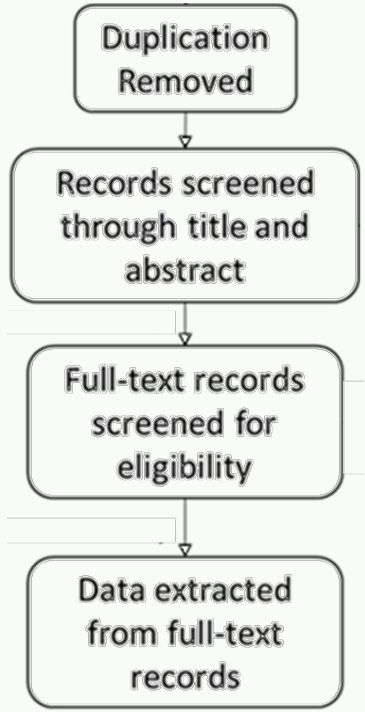
1. Conduct **in silico** modeling work
2. Conduct **in vitro** molecular biology studies
3. Conduct **in vivo** laboratory exposures
4. Evaluate existing data and literature

Incorporating Existing Evidence to Support NAMs Development

Center for evidence-based management

- Systematic literature review utilizes transparent methods to collect data, critically assess research studies, and synthesize findings
- Treats a literature search like an experimental study, providing protocols and details such that the review may be independently replicated.



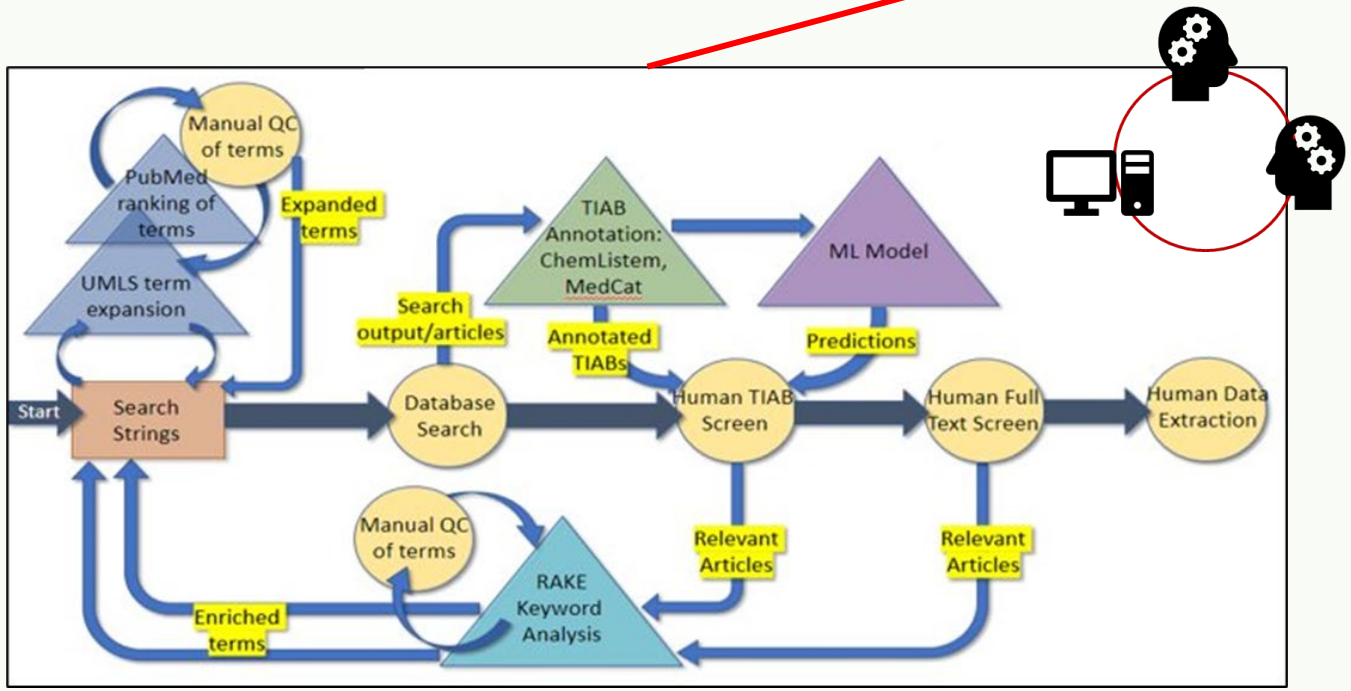


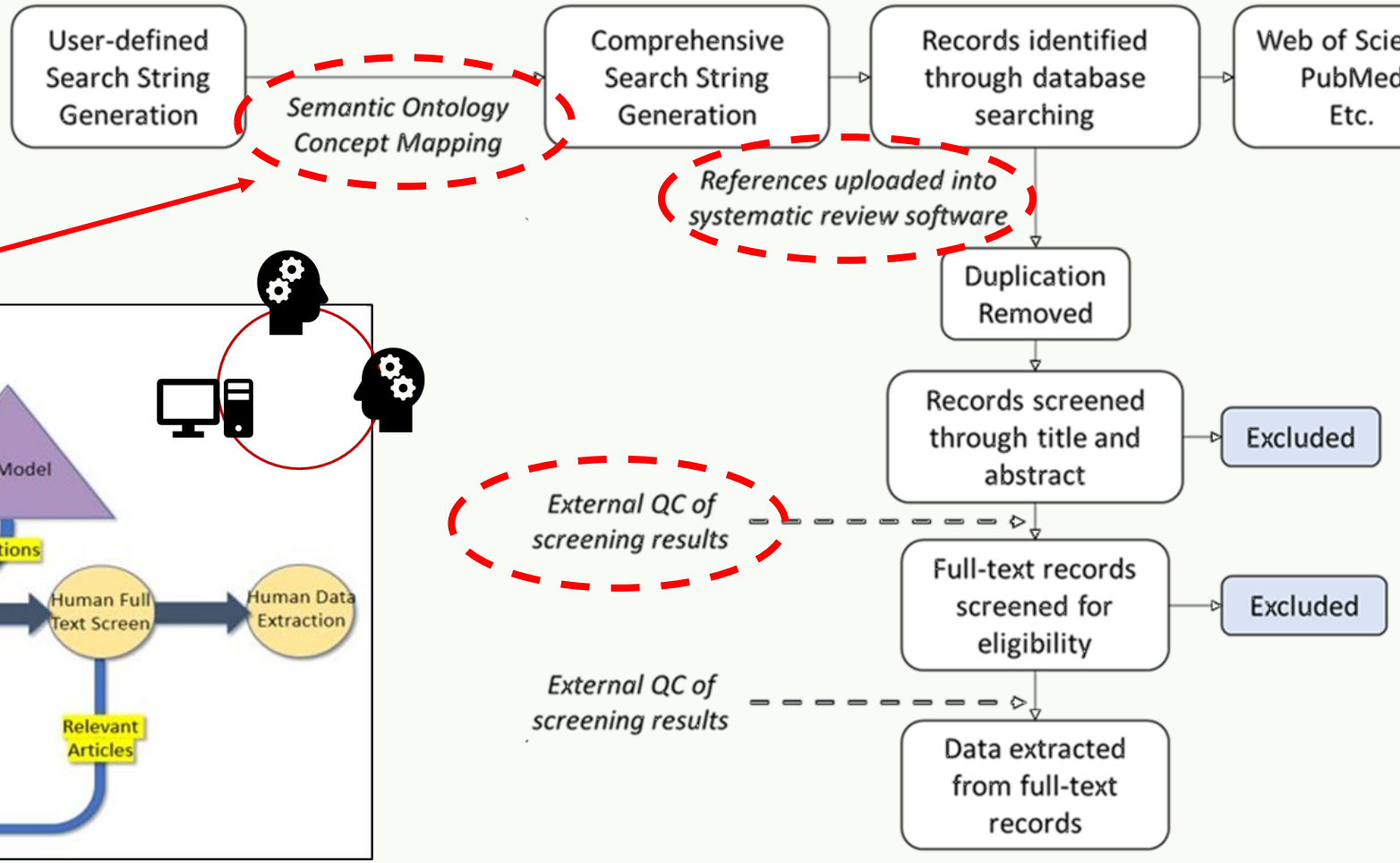
Compared to traditional literature reviews, systematic review methods;

1. Reduce testing resources by broadly sampling existing data
2. Increase transparency, objectivity, and reproducibility of information used to reach a conclusion
3. Reduce the influence of reviewer bias and error in the evaluation process

Emerging tools for Systematic Literature Review

- Advances in data science have contributed to new tools designed to decrease bias in systematic reviews, as well as the resources required





Case Study: Cross-Species Extrapolation of the Androgen Receptor

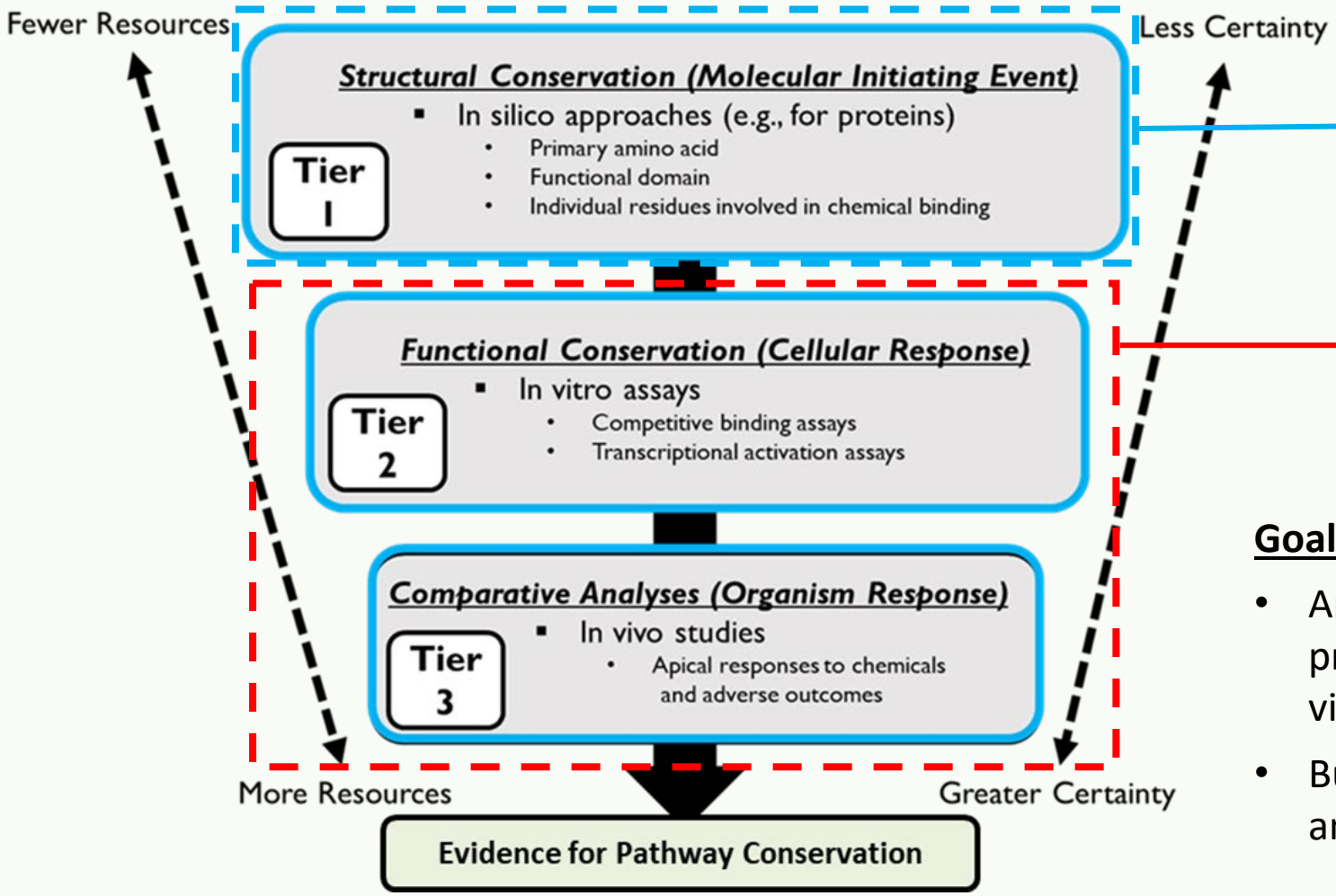
U.S. EPA ToxCast Program:

- 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*)

Can we expect compounds that interact with the mammalian androgen receptor (AR) to also interact with the AR in other species?

Case Study: Cross-Species Extrapolation of the Androgen Receptor



Goals

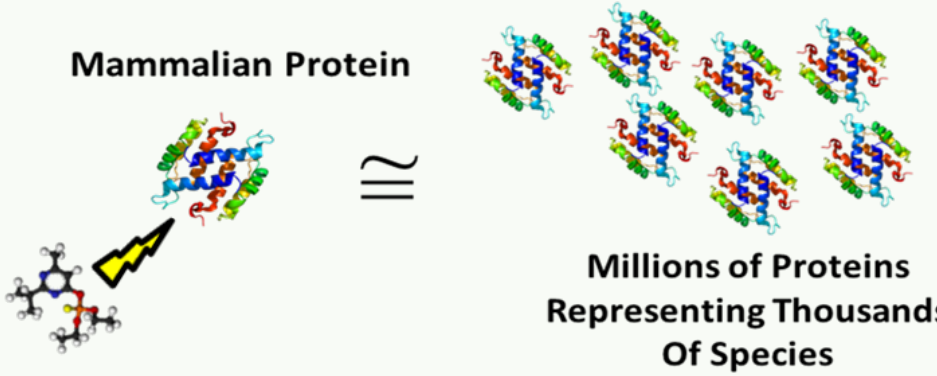
- Anchor SeqAPASS-derived molecular-based predictions of species susceptibility with existing in vitro and in vivo data
- Build weight of evidence for the extrapolation of androgenic responses across species.

Adapted from Ankley et al. 2016

SeqAPASS: Sequence Alignment to Predict Across Species Susceptibility

- A NAM used to predict biological pathway conservation across taxa and extrapolate from model species to untested species, is the US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool
- Online, publicly available tool rapidly evaluates protein sequences across thousands of diverse species

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

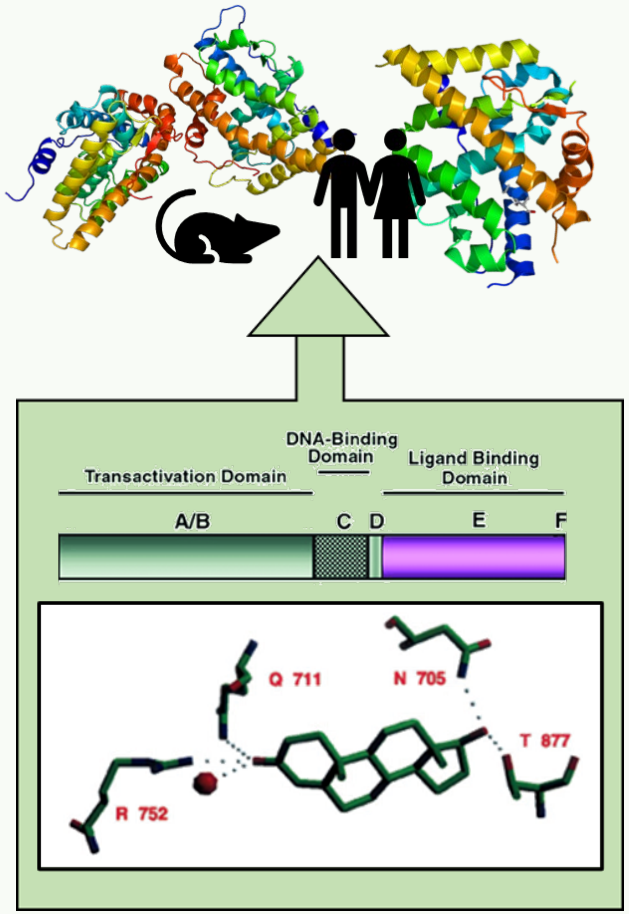


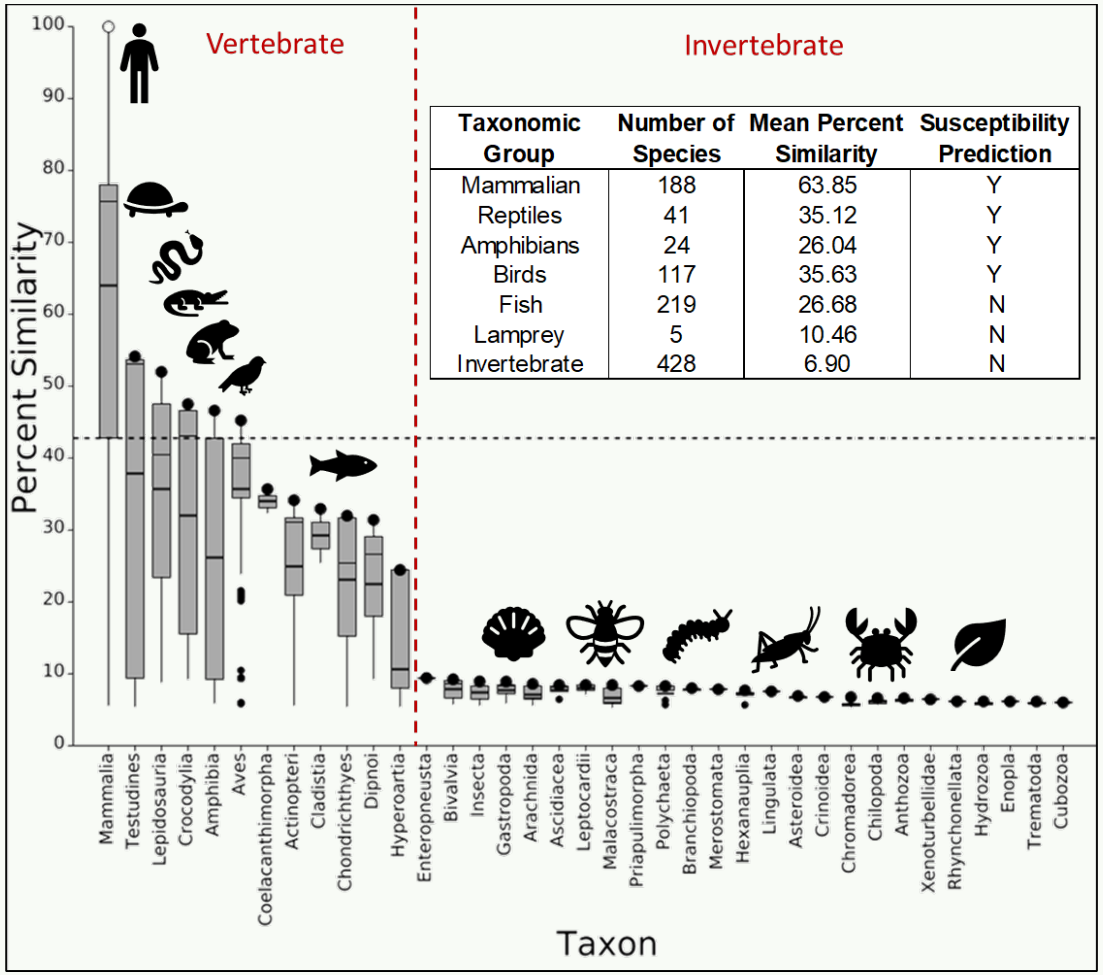
SeqAPASS Applications

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

Case Study: Cross-Species Extrapolation of the Androgen Receptor

- SeqAPASS predicts that vertebrate species with available data share similar suseptibility to compounds interacting with the AR ligand binding domain

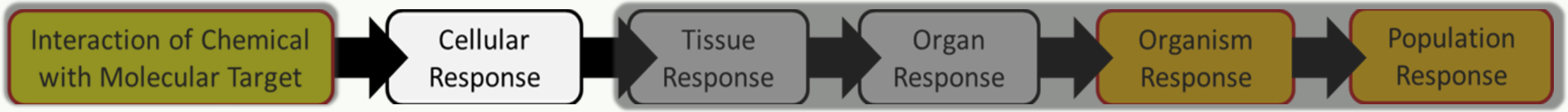




Taxonomic Group	Number of Species	Mean Percent Similarity	Susceptibility Prediction
Mammalian	188	63.85	Y
Reptiles	41	35.12	Y
Amphibians	24	26.04	Y
Birds	117	35.63	Y
Fish	219	26.68	N
Lamprey	5	10.46	N
Invertebrate	428	6.90	N

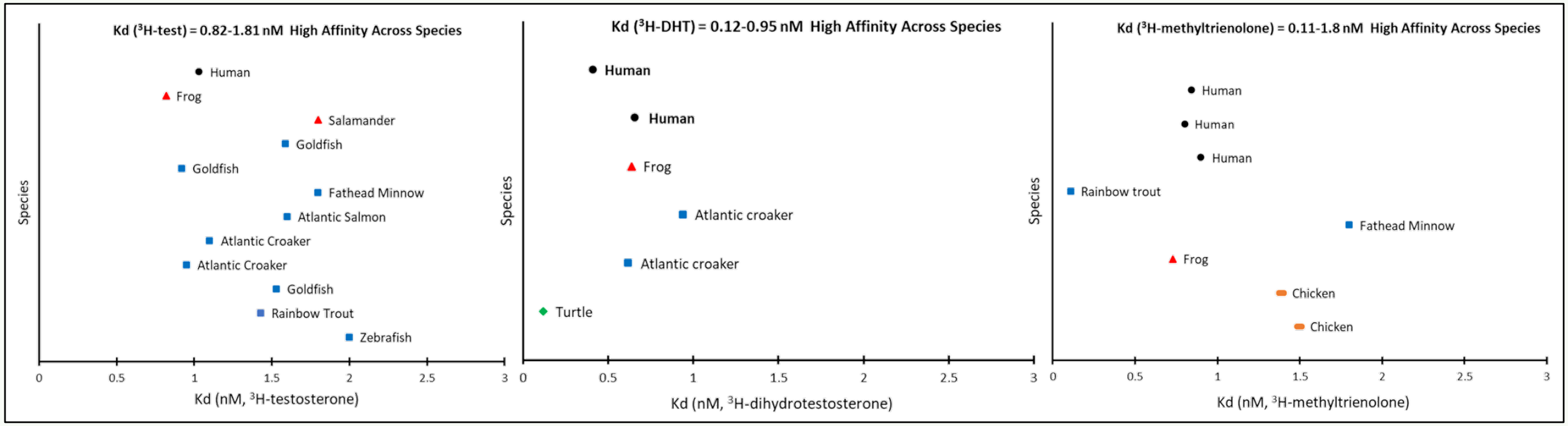
Common Name	Similar Susceptibility
Human	Y
Rhesus monkey	Y
Rabbit	Y
Pig	Y
Black rat	Y
Mice	Y
Mainland tiger snake	Y
Western terrestrial garter snake	Y
Western painted turtle	Y
Japanese quail	Y
Chicken	Y
Zebra finch	Y
Chinese alligator	Y
Tropical clawed frog	Y
African clawed frog	Y
Reedfish	Y
Gray bichir	Y
Rainbow trout	Y
Fathead minnow	Y
Zebrafish	Y
Japanese medaka	Y
Little skate	Y
West African lungfish	Y

Case Study: Cross-Species Extrapolation of the Androgen Receptor

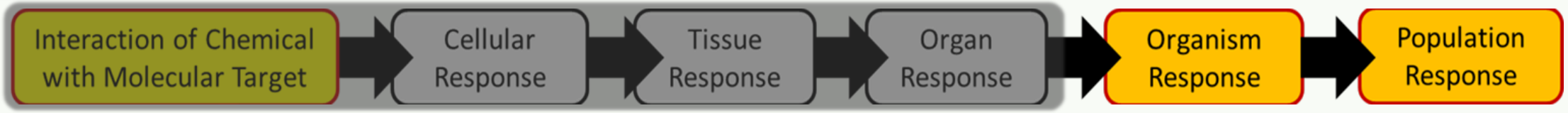


Mammal
Fish
Amphibian
Reptile
Bird

- Preliminary results suggest functional conservation of AR across vertebrate species for three high-affinity AR ligands



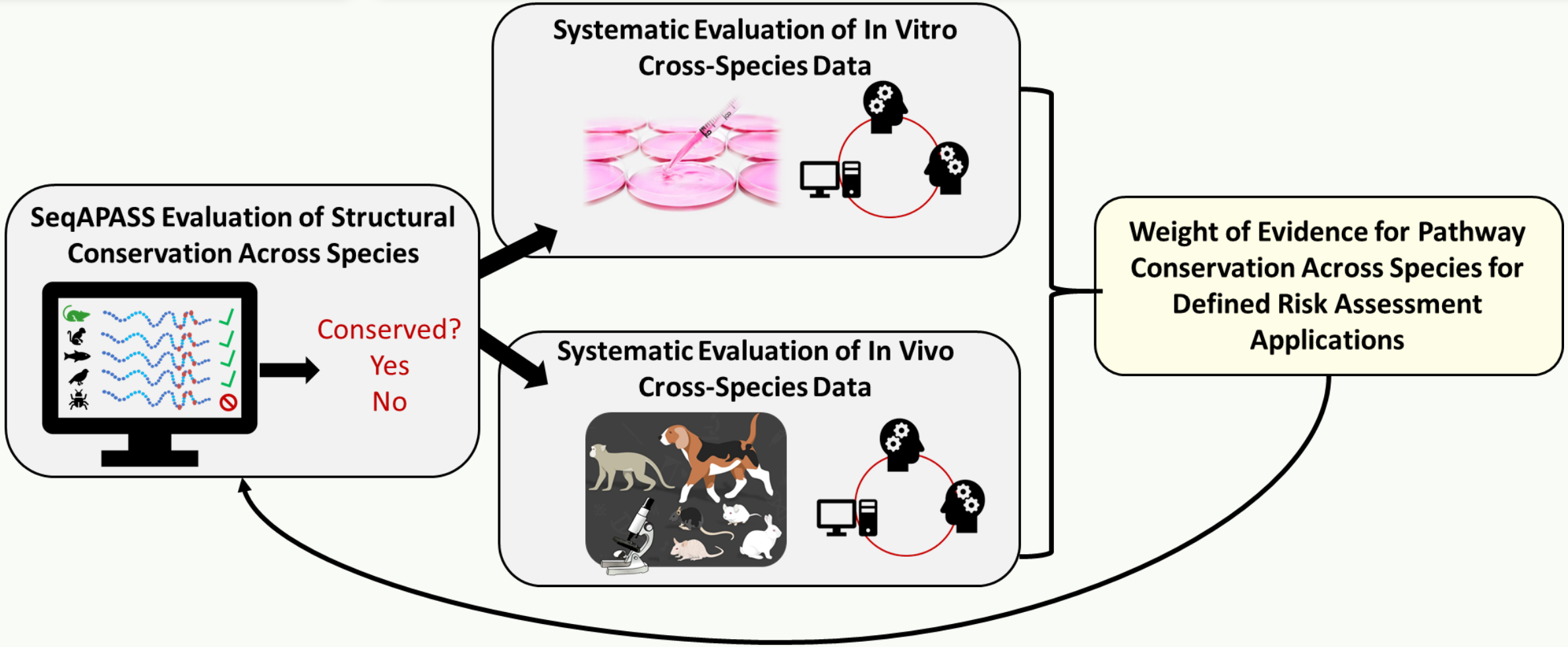
Case Study: Cross-Species Extrapolation of the Androgen Receptor



- 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

Case Study: Cross-Species Extrapolation of the Androgen Receptor



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

Systematic Review in NAMs Research: Going Forward

Through the AR case-study, we demonstrated;

1. A framework for understanding pathway conservation for endocrine targets across species
2. A strategy to bridge the gap between existing NAMs and current systematic review practices.

- Incorporating NAMs into safety evaluation and risk assessment requires methods that are not only effective and reliable, but also transparent and scientifically-defensible
- When there is a sufficient body of published literature, using existing evidence, provides a mechanism to reduce the need for additional animal testing and potentially increase the rate of chemical review

- Systematic reviews provide a transparent, methodologically rigorous and reproducible means of summarizing the available evidence
- Now a well-established approach in many research fields with a growing data curation toolbox, systematic review is receiving increased attention as a tool for answering toxicological questions

EPA

Collaborators

Carlie LaLone (USEPA-ORD)
Scott Lynn (USEPA-OCSPP)
Kristan Markey (USEPA-ORD)

ORD EDSP

BATTELLE
It can be done

ILS
Advancing Science, Improving Lives

Thanks!

Any questions?

Email: Vliet.Sara@epa.gov

ORISE
The Oak Ridge Institute for Science and Education

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!

LaLone.Carlie@epa.gov
Blatz.Donovan@epa.gov