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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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1392/P472

Background

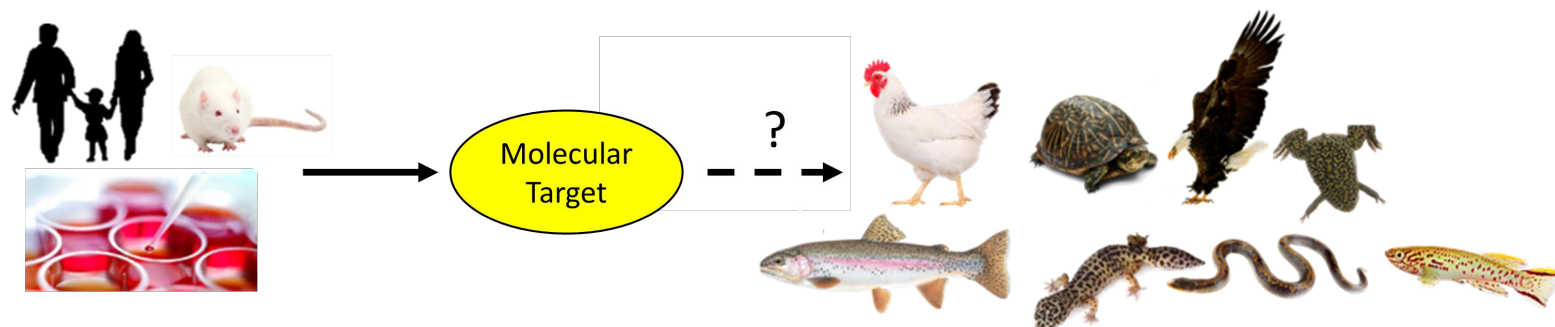
EPA & the Endocrine Disruptor Screening Program (EDSP)

- Tasked with evaluating thousands of chemicals for potential endocrine bioactivity to protect human health and wildlife
- Traditional methods rely on resource-intensive whole-animal testing in which animals are exposed and any endocrine effects are observed
- 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



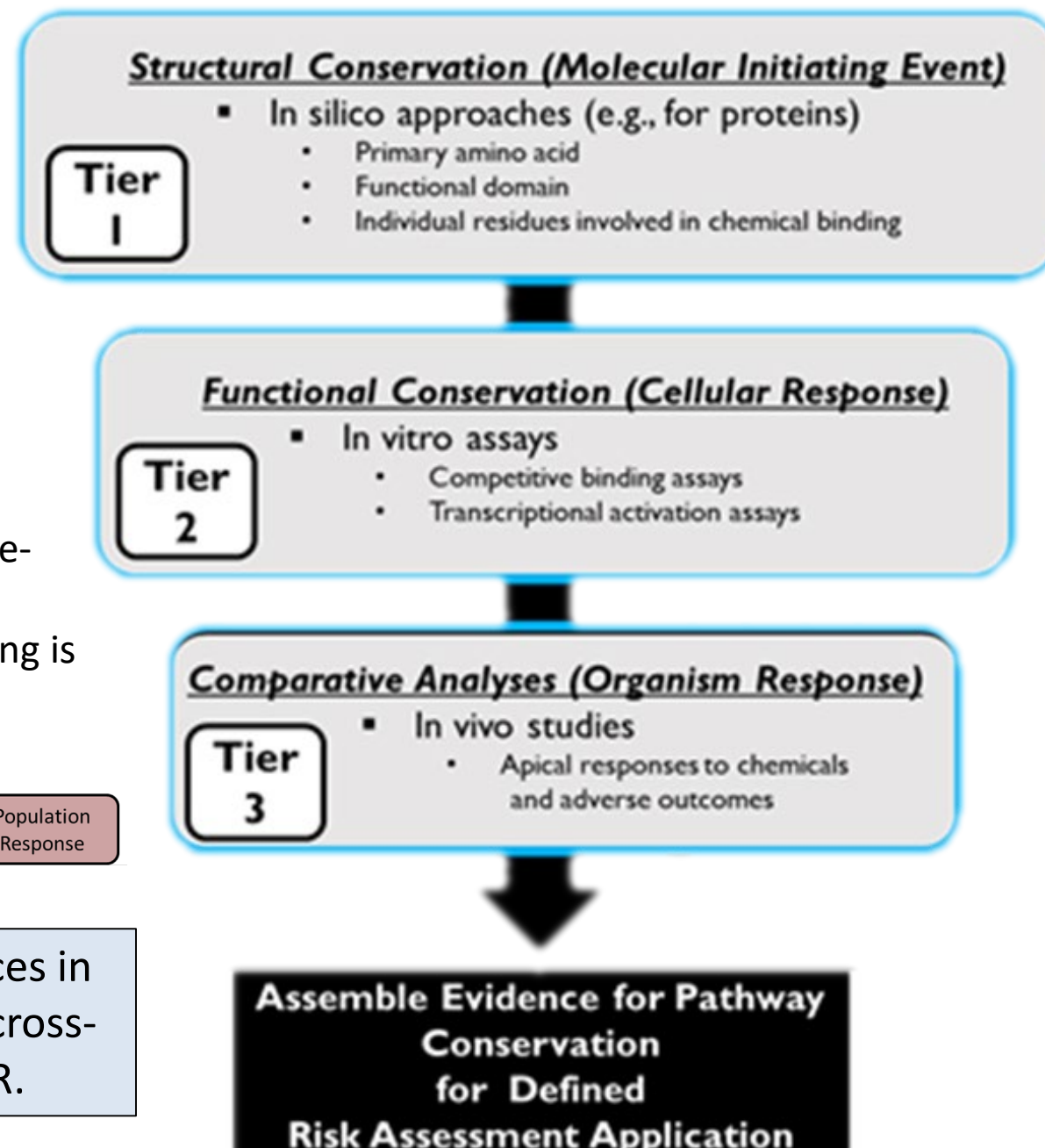
U.S. EPA Toxicity Forecaster ToxCast Program:

- Screens thousands of chemicals in mammalian-based high throughput assays for potential bioactivity
- Rapid, automated high-throughput screening assays using mammalian cell lines
- Platform allows for the prediction of chemical toxicity, prioritization of chemicals for further testing, and identification of putative molecular target
- However, it remains unclear if these mammalian-based screening approaches reasonably reflect potential impacts on non-mammalian vertebrates
- To understand chemical susceptibility across species, we can evaluate the conservation of chemical-molecular interactions, cellular responses, and organismal outcomes across taxa



Hierarchical Framework for Evaluating Pathway Conservation

- Previously published Hierarchical Framework for Evaluating Pathway Conservation
- Uses available tools and existing data to assemble evidence for pathway conservation that can be used to define risk assessment applications



Androgen Receptor (AR): An Important Endocrine Target

- The androgen receptor (AR) is an important endocrine target for many environmental and industrial chemicals
- Androgenic activity is observed at environmental chemical concentrations
- Aside from ER α , AR is an endocrine target with a large base of pre-existing structural, molecular target, and toxicity data
- Understanding of the chemicals that can disrupt androgen signaling is still growing and evolving



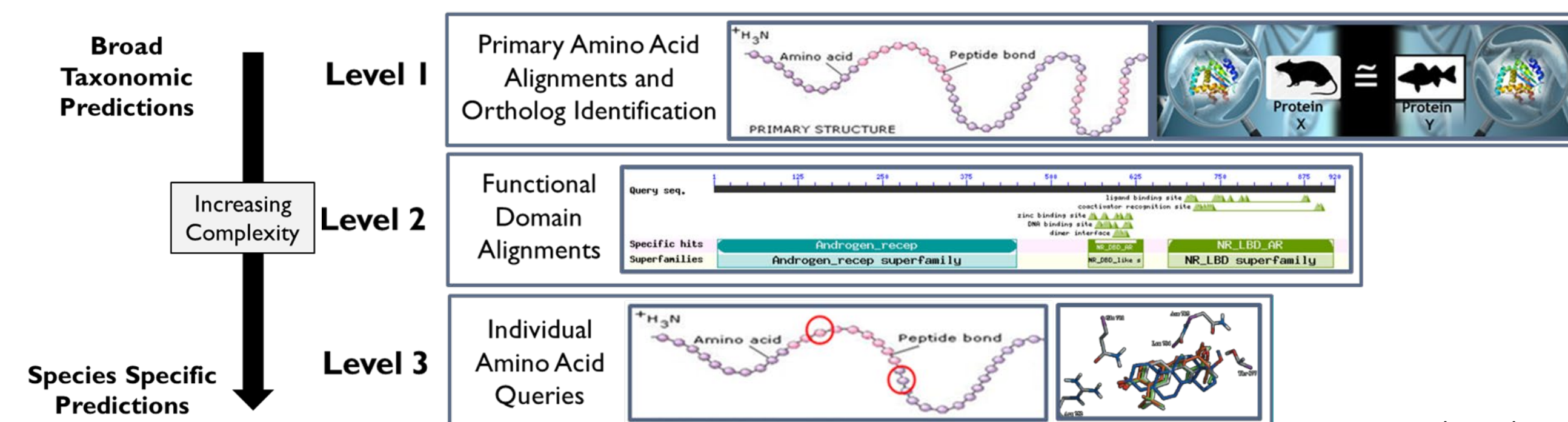
Objective: Leverage existing data and technological advances in data curation science to enable a robust evaluation of the cross-species comparability of chemical interactions at the AR.

U.S. Environmental Protection Agency
Office of Research and Development

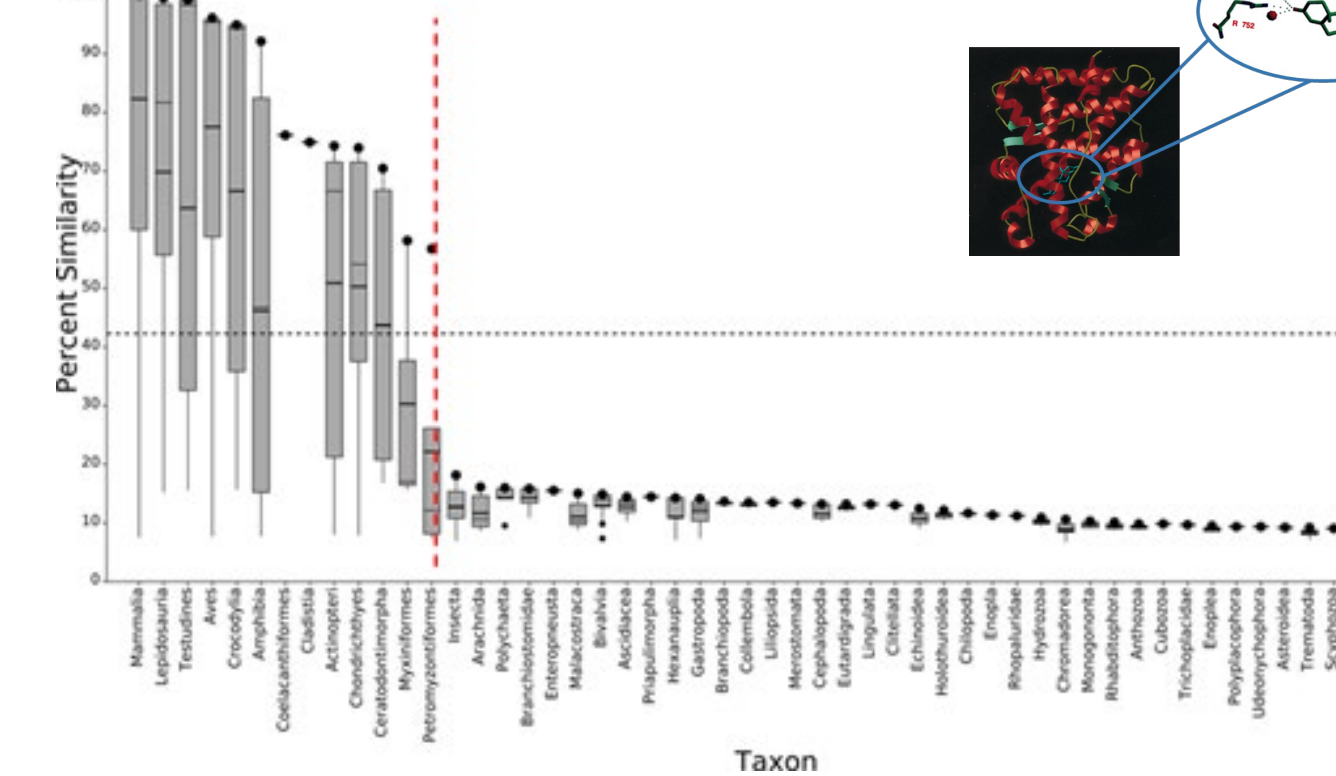
Tier 1: In-Silico Evaluation of Structural Conservation

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool

- Leverages publicly-available databases and tools (National Center for Biotechnology and Information)
- Uses existing knowledge of protein sequence and ligand binding to make predictions of chemical susceptibility across species



SeqAPASS Level 2: Similarity of AR Ligand Binding Domain Across Taxa



- Evaluated entire primary amino acid sequence, sequence of ligand binding domain, and conservation of critical amino acids involved in AR-ligand binding
- Results across all three levels of analysis indicate conservation of AR and critical binding residues across vertebrate species

| Class Name | Level 1 Shared Susceptible? | Level 2 Shared Susceptible? | Level 3 | |
|-------------------|-----------------------------|-----------------------------|------------------------------------|-----------------------------------|
| | | | Species with Shared Susceptibility | Species not Similarly Susceptible |
| Mammalia | Yes | Yes | 106 | 0 |
| Lepidosauria | Yes | Yes | 3 | 0 |
| Testudines | Yes | Yes | 3 | 0 |
| Aves | Yes | Yes | 33 | 0 |
| Crocodylia | Yes | Yes | 3 | 0 |
| Amphibia | Yes | Yes | 5 | 0 |
| Coelacanthiformes | Yes | Yes | 2 | 0 |
| Cnidaria | Yes | Yes | 1 | 0 |
| Actinopteri | Yes | Yes | 88 | 1 |
| Chondrichthyes | Yes | Yes | 3 | 0 |
| Ceratodontomorph | Yes | Yes | 2 | 0 |

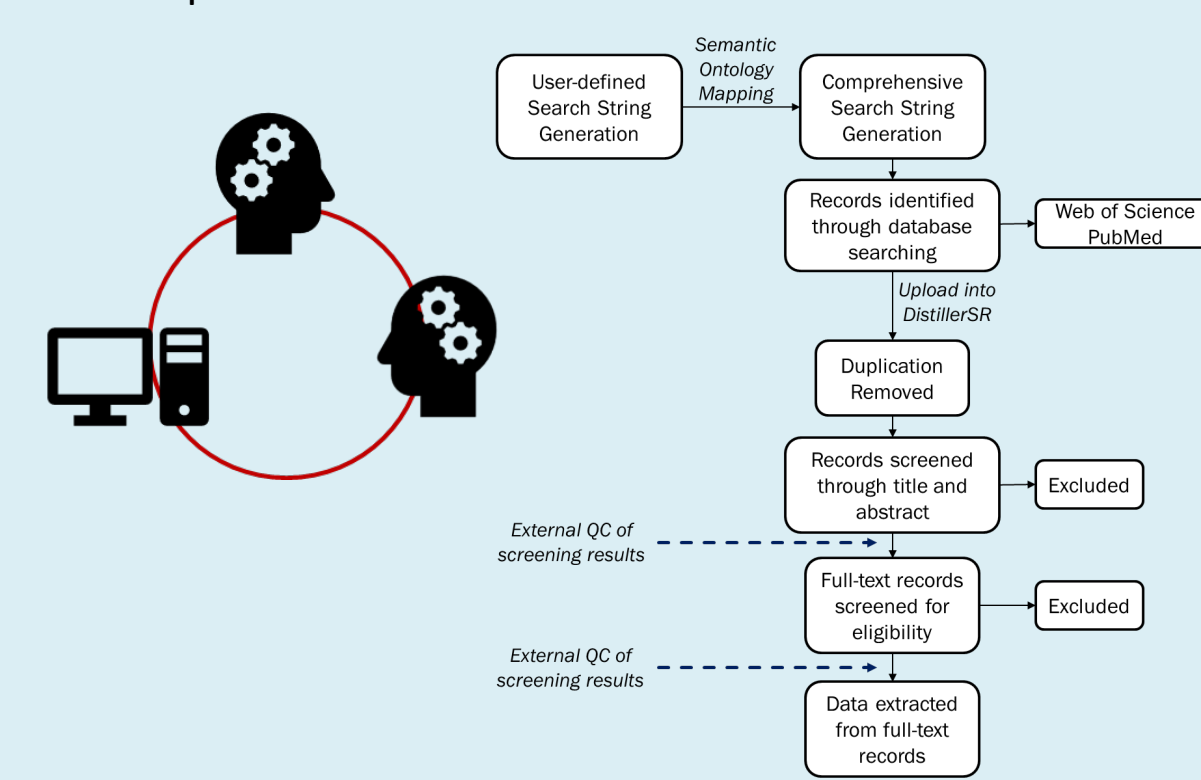
Incorporation of Technical Advances and Tools for Improved Systematic Review

Semantic Ontology Concept Mapping

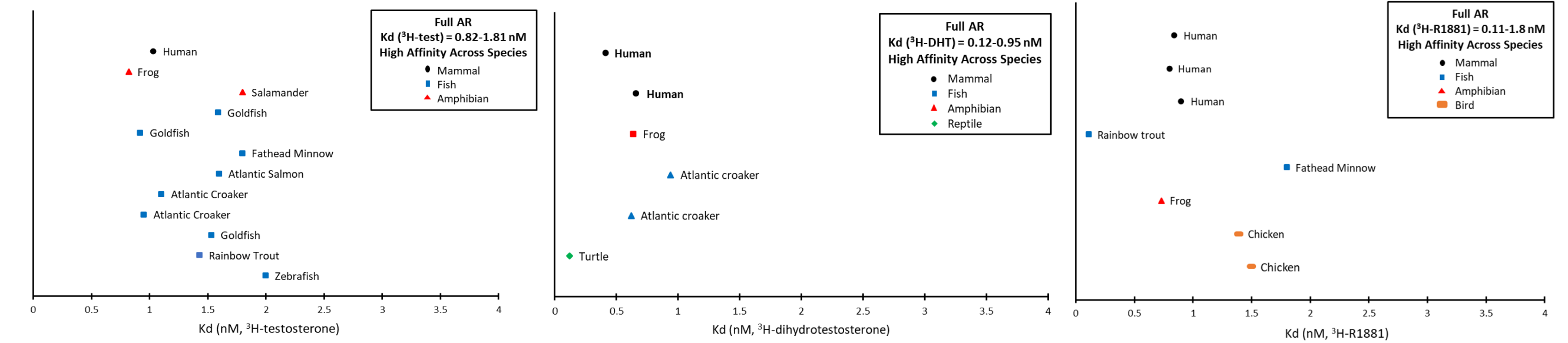
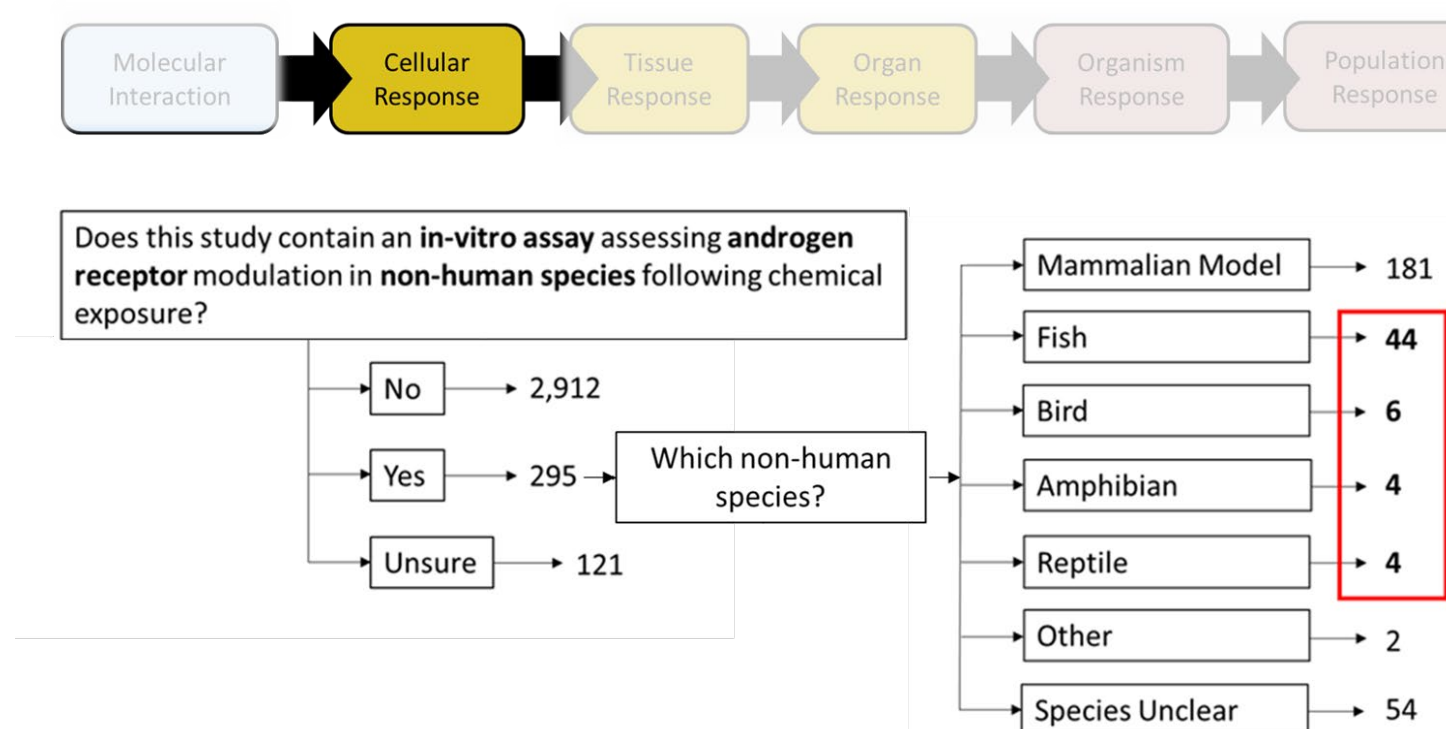
- Using accurate search terms is essential for conducting an unbiased, comprehensive survey of the literature landscape
- Scientific language, however, is often non-standard, redundant, and mischaracterized
- Semantic mapping approaches can develop comprehensive literature search strings by expanding the vocabulary and knowledge to include related categories and word

DistillerSR Systematic Review Software

- Collaborative web-based systematic literature review software
- Provides platform for article characterization, evaluation, and data collection
- Facilitates Quality Assurance/Quality Control and incorporates blind "reviews" of article screens



Tier 2: In-Vitro Evaluation of Cellular Responses



- High saturable binding is observed across species and taxonomic groups with available for three common AR ligands
- Results suggest functional conservation of AR across vertebrate species

Tier 3: In-Vivo Evaluation of Organismal Responses

| Chemical | Vertebrate Class | | | |
|------------------------|--|---|---|--|
| | Fish | Reptile | Amphibian | Bird |
| Methyltestosterone | • Sex reversal of females • Reduced G.S.I | • Altered population sex-ratios towards male-biased populations • Masculinized gonad tissue • Development of male secondary sex characteristics | • Altered population sex-ratios towards male-biased populations | • Reduced egg laying in females |
| Testosterone | • Sex reversal of females | • Masculinized gonad tissue • Development of male secondary sex characteristics | • Altered population sex-ratios towards male-biased populations | • Cloacal gland induction • Increase in of crowing behavior |
| 17 β -trenbolone | • Reduced circulating vitellogenin • Reduced circulating E2 • Masculinization of female fish reproductive tissues • Development of male secondary sex characteristics | • Masculinized gonad tissue • Altered population sex-ratios towards male-biased populations | • Masculinized gonad tissue • Development of male secondary sex characteristics • Altered population sex-ratios towards male-biased populations | • Cloacal gland induction characteristics • Altered population sex-ratios towards male-biased populations |

- Exposure to a number of AR-binding compounds results in organismal effects across vertebrate taxa
- Although exact measure of androgenic effects vary across taxa, results suggest conservation of AR responsiveness across species

Summary: A Weight-of-Evidence for AR Conservation

- Evaluation of the protein structure of AR across species indicates conservation of residues crucial for AR-ligand binding
- Preliminary in vitro binding data suggests strong similarities across non-mammalian vertebrate species and taxonomic groups for high-affinity AR-ligands
- Preliminary comparisons of in vivo studies demonstrate a degree of cross-species responsiveness to strongly androgenic compounds.
- Overall, this data suggests that mammalian-based androgen receptor screening approaches reasonably reflect potential impacts on non-mammalian vertebrates



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