A Weight-of-Evidence Approach for Androgen Receptor Conservation Across Vertebrate Species

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Background

Endocrine Disruptor Screening Program (EDSP)[1]:

- Tasked with evaluating thousands of chemicals for potential endocrine bioactivity to protect human health and wildlife
- Traditional evaluation methods rely on resource-intensive whole-animal testing
- New Approach Methodologies are needed to rapidly prioritize and screen chemicals for potential endocrine bioactivity while reducing animal testing

Traditional Methods
- Whole-Animal Assays
- Resource- and time-Intensive
- Observations of toxic effects in whole organisms

Technological Advances

Modern Methods
- In-Vitro and In-Silico Methods
- Reductions in animal use
- Focus on molecular target and prediction of toxic effect

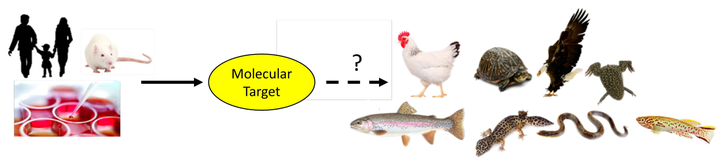
U.S. EPA Toxicity Forecaster (ToxCast) Program[2]:

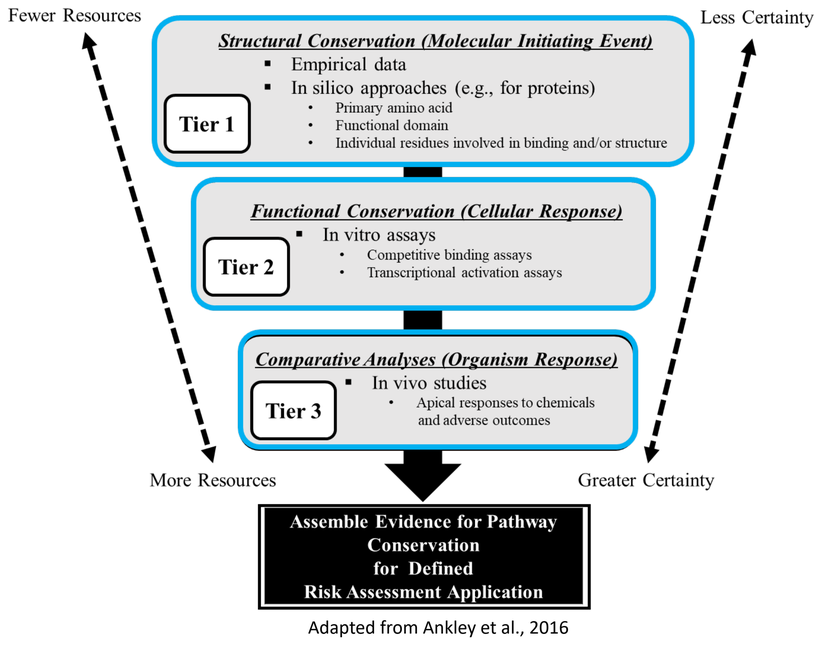
- Rapid, automated high-throughput screening assays using mammalian cell lines for the prioritization of chemicals for further testing and identification of putative molecular target
- Whether or not these mammalian-based screening approaches reasonably reflect potential impacts on non-mammalian vertebrates remains unclear
- Evaluating the conservation of chemical-molecular interactions, cellular responses, and organismal outcomes across taxa can facilitate an understanding of chemical susceptibility across species

Chemical → Molecular Interaction → Cellular Response → Tissue Response → Organ Response → Organism Response

Hierarchal Framework for Evaluating Androgen Receptor Conservation:

- Previously published Hierarchal Framework for Evaluating Pathway Conservation[3] uses available tools and existing data to assemble evidence for pathway conservation
- Androgen receptor (AR) is an important endocrine target for a number of environmental chemicals
- Knowledge of chemicals that can disrupt AR signaling is still growing
- A large base of pre-existing structural, molecular target, and toxicity data exists for AR





Adapted from Ankley et al., 2016

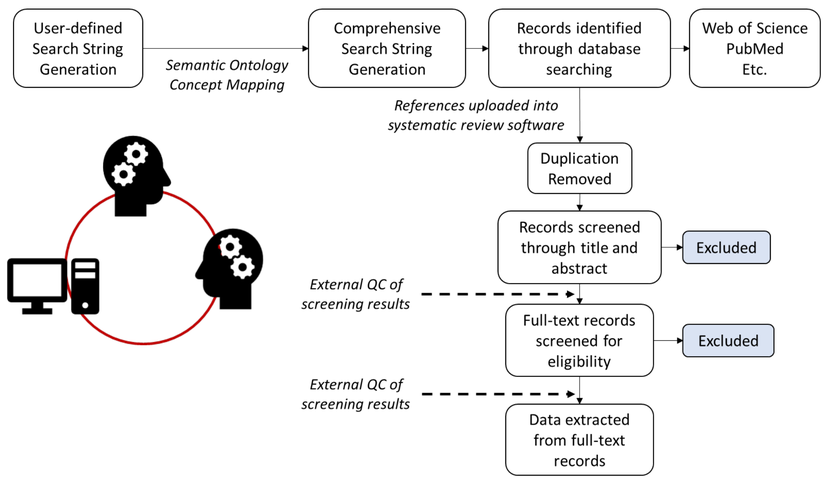
Incorporation of Technical Advances and Tools for Improved Systematic Review:

Semantic Ontology Concept Mapping

- Using accurate search terms is essential for conducting an unbiased, comprehensive survey of the literature landscape
- Scientific language is often non-standard, redundant, and mischaracterized
- Semantic ontology concept mapping approaches can develop comprehensive literature search strings by expanding the vocabulary and knowledge
- At the endpoint level, this technique can be used as a point of integration and promote consistent interpretation as well as interoperability with other relevant databases.

Systematic Review Software

- Collaborative web-based systematic literature review software provides a platform for unbiased article evaluation, and data collection
- Facilitates blind Quality Assurance/Quality Control

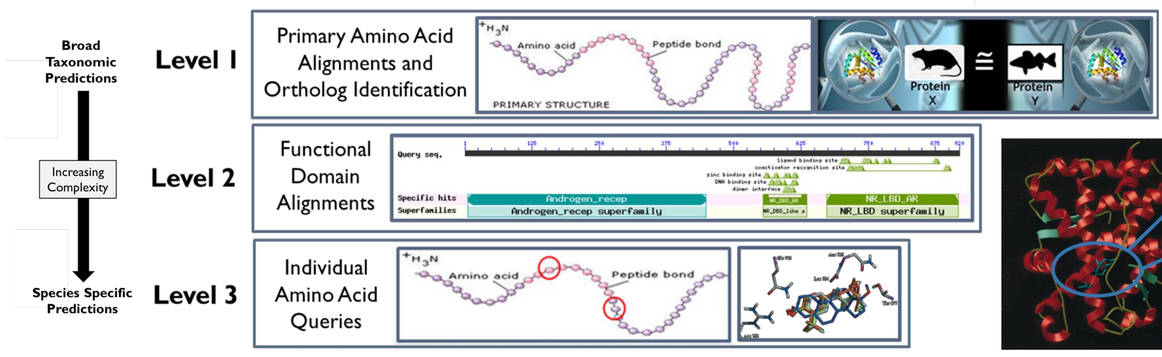


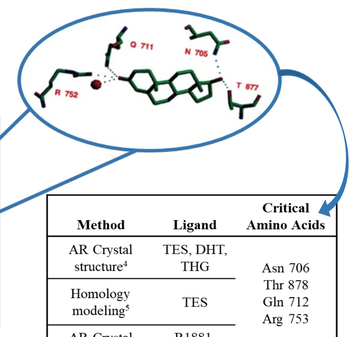
Study Objective: Leverage existing data and technological advances in data curation science to enable a robust evaluation of the cross-species comparability of chemical interactions at the AR

Tier 1: In-Silico Evaluation of Structural Conservation

Sequence Alignment to Predict Across Species Susceptibility Tool[4,5] (SeqAPASS; seqapass.epa.gov/seqapass):

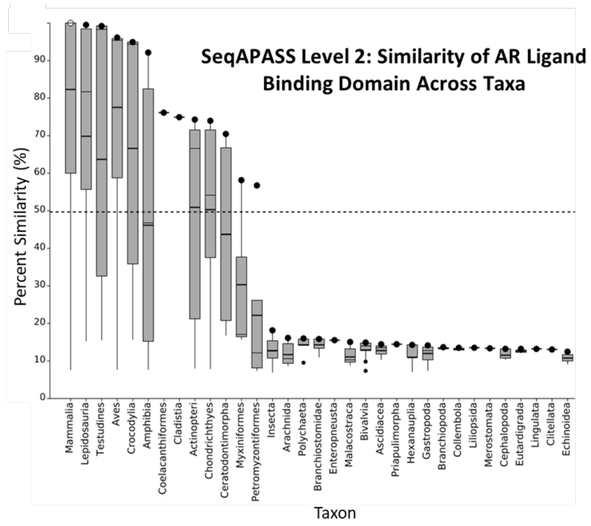
Molecular Interaction → Cellular Response → Tissue Response → Organ Response → Organism Response





Method	Ligand	Critical Amino Acids
AR Crystal structure[4]	TES, DHT, THG	Asn 706, Thr 878, Gln 712, Arg 753
Homology modeling[5]	TES	
AR Crystal structure[6]	R1881, LGD2226	

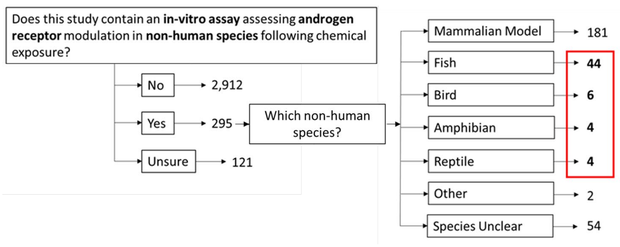
- Leverages publicly-available databases and tools (National Center for Biotechnology and Information) and uses existing knowledge of protein sequence and ligand binding to make predictions of chemical susceptibility across species

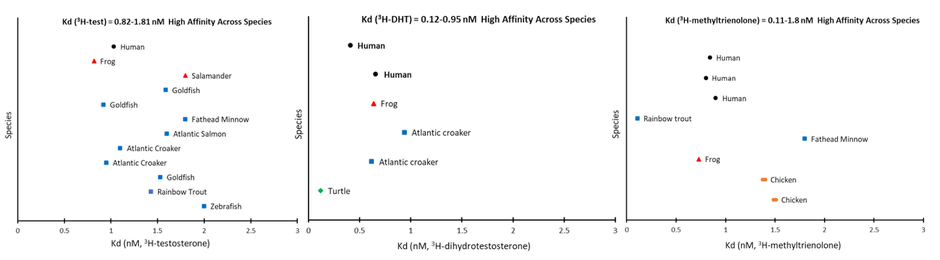


Class Name	Level 1 Shared Suseptible?	Level 2 Shared Suseptible ?	Level 3: Species with Shared Suseptibility	Level 3: Species not Similarly Suseptible	Level 3 Shared Suseptible ?
Mammalia	Yes	Yes	106	0	Yes
Lepidosauria	Yes	Yes	3	0	Yes
Testudines	Yes	Yes	3	0	Yes
Aves	Yes	Yes	33	0	Yes
Crocodylia	Yes	Yes	3	0	Yes
Amphibia	Yes	Yes	5	0	Yes
Coelacanthiformes	Yes	Yes	2	0	Yes
Cladistia	Yes	Yes	1	0	Yes
Actinopteri	Yes	Yes	88	1	Yes/No
Chondrichthyes	Yes	Yes	3	0	Yes
Ceratodontimorpha	Yes	Yes	2	0	Yes

- Results across all three levels of analysis suggest conservation of AR and critical amino acid residues across vertebrate species

Tier 2: In-Vitro Evaluation of Cellular Responses





Molecular Interaction → Cellular Response → Tissue Response → Organ Response → Organism Response → Population Response

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

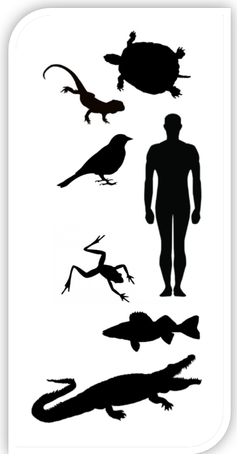
Mammal, Fish, Amphibian, Reptile, Bird

Tier 2: In-Vitro Evaluation of Cellular Responses

Molecular Interaction → Cellular Response → Tissue Response → Organ Response → Organism Response → Population Response

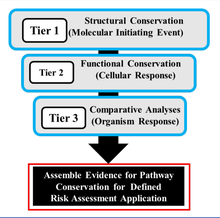
- Preliminary results suggest conservation of AR responsiveness to high-affinity androgens across species

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



Summary: A Weight-of-Evidence for AR Conservation

- Evaluation of the AR protein sequence suggests conservation AR-ligand binding across vertebrate species with available data
- Preliminary comparisons of in vitro binding data and in vivo studies suggests strong similarities across taxonomic groups for high-affinity AR-ligands
- Overall, this preliminary data suggests that mammalian-based androgen receptor screening approaches, such as the ToxCast program, can reasonably reflect potential impacts on non-mammalian vertebrates
- Future work will compare all results from in vitro and in vivo systematic review across species and chemicals with different binding affinities (i.e. weak, moderate, and high affinity)



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