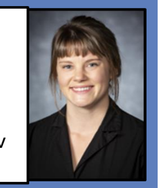
Advanced Systematic Review for Understanding Cross-Species Conservation of Androgen Receptor-Based Chemical Toxicity

P130

Sara M.F. Vliet [a], Kristan J. Markey [b], Scott G. Lynn [c], Kelsey Vitense [a], Carlie A. LaLone [d]

[a]U.S. EPA, ORD/CCTE/SCDCD, Duluth, Minnesota; [b]U.S. EPA, ORD/CPHEA/CPAD, Washington, DC; [c]U.S. EPA, OCSPP/OPP, Washington, DC; [d]U.S. EPA, ORD/CCTE/GLTED, Duluth, Minnesota

Sara Vliet, PhD
vliet.sara@epa.gov
218-529-5022



www.epa.gov

Introduction

U.S. EPA & the Endocrine Disruptor Screening Program (EDSP):

- Tasked with evaluating 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

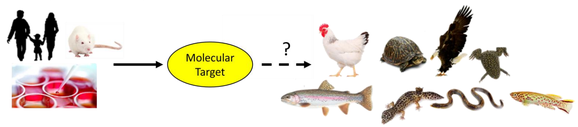
Technological Advances

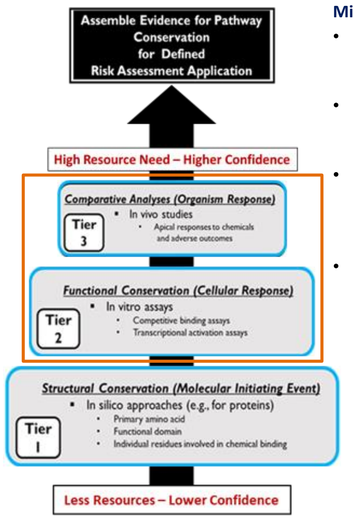
Modern Methods

- 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[1]:

- 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
- **There is a need to build confidence that these mammalian-based screening approaches reasonably reflect potential impacts on non-mammalian vertebrates**





Mining Existing Data:

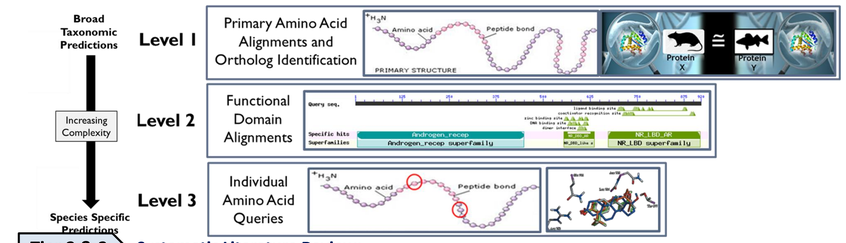
- Conducting experimental studies to fill these data gaps can be cost prohibitive and resource intensive
- For many molecular targets, a large base of pre-existing structural, molecular target, and toxicity data exists
- This existing evidence can be mined, aggregated, and assessed to compile weights-of-evidence without the need for additional studies
- Advances in data curation technology continue to increase the power and efficiency of utilizing existing data within scientific literature

Study Objective: Demonstrate the utility of combining approaches in bioinformatics and data curation science to enable a more robust evaluation of the cross-species comparability of chemical interactions at the AR

Methods

Tier 1 **Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; seqapass.epa.gov) Tool:**

- Web-based SeqAPASS tool was used to mine NCBI protein databases (Nat. Center for Biotechnology and Information) and rapidly align and compare AR conservation across species with available data
- SeqAPASS-generated predictions of shared chemical suseptibility were compared to empirical data

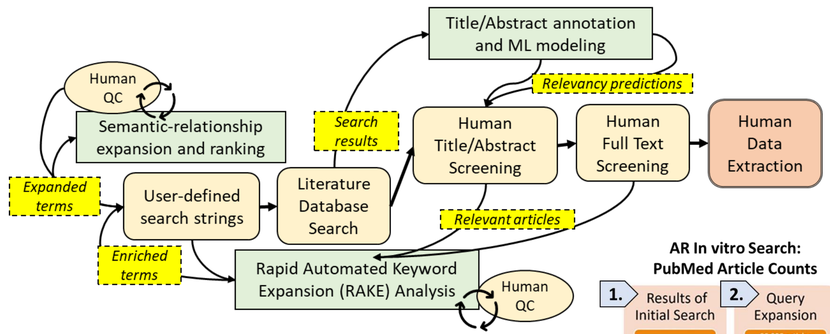


Tier 2 & 3 **Systematic Literature Review:**

Question (PECO Statement): In non-mammalian vertebrates in a controlled laboratory setting (in vitro/in vivo), do androgenic responses to discrete chemical exposures occur relative to non-exposed?

Sub-Question: Are responses elicited by chemicals which interact with the androgen receptor in mammalian test system, predictive of responses in non-mammalian vertebrates?

- Conducted a large-scale systematic literature review of in vivo and in vitro AR-mediated toxicity data
- Employed query expansion techniques to generate unbiased and comprehensive search strings
- Developed machine-learning (ML) models to facilitate rapid and unbiased literature screening



Include/Exclude Modeling Approach

- 1 question
- 1 classifier

1. Does this study contain an in-vitro assay assessing androgen receptor modulation in non-human species following chemical exposure?
   - Yes
   - No
   - Unclear

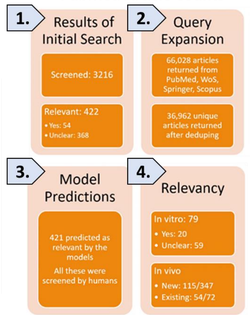
Yes OR Unclear moves forward

Inventory Modeling Approach

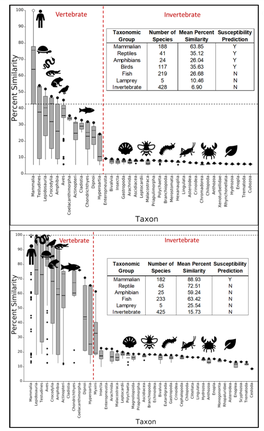
- 4 separate questions
- 4 separate classifiers

Chemical	Androgen receptor modulation	Species Group	Type of Study
Yes No Unclear	Yes No Unclear	Human Mammal Fish Bird	In-Vivo In-Vitro Epi Clinical

AR In vitro Search: PubMed Article Counts



Results

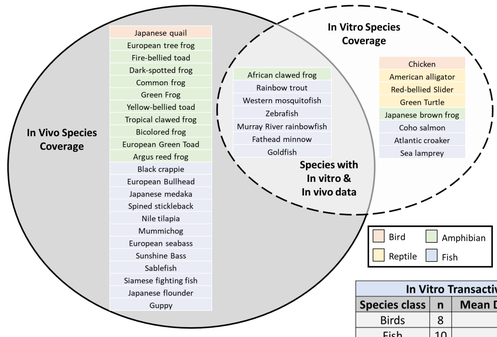


- Evaluated entire primary amino acid sequence, sequence of ligand binding domain, and conservation of five critical amino acids involved in AR-ligand binding
- Results across all levels of analysis indicate conservation of AR sequence and amino acid residues across vertebrates

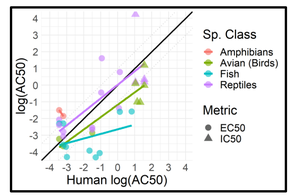
Total Match · Partial Match · Not a Match · Susceptible Yes · Susceptible No · Common Model Organism

Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5
Human	Y					
House mouse	Y					
Norway rat	Y					
Painted turtle	Y					
Eastern brown snake	Y					
American alligator	Y					
Nile crocodile	Y					
African clawed frog	Y					
Japanese quail	Y					
Golden eagle	Y					
Fathead minnow	Y					
Zebrafish	Y					
Turquoise killifish	Y					

Chemical Name	Amphibian		Bird		Fish		Reptile	
	In Vitro	In Vivo	In Vitro	In Vivo	In Vitro	In Vivo	In Vitro	In Vivo
11-Ketotestosterone								
18-Trenbolone								
17-Methyltestosterone								
Androstenedione								
5α-Dihydrotestosterone								
Bicalutamide								
Cyproterone acetate								
Flutamide								
Hydroxyflutamide								
Levonorgestrel								
Methoxychlor								
Methyltrienolone								
Nandrolone								
Norethindrone								
o,p'-DDT								
p,p'-DDE								
Testosterone								
Vinclozolin								



In Vitro Transactivation Assays		
Species class	n	Mean Difference (log10 uM)
Birds	8	-1.12
Fish	10	-1.27
Reptiles	14	0.20



- Across all extracted data, fish species were the most data-rich vertebrate class
- Across in vitro transactivation data for AR, birds and fish demonstrate lower average AC50 values relative to human values

Summary: Towards a Weight-of-Evidence for AR Conservation

- The current state of the AR data landscape demonstrates a need for increased toxicity data for non-model species
- Structural evaluation of AR protein conservation using the SeqAPASS tool provides lines of evidence for conservation among 1020 vertebrate species, the majority of which have no empirical toxicity data available.
- Inclusion of query expansion techniques and machine-learning models can assist in conducting comprehensive, efficient, and unbiased systematic literature reviews of toxicity data
- Preliminary comparative data suggest non-mammalian vertebrate share responsiveness to AR-targeting compounds
- However, preliminary point of departure comparisons suggest that some species, such as birds and fish, may demonstrate increased sensitivity to AR-targeting compounds relative to mammalian test systems
- Overall, these results indicate that further development of NAMs for screening toxicity in diverse, non-mammalian species may be useful for capturing AR activity due to differences in sensitivity

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

The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

SOT Annual Meeting, March 27th – 31st 2022, San Diego, CA