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

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*The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the US EPA.*
The Need for New Approach Methods (NAMs)

- The US Environmental Protection Agency’s mission is to protect human and environmental health and ensure that chemicals in the marketplace are reviewed for safety.

- 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)

- In Silico & Bioinformatics
- In Vitro Assays
- High-Throughput Screening
- Omics
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

- How well do surrogate species represent species of concern?

- Can we expect chemicals that interact with mammalian receptors to also interact with receptors of other species we want to protect?

- High throughput screening assays (US EPA ToxCast) rapidly test chemicals using mammalian cell lines to identify those most likely to interfere with biological pathways and identify potential molecular targets

- Extrapolation from mammalian species to other species of concern is essential
SeqAPASS: Sequence Alignment to Predict Across Species Susceptibility

- Online, free, 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

https://seqapass.epa.gov/seqapass/
SeqAPASS: The Basics

Flexible Analysis Based On Available Data

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Primary Amino Acid Sequence Alignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 2</td>
<td>Conserved Functional Domain Alignments</td>
</tr>
<tr>
<td>Level 3</td>
<td>Critical (Close Contact) Amino Acid Conservation</td>
</tr>
</tbody>
</table>

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. Across all three levels, SeqAPASS results suggest conservation of AR across vertebrate species

2. 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

3. Line of evidence for pathway conservation

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th># of Spp.</th>
<th>Shared Susceptibility</th>
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</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>117/1</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Lizards, Snakes</td>
<td>11</td>
<td>Yes</td>
</tr>
<tr>
<td>Turtles</td>
<td>3</td>
<td>Yes</td>
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<tr>
<td>Birds</td>
<td>58</td>
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</tr>
<tr>
<td>Crocodiles, Alligators</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>Amphibians</td>
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<td>Yes</td>
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<tr>
<td>Coelacanths</td>
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<td>Yes</td>
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<td>Eel-shaped</td>
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<td>Yes</td>
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<tr>
<td>Bony Fish</td>
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<tr>
<td>Sharks, Rays</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>Lungfish</td>
<td>2</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Evaluating Existing Data to Extrapolate High-Throughput Androgen Receptor Screening Data Across Species

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

- Current systematic review practices and advances in data science can improve this workflow
- Existing in vivo and in vitro data from vertebrate species exposed to known androgenic compounds provides additional lines of evidence for conservation of the AR pathway across species
Incorporation of Technical Advances and Tools for Improved Systematic Review

- Development of machine learning models to screen through identified articles and identify possible errors (compared with external review by scientists)
  - E.g. Does the "computer" agree with the human that this article is relevant/irrelevant?

- 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 mapping approaches can develop comprehensive literature search strings by expanding vocabulary based on knowledge of related concepts

- Collaborative web-based systematic literature review software provides a platform for unbiased article evaluation and data collection, enabling external review by scientists

- Systematic review software and tools for Quality Assurance/Quality Control, leading to transparent and reproducible reviews
Evaluating Existing Data to Extrapolate High-Throughput Androgen Receptor Screening Data Across Species

- Systematic Evaluation of In Vitro Cross-Species Data
  - SeqAPASS Evaluation of Structural Conservation Across Species
    - Conserved? Yes No
  - Weight of Evidence for Pathway Conservation Across Species for Defined Risk Assessment Applications

- Systematic Evaluation of In Vivo Cross-Species Data

- Apply pathway to other targets of interest
- Repeat process to account for the emergence of new information
- Inform future computational predictions
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 technological advances in systematic review and data science, we demonstrated a framework for the rapid and efficient curation of existing data.

3. 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.
Anyone can use SeqAPASS to help inform their own research questions! If you are interested in using SeqAPASS we are happy to help!

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