

# Ecotoxicology Alternatives

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Michelle Embry, PhD

Deputy Director, Health and Environmental Sciences Institute (HESI)

[membry@hesiglobal.org](mailto:membry@hesiglobal.org)

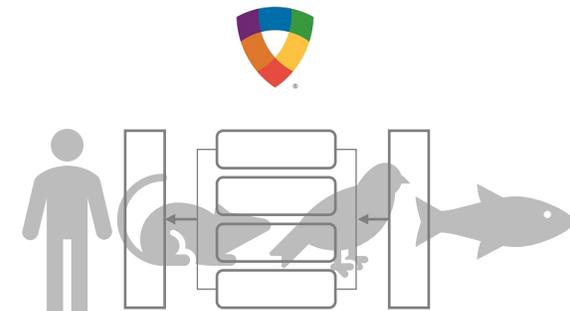
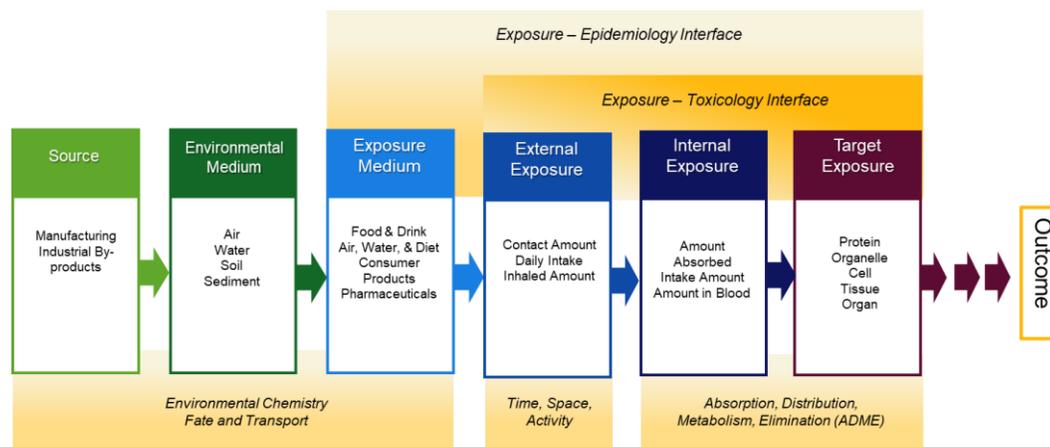


HESI®

# HESI Next Generation Ecological Risk Assessment Committee

**MISSION:** To develop, refine, and communicate the scientific tools and approaches needed to support ecological risk assessment around the globe, with a focus on alternative, non-animal testing methods. This mission will include but is not limited to the following:

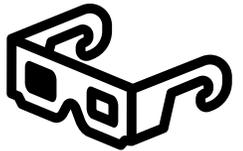
- Tools to evaluate the toxicokinetics of organic chemicals that facilitate the connection between exposure and hazard (e.g., ADME & bioaccumulation)
- Use and development of alternative methods for ecological assessment in various contexts (e.g., screening, prioritization, classification, risk assessment), sectors (e.g., pharmaceutical, agrochemical, chemical, personal care/cosmetics), and regulatory jurisdictions.



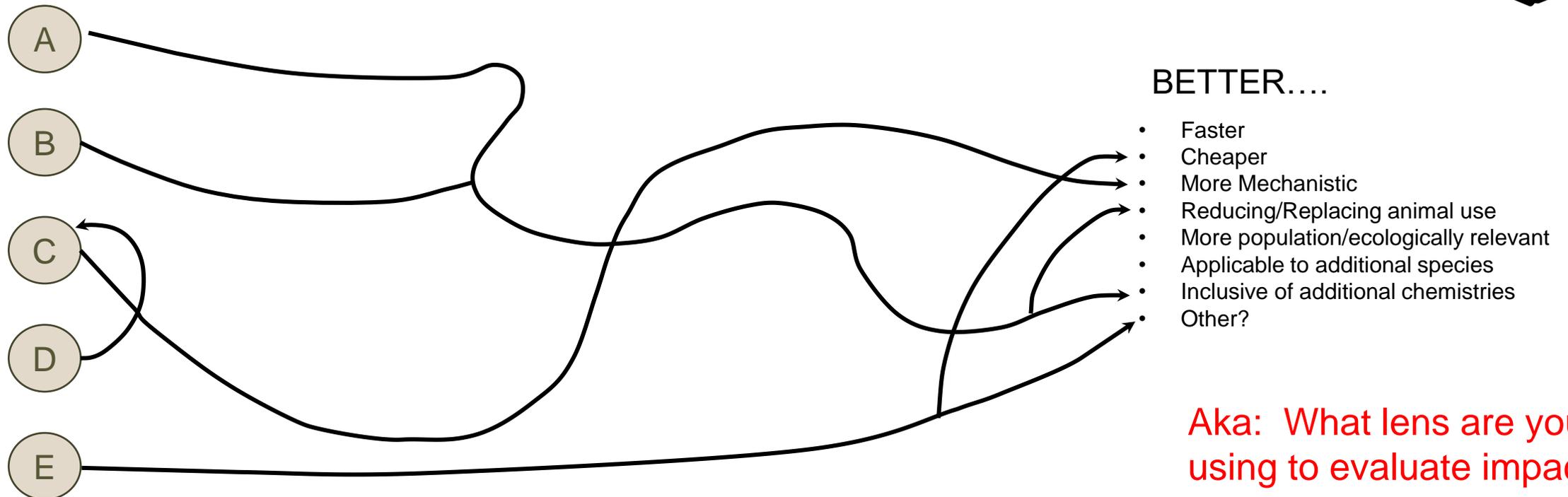


# NAMs: Not a linear path and not a single destination.....

Overall: Want to do a BETTER job of predicting potential ecological impacts



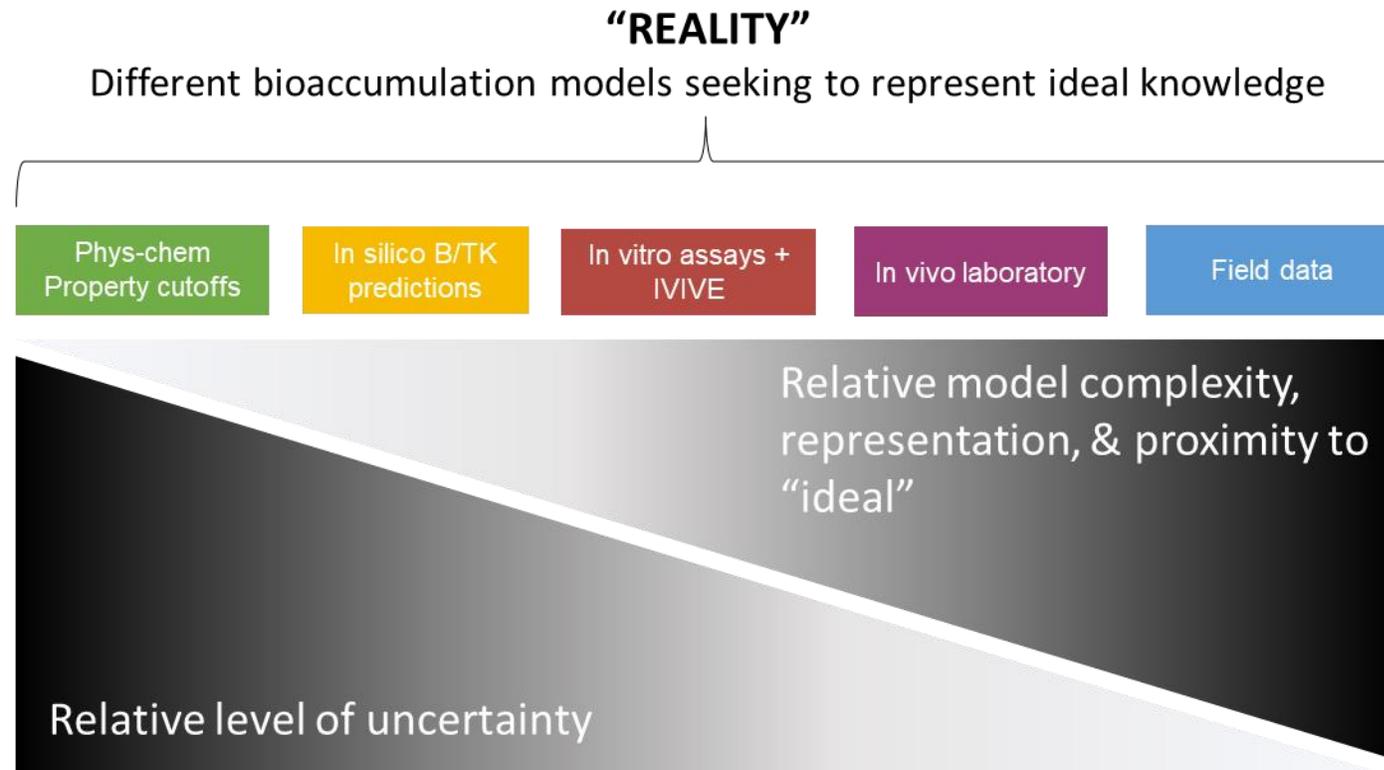
Methods / approaches



**Aka: What lens are you using to evaluate impact?**



# Balance between reality & ideality.....





# OECD IATA Case Studies Project

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OECD Home > Chemical safety and biosafety > Assessment of chemicals > Integrated Approaches to Testing and Assessment (IATA)

## Integrated Approaches to Testing and Assessment (IATA)

**Integrated Approaches to Testing and Assessment (IATA)**

### Latest news



Watch the webinar replay on the Integrated Approaches to Testing and Assessment concepts and OECD Case Studies | Presentations

New case studies have been published! Find out more information here and in the "Case Studies on IATA" section below.

## Developing Integrated Approaches for Testing and Assessment (IATAs) in order to support chemical safety

Minimising environmental and human health risks from chemicals is a critical regulatory issue. The assessment of environmental and human health risk requires the identification, compilation and integration of information on the chemical hazards, exposure, and the relationships between exposure, dose and adverse effects.

Advances in testing methods, biotechnology and computational models are paving the way for major improvements in how scientists evaluate the risks posed by potentially toxic chemicals. These advances enable toxicity testing that is faster, less expensive, and more relevant to human responses than traditional toxicity testing methods. These new methods also rely on *in silico*, *in chemico* and *in vitro* approaches that reduce the need for animal testing.

## Case Studies on IATA

The OECD IATA Case Studies Project allows countries to share and explore the use of novel methodologies in IATA for evaluating the safety of chemicals within a regulatory context. In the interactive reports below, you will find:

- The total number of case studies by endpoints, assessment type and IATA topics
- The full list and links to the case studies
- The consideration documents capture learnings and lessons from the review experience.

The OECD IATA Case Studies Project allows countries to share and explore the use of novel methodologies in IATA for chemical hazard characterisation within a regulatory context. Find all the published case studies in the interactive table below.

Year	No.	Title	Key words provided by the authors	ADP (When a case study includes a ADP that has a ADP-Web No., the ADP-Web No. is listed.)	Other ADP-Web number	Uncertainty reporting	NAMs	Low/no toxicity
2022	1	<a href="#">Case Study on the use of Integrated Approaches for Testing and Assessment for the assessment of mechanotoxicity: Prediction of a Next Generation Risk Assessment Framework</a>	<ul style="list-style-type: none"> <li>NORA framework with inconsistent NAMs</li> <li>Key OMs resulted in inconsistent predictions</li> <li>POD using WAF</li> <li>Model calculated by PoD</li> <li>Revised NORA framework</li> </ul>	X				
2022	2	<a href="#">Case Study on the use of Integrated Approaches for Testing and Assessment for the assessment of mechanotoxicity: "Case Study on the use of Integrated Approaches for Testing and Assessment for the assessment of mechanotoxicity"</a>	<ul style="list-style-type: none"> <li>Two rule-based OMs for eye hazard identification (TG467)</li> <li>Four eye-equivalent liquids with the different L<sub>50</sub> A<sub>50</sub></li> <li>DAL-2: <i>in vitro</i> only</li> <li>Same conclusion with little uncertainty</li> <li>Feasibility and reliability of the TG 467</li> </ul>	X				
2021	1	<a href="#">Case study on the integration of <i>in vitro</i> data in the developmental neurotoxicity based assessment and characterisation using D<sub>NT</sub>-VNI</a>	<ul style="list-style-type: none"> <li><i>In vitro</i> developmental neurotoxicity testing battery (D<sub>NT</sub>-VNI)</li> <li>Permethrin</li> <li><i>In vivo</i> developmental neurotoxicity study</li> </ul>	X		X	X	
2021	2	<a href="#">Case study on the integration of <i>in vitro</i> data in the developmental neurotoxicity based assessment and characterisation using D<sub>NT</sub>-VNI</a>	<ul style="list-style-type: none"> <li><i>In vivo</i> developmental neurotoxicity testing battery (D<sub>NT</sub>-VNI)</li> <li>Permethrin</li> <li><i>In vivo</i> developmental neurotoxicity study</li> </ul>	X		X	X	X
2021	3	<a href="#">Case study on the use of Integrated Approaches for Testing and Assessment for the assessment of mechanotoxicity: "Case Study on the use of Integrated Approaches for Testing and Assessment for the assessment of mechanotoxicity"</a>	<ul style="list-style-type: none"> <li>D<sub>NT</sub> - developmental neurotoxicity</li> <li>Permethrin</li> <li>Same endpoints</li> <li>Zelrafach</li> </ul>	X		X	X	
2021	4	<a href="#">Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity based characterisation of permethrin</a>		X		X	X	
2021	5	<a href="#">Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity based characterisation of permethrin and the metabolite permethrin-epoxide</a>		X		X	X	
2021	6	<a href="#">Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity based characterisation of permethrin and the metabolite permethrin-epoxide: "Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity based characterisation of permethrin and the metabolite permethrin-epoxide"</a>	<ul style="list-style-type: none"> <li>Hazard characterisation of BPA and alternatives</li> <li>Transcription points of departure</li> <li><i>In vivo</i> and <i>in silico</i> weight of evidence</li> </ul>	X		X	X	

# Scope

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Ongoing work in the new approach methodology (NAM) / alternatives space across species / taxa

NAMs covers a broad space:

- *Exposure and toxicity*
- *QSARs / models*
- *Extrapolations & data distributions*
- *In vitro*
- *In vivo testing on non-protected taxa*
- *Optimized in vivo testing*

\*\*There is a fundamental need for foundational information to support NAM development & use\*\*

- Chemical property information
- Understanding of test systems
- Physiological information
- Behavioral / ecological information
- ADME parameters
- Databases of existing in vivo data
- Etc.

# Alternatives in Ecological Risk Assessment: Focus on birds & fish

	In silico	In vitro / in vivo NPLS	In vivo
	<ul style="list-style-type: none"> <li>• Toxicity QSARs (e.g., ECOSAR)</li> <li>• TK QSARs</li> <li>• ecoTTC</li> <li>• Read-across</li> <li>• Species sensitivity distributions</li> <li>• Acute to chronic ratios</li> <li>• Receptor binding QSARs (mostly EDC)</li> </ul>	<ul style="list-style-type: none"> <li>• RTGill W1 (OECD TG 249)</li> <li>• Fish embryo toxicity assay (OECD TG 236)</li> <li>• In vitro TK (OECD TG 319 A/B)</li> <li>• Algae / daphnia tests</li> <li>• Hyalella BCF</li> </ul>	<ul style="list-style-type: none"> <li>• Threshold approach</li> <li>• Modified BCF (OECD TG305)</li> <li>• Ongoing work to evaluate need for multiple fish species (USEPA)</li> <li>• Ongoing work to look across ELS / FFLC</li> <li>• Reduction in control fish needed (OECD TG210)</li> </ul>
	<ul style="list-style-type: none"> <li>• Ongoing work to develop models for extrapolation in birds</li> <li>• Interest in QSAR development</li> <li>• Database development (several ongoing) – Kuo et al bioaccumulation / TK just published</li> </ul>	<ul style="list-style-type: none"> <li>• Bird hepatocytes &amp; S9 – work via U of Sask on a TK method</li> <li>• Some bird cell lines available</li> <li>• Ongoing work on bird ELS (embryo tests)</li> </ul>	<ul style="list-style-type: none"> <li>• Evaluation of utility of bird sub-acute tests</li> <li>• Evaluation of number of animals for acute toxicity tests</li> <li>• Ongoing work on acute dietary → repro tests</li> </ul>

# Highlight of ongoing efforts -- birds

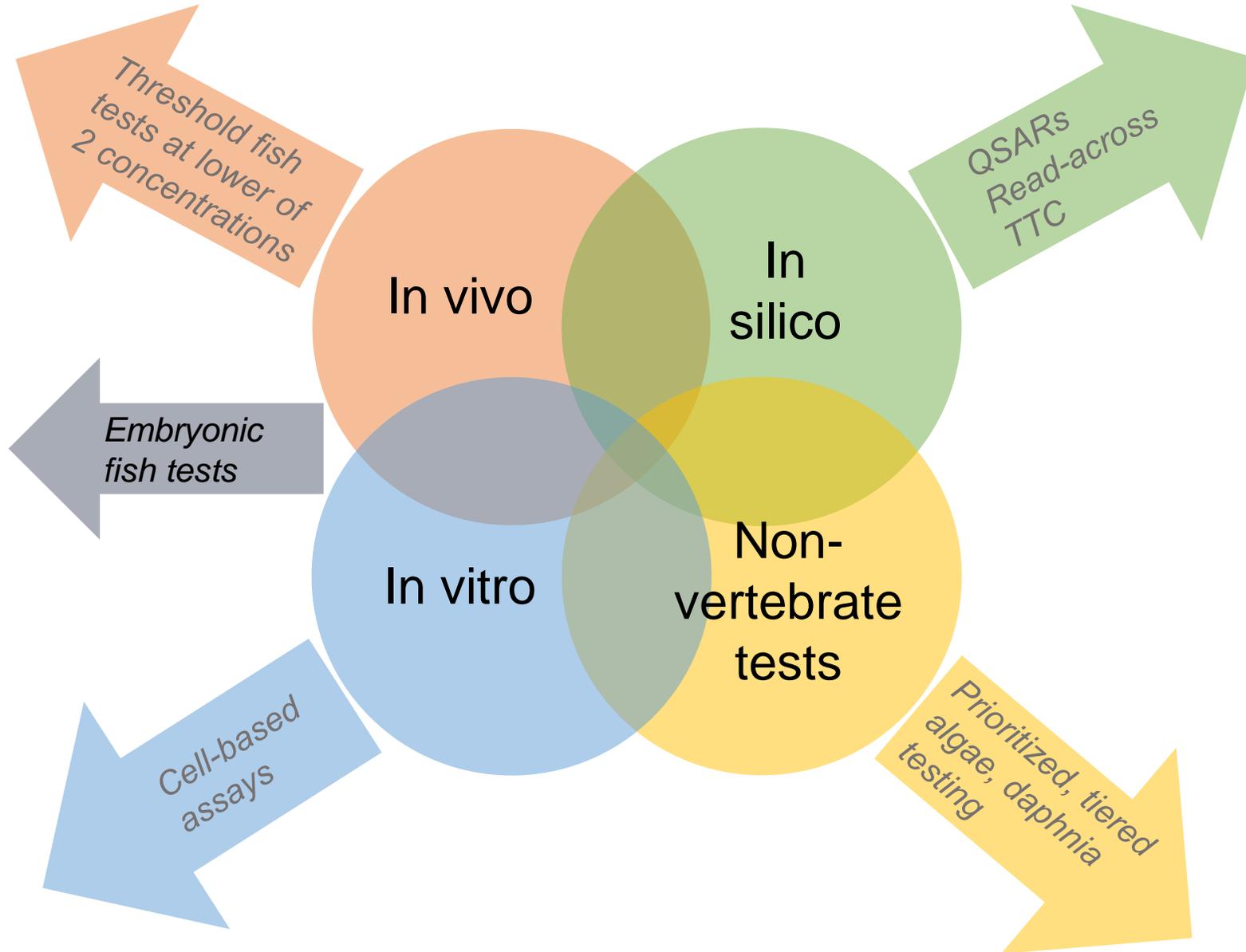
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- Retrospective evaluation of existing test data:
  - *USEPA pesticide office evaluation of avian sub-acute dietary test*
  - *Hilton et al., 2019. 10.1016/j.yrtph.2019.03.013*
- Refinement of OECD TG 223 (avian acute oral): reduction in animal numbers based on retrospective analysis
  - *Reduction of numbers of birds needed from 40 to ~15*
  - *Edwards et al., 2017. 10.1002/ieam.1930*
- Collection of avian bioaccumulation and TK information
  - *Can inform bioaccumulation & species extrapolation*
  - *Kuo et al., 2022. 10.1007/s44169-022-00008-8*
- Further development of bird in vitro models (focus on TK)
  - *Within HESI Committee – U of Sask. (M. Brinkmann) – in vitro TK approach*
  - *EcoTox Chip Project: <https://www.ecotoxchip.ca/>*

CRACK-IT Proposal for  
bird acute & chronic  
submitted to NC3Rs!

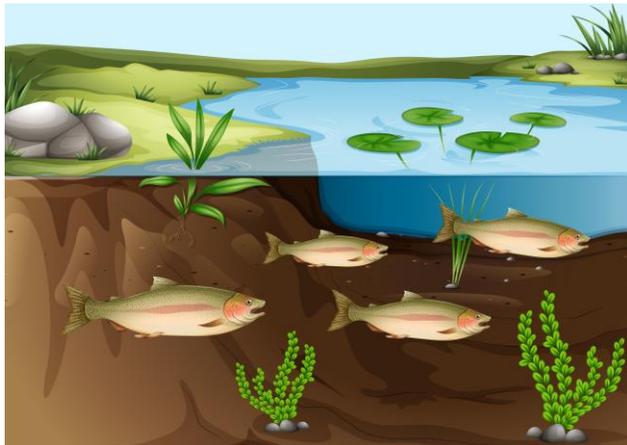


# The Scope of Alternatives: Fish Toxicity

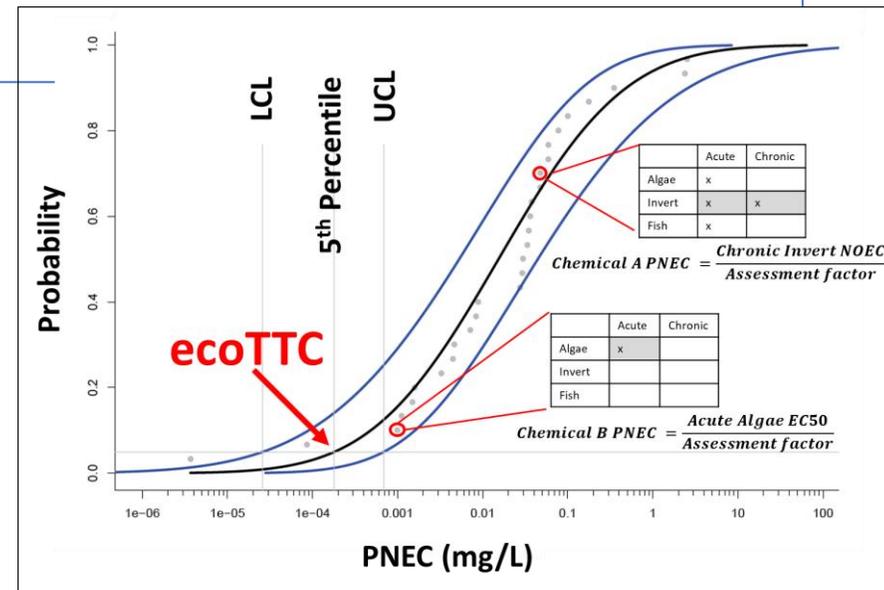


# Ecological Threshold of Toxicological Concern (ecoTTC)

- Leverages the concept used for decades in human health
- Concept is to leverage existing *in vivo* data to identify *de minimis* values for groups of chemicals that can be used to address gaps for chemicals with little or no data
- Originally applied to assess chemicals present in low levels (e.g., food contact materials, flavorings, impurities)
- **Based on grouping of chemicals**

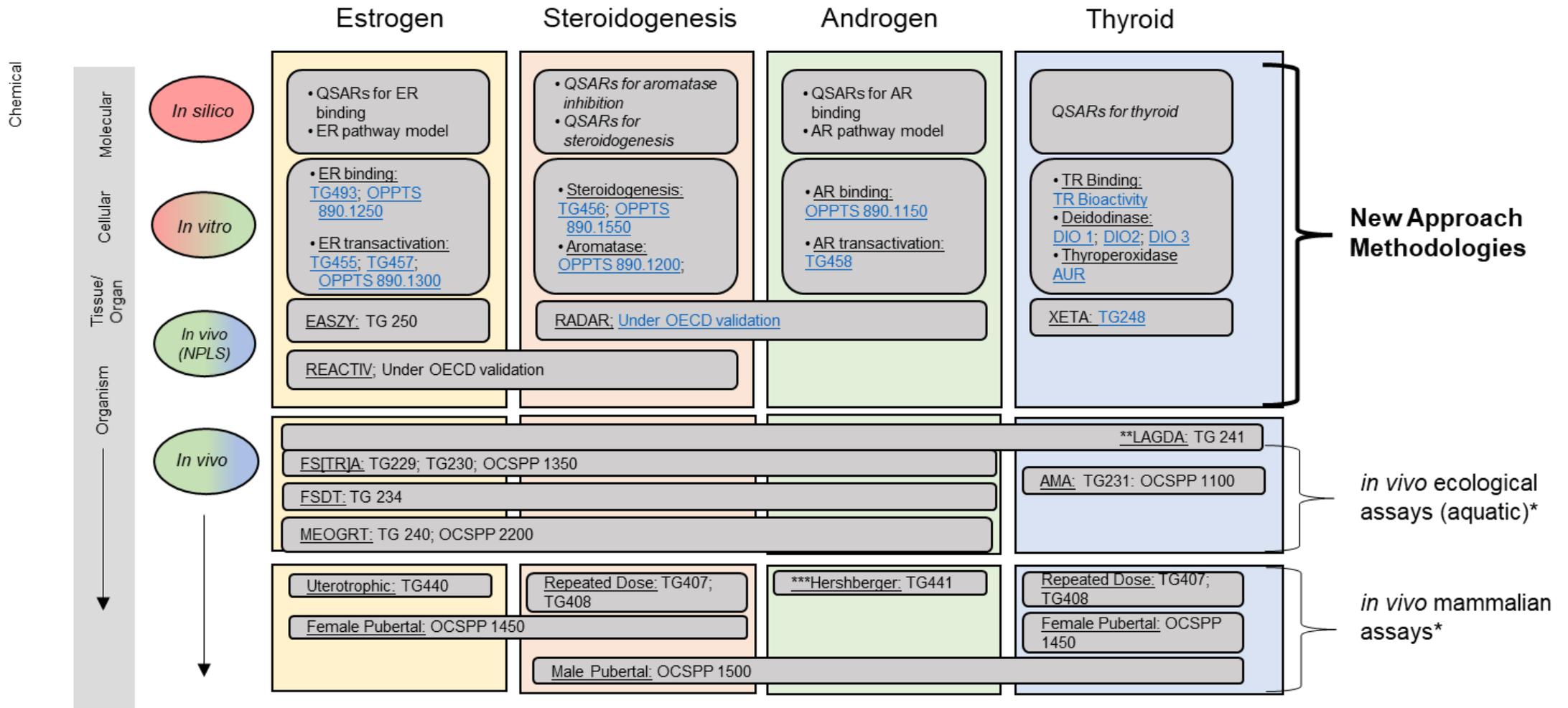


[www.envirottoxdatabase.org](http://www.envirottoxdatabase.org)





# Endocrine endpoints



# Fish RT-gill W1 Cell Line Assay



published in April 2019

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**ISO 21115:2019** [Preview](#)

Water quality -- Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1)



published in June 2021



**Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay**

The RTgill-W1 cell line assay describes a 24-well plate format fish cell line acute toxicity test using the permanent cell line from rainbow trout (*Oncorhynchus mykiss*) gill, RTgill-W1. After 24 h of exposure to the test chemical, cell viability is assessed based on three fluorescent cell viability indicator dyes, measured on the same set of cells. Resazurin enters the cells in its non-fluorescent form and is converted to the fluorescent product, resorufin, by mitochondrial, microsomal or cytoplasmic oxidoreductases. A reduction in the fluorescence of resorufin indicates a decline in cellular metabolic activity. [More](#)

Published on June 18, 2021    Also available in: [French](#)

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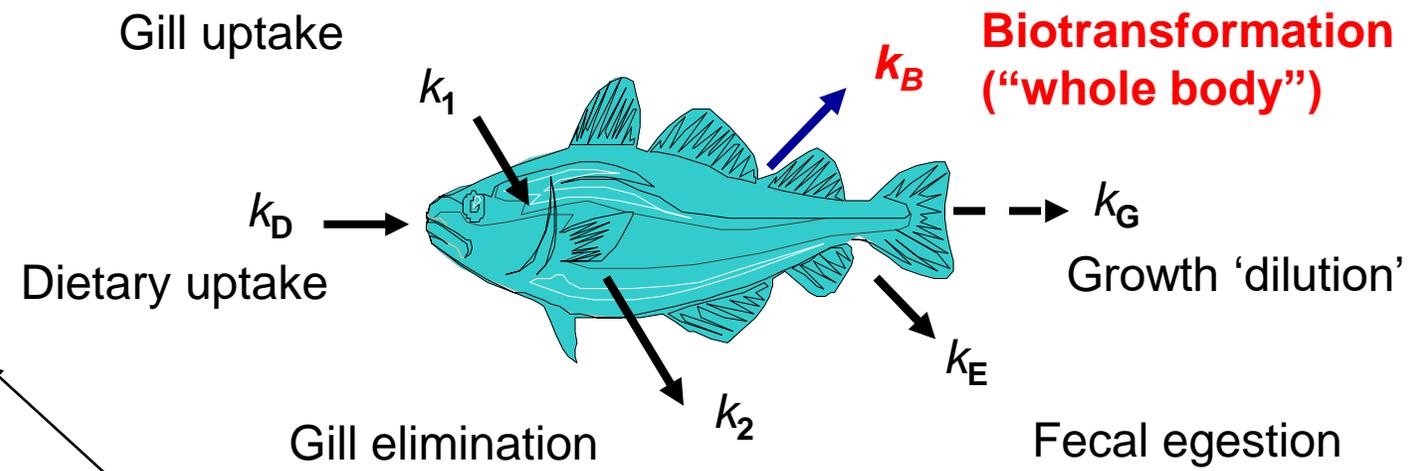






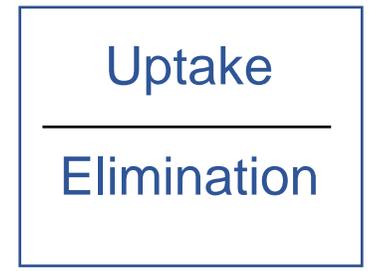
# Bioaccumulation

- **In silico models**
  - Several validated fish *in silico* (QSAR) models for predicting  $k_B^*$
- **In vitro methods**
  - OECD TG319 A/B



Mass Balance Equation

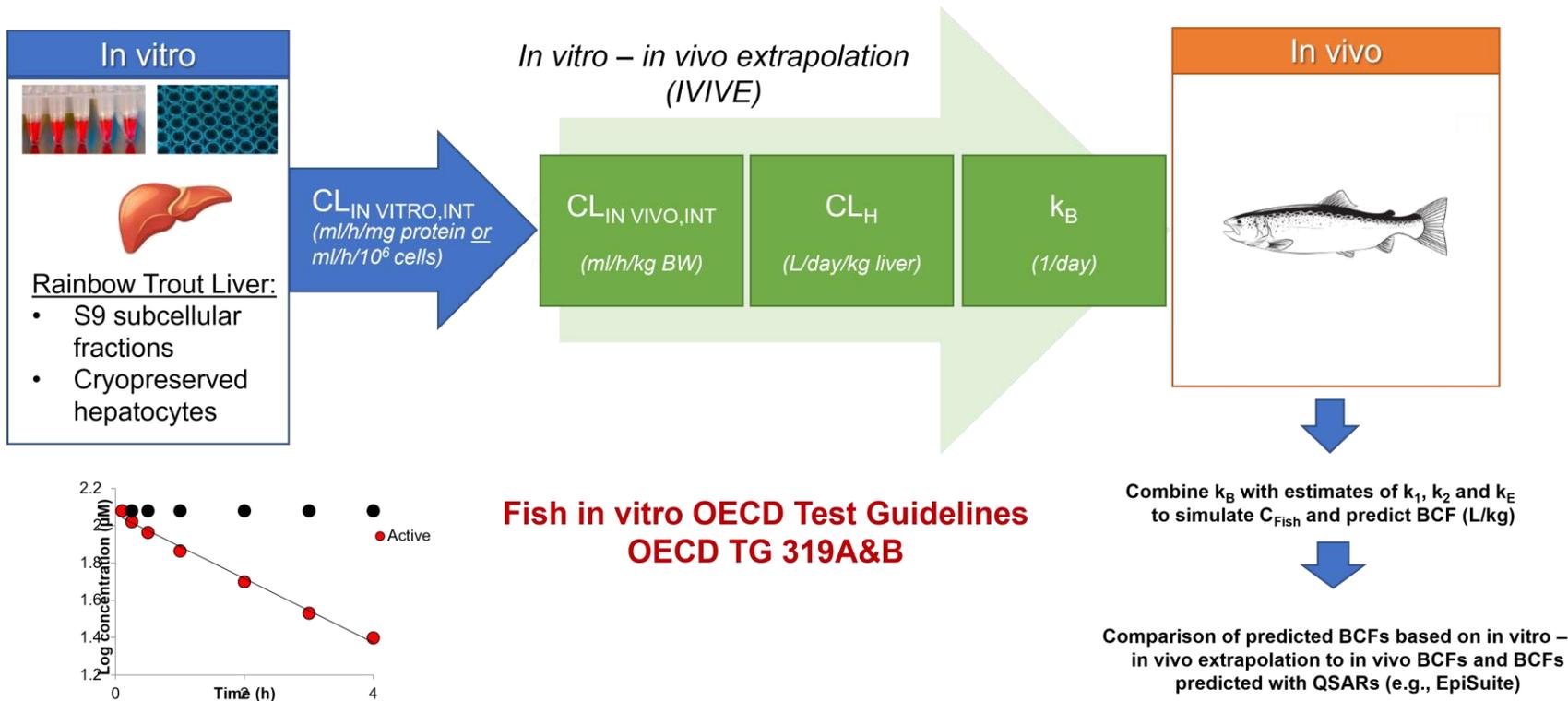
$$C_{\text{Fish}} = \frac{[(k_1 C_W) + (k_D C_D)]}{(k_2 + k_E + k_B + k_G)}$$



*\*In silico fish kB references:*  
Papa et al., 2014: 10.1016/j.scitotenv.2013.10.068  
Brown et al., 2012: 10.1021/es301182a  
USEPA EPISuite  
Arnot et al., 2009: 10.1897/08-289.1



# In vitro fish biotransformation



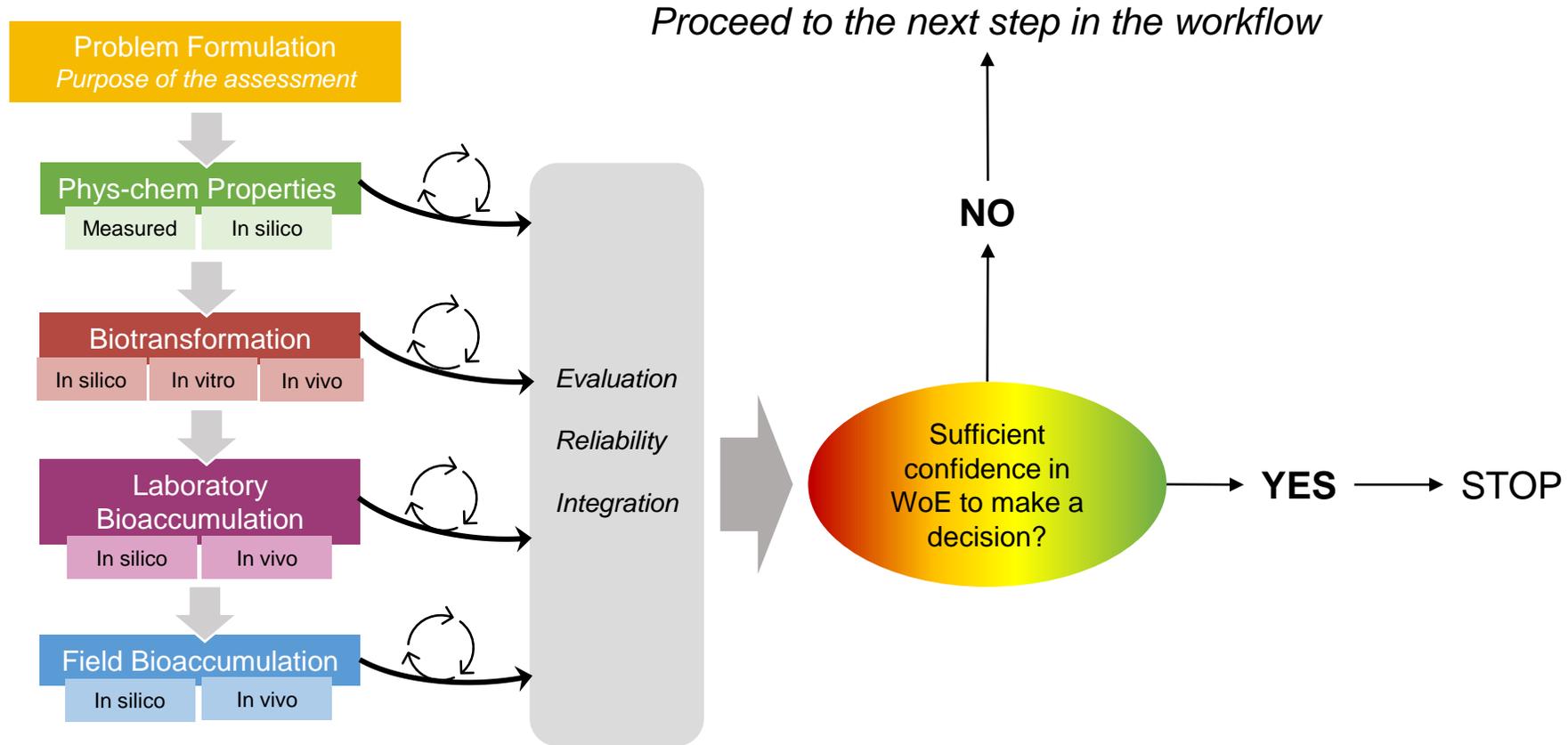
- Evaluated in >250 unique chemistries across various classes
- Generation of data is allowing better understanding of utility & application of the method
- Integration into WoE



In vitro bird assay under development!



# NAMs within IATAs.....



# Contact Information

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[www.envirotoxdatabase.org](http://www.envirotoxdatabase.org)

**Michelle Embry**

**membry@hesiglobal.org**

