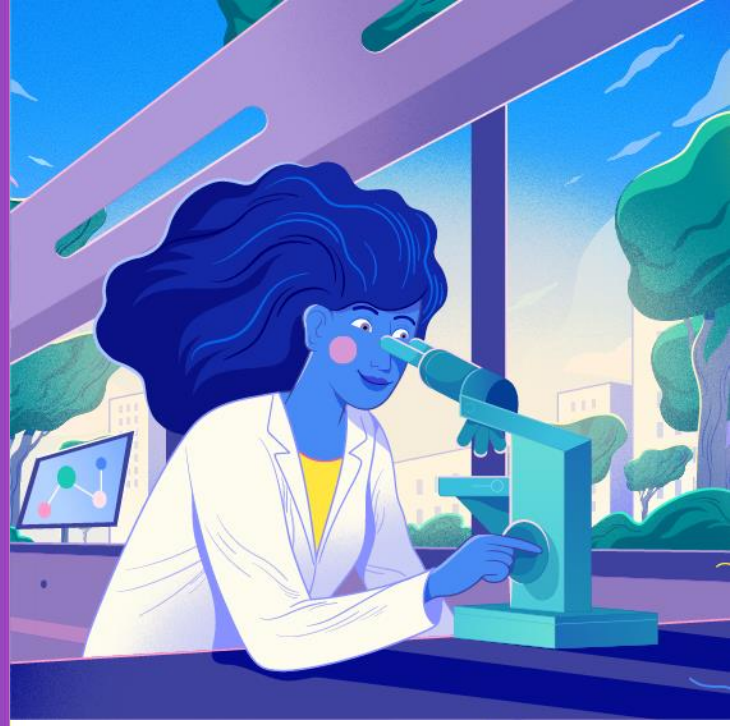
Developing functional extrapolation approaches across species to inform ERA



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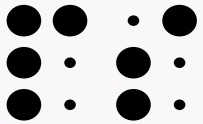
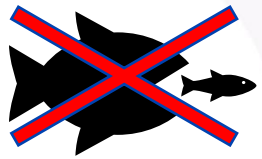
Safety science: what can we do better?

Ensuring that the use of ingredients in our products is **safe** for the receiving environment **YET...**

Increasing number of chemicals



Limited availability of toxicity data



Moving away from animal tests

...THUS increased need to rely on NAMs, mechanistic and predictive data to support Environmental Risk Assessment

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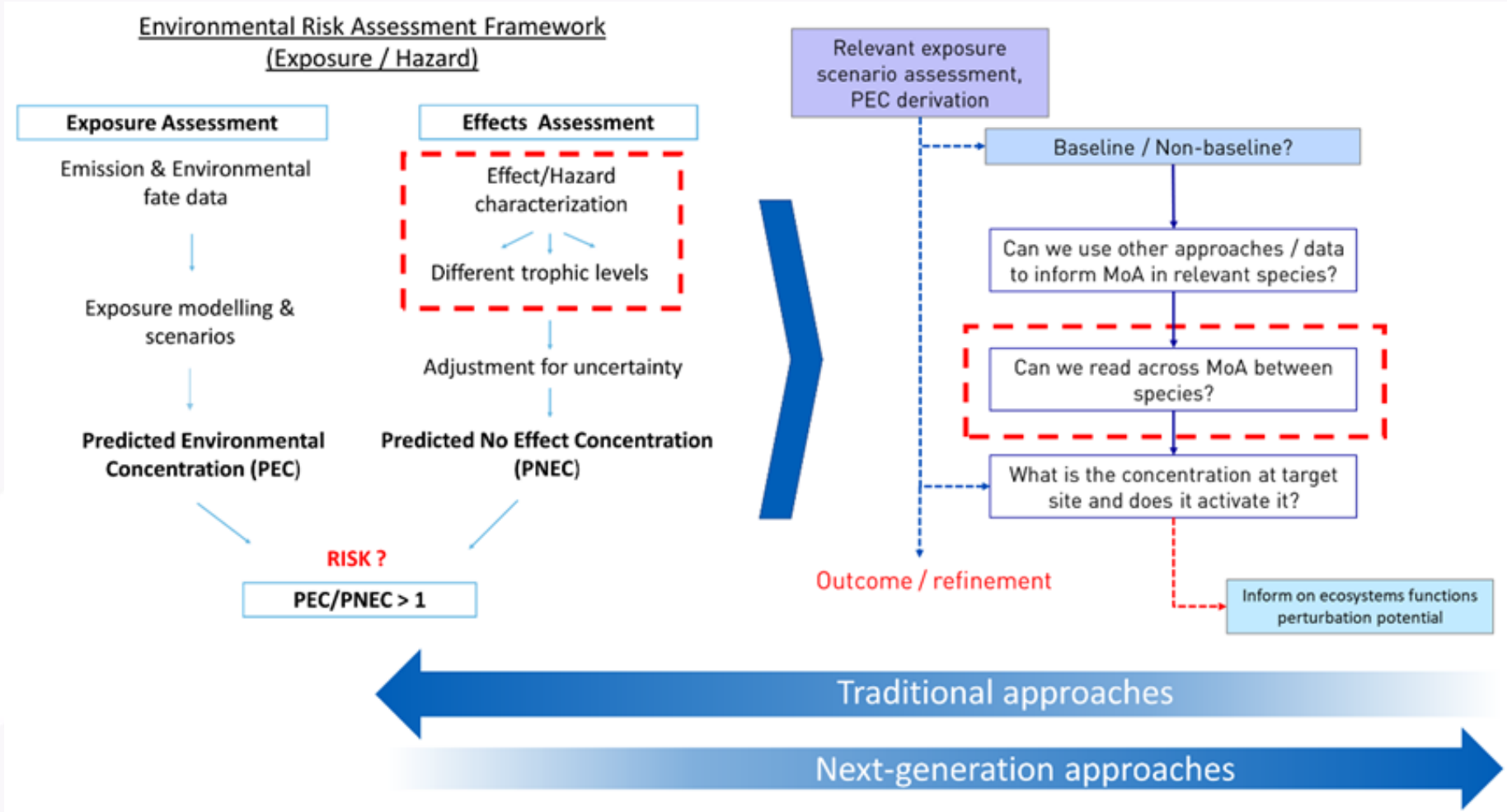
ERA: opportunities for evolving current approaches

Environmental Risk Assessment is driven **by exposure,**

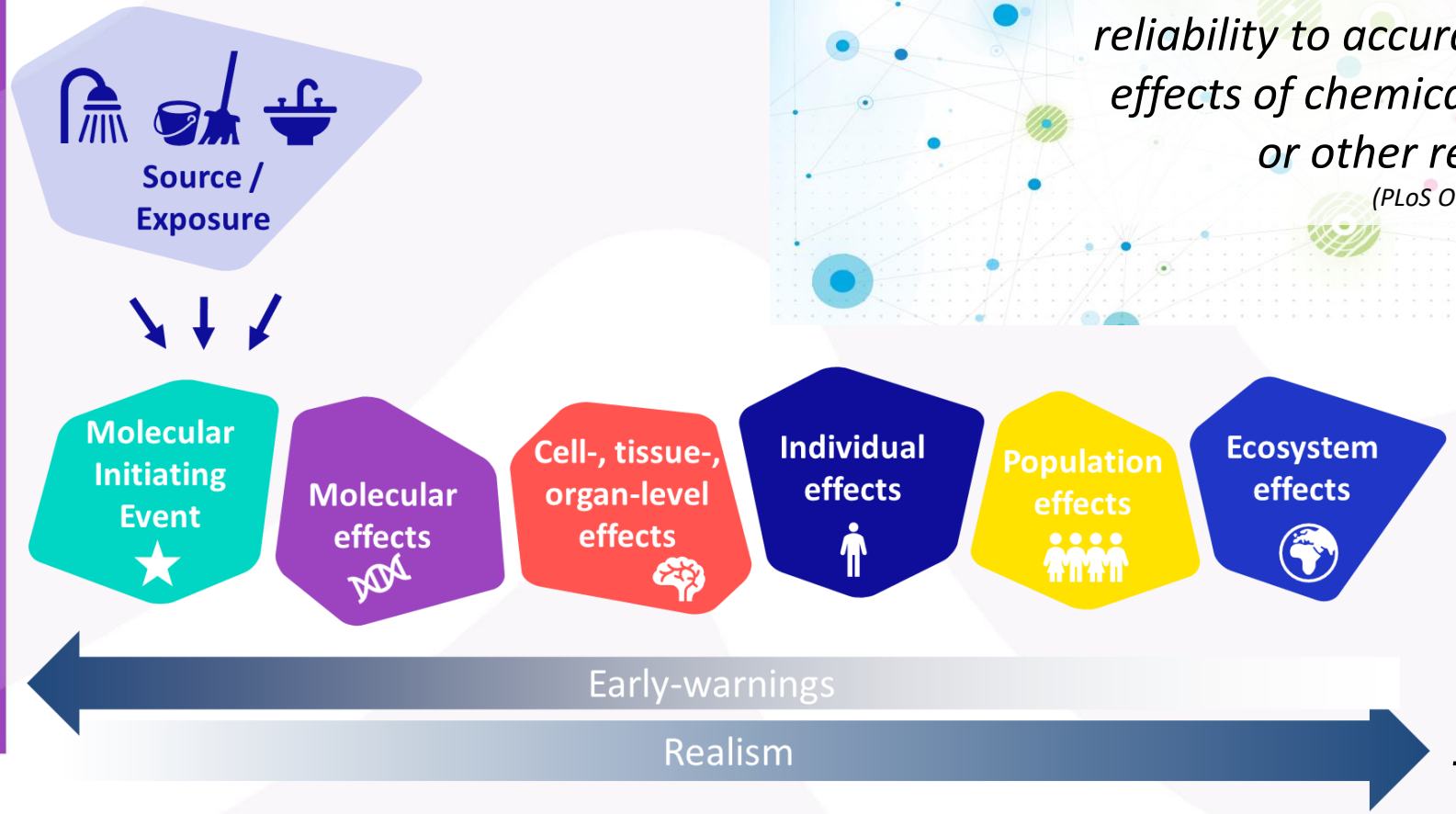
but evaluation of hazard can be improved, especially for specifically-acting chemicals



Cross species extrapolation – what do we know

"Cross-species extrapolation of biological processes represents a cornerstone in […] toxicology, intimately dependent on its reliability to accurately predict […] harmful effects of chemical substances in humans or other recipient species"

(PLoS ONE 9(10):e110467)



Critical to discern the conservation in physiological processes across species to predict response patterns and toxicity outcomes for specific MoAs in the environment.

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Mechanistic-based approaches in ERA: where are the opportunities?

Leveraging on the integrated use of all available data in a WoE approach to frame a mechanistically-driven testing strategy and hazard characterization.

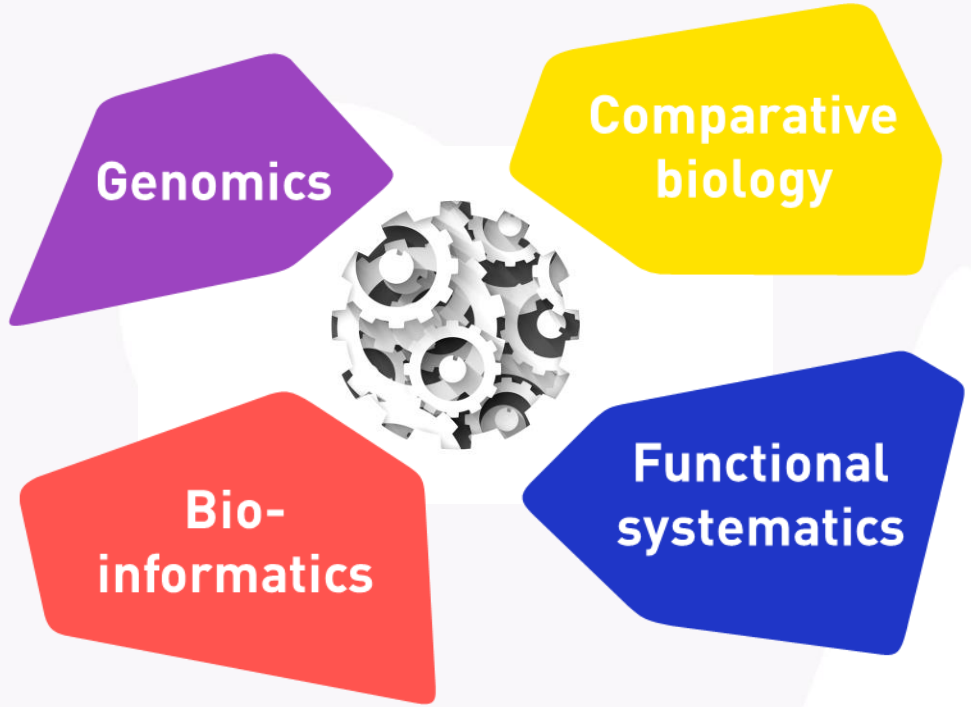
QSARs/*in-silico* alerts →

Pathways/AOPs →

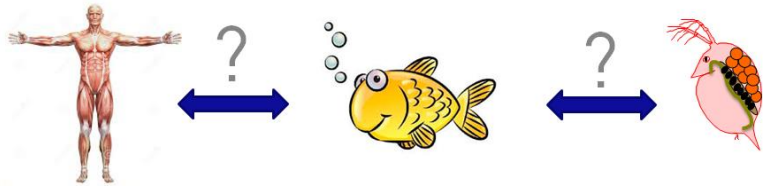
MoAs →

orthology →

functional annotation →



Can we use *in silico/in vitro* human data to understand potential effects in other species?



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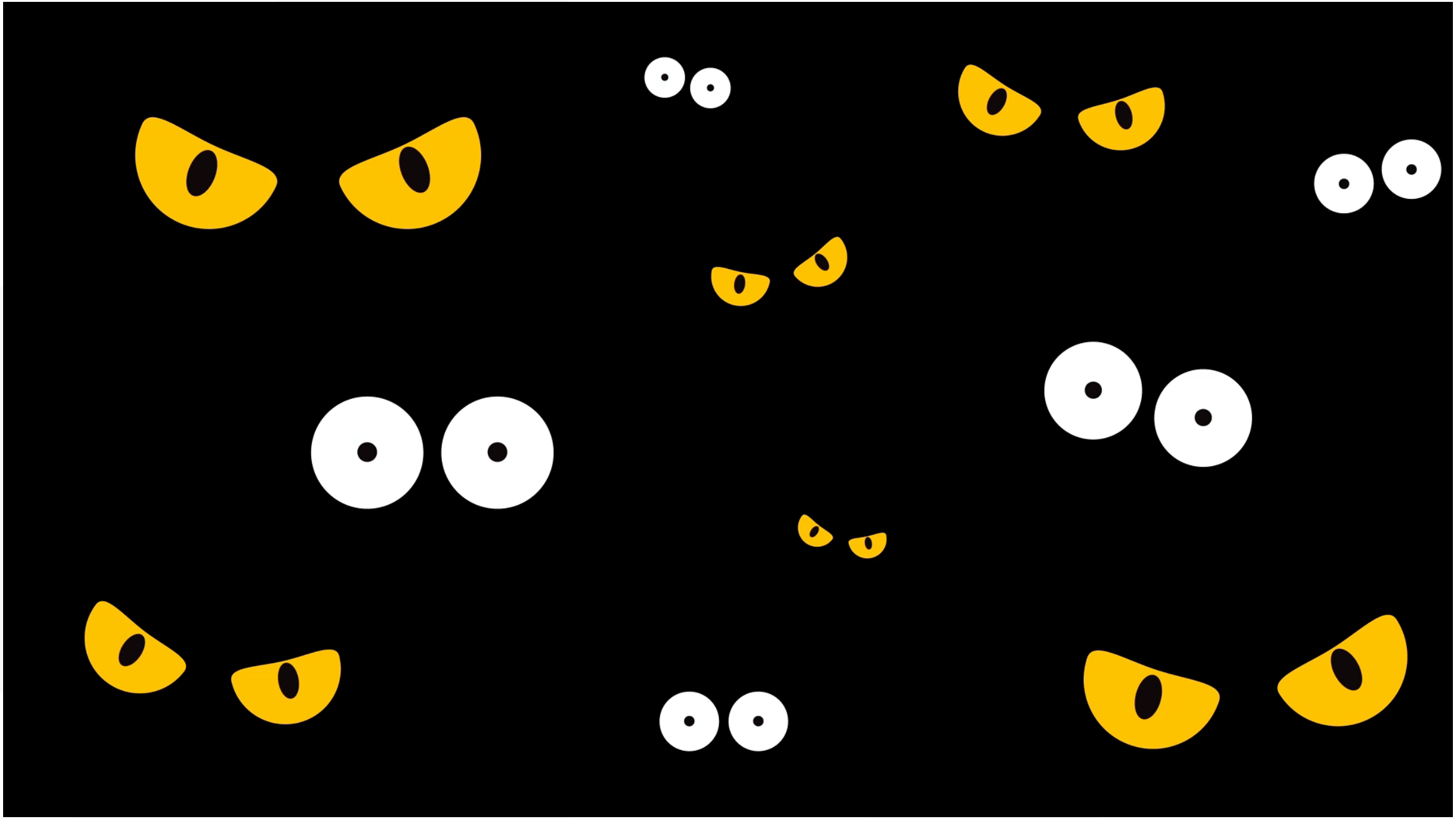


Genes to Pathways – Species Conservation ANalysis (G2P-SCAN)

Aim:

to integrate and socialize a number of existing databases to provide evidence of the conservation and functional coverage across 6 model species

Input: Human gene(s)

Step 1:
Mapping human gene(s) to their known biological pathways

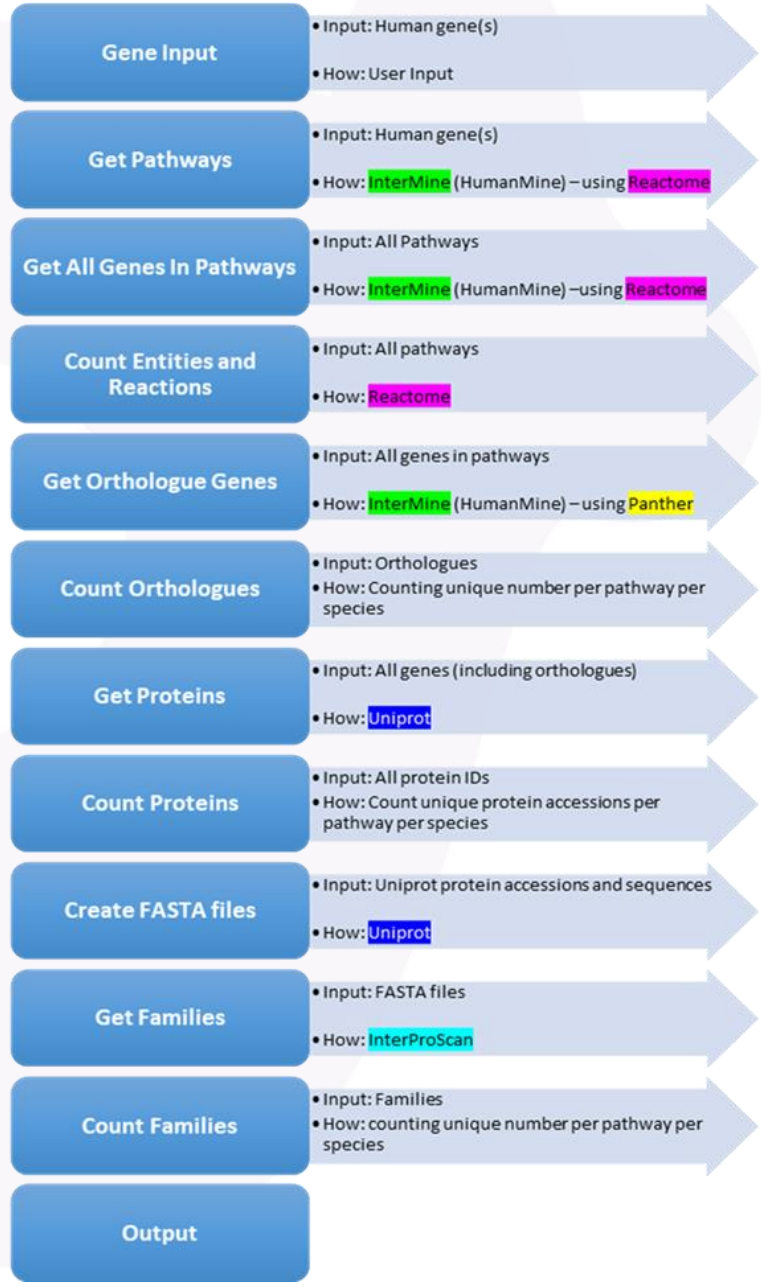
Step2:
Using orthology to assess the similarity across species

per each pathway: Retrieve all genes mapped → Finding all orthologues in other species: Mouse, Rat, Fish, Worm, FruitFly, Yeast

Step 3:
Retrieving information to infer gene functionality

Step 4:
Data analysis

Output: overview of all identified pathways and genes/proteins/families



Choose a CSV file with ENS ids

Browse... No file selected

Species

- ☑ Rat (R. norvegicus)
- ☑ Mouse (M. Musculus)
- ☑ Zebrafish (D. rerio)
- ☑ Worm (C. Elegans)
- ☑ Fly (D. Melanogaster)
- ☑ Yeast (S. cerevisiae)

Filter Orthologues

- ☐ Search only Least Divergent Orthologues

Run Pathways

- ☑ Parental
- ☑ Intermediate
- ☑ Terminal

Gene Summary
(X) Inputted Genes:
- XXX
- XXY
- XYY

(X-Y) Genes mapped to Pathways.
(Y) is/are not mapped to any Pathways
- XYY

(Z) Is/ are not found
- XXX

Run Selection
Model Organisms
- Rat (R. norvegicus)
- Mouse (M. Musculus)
- Zebrafish (D. rerio)
- Worm (C. Elegans)
- Fly (D. Melanogaster)
- Yeast (S. cerevisiae)

Orthologues
- All Orthologues

Pathways
- Terminal

Tool Versions
GTP – 1.0.0
Reactome – v72
Uniprot – XX
InterProScan - 5.45-80.0

Run Time – date/time
Run duration – 1hr

Results

View by Category

Switch to Species | Download

Genes | Proteins | Families | Entities/Reactions

Show as %

Pathway Name	Human				Rat			Mouse			Zebrafish			Worm			Fly			Yeast		
	Gene Matched	Coverage	Input Found	#Genes	Input Found	#Total Orthologues	#Unique Orthologues	Input Found	#Total Orthologues	#Unique Orthologues	Input Found	#Total Orthologues	#Unique Orthologues	Input Found	#Total Orthologues	#Unique Orthologues	Input Found	#Total Orthologues	#Unique Orthologues	Input Found	#Total Orthologues	#Unique Orthologues
A																						
-B																						
--C																						
D																						

Click on link embedded in pathway name to Reactome page

Hover to see which genes are found.

- **Technical validation**
  (manual vs tool)
- **Scientific validation**
  (5 Case-studies)

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Novelty, Drivers and Benefits

Transparency and Consistency in results

- Based on existent and reliable public databases
- Time-saving
- Flexible and user-friendly

Multiple applications

- Identifying level of conservation across species at different levels
- Providing increased understanding of (toxicity) mechanisms across species
- Informing on the potential susceptibility space of chemicals in the environment

Promotion of an integrated framework for RA without the use of animal-data

- maximising the use of available data and resources
- bridging the gaps between Human vs Eco

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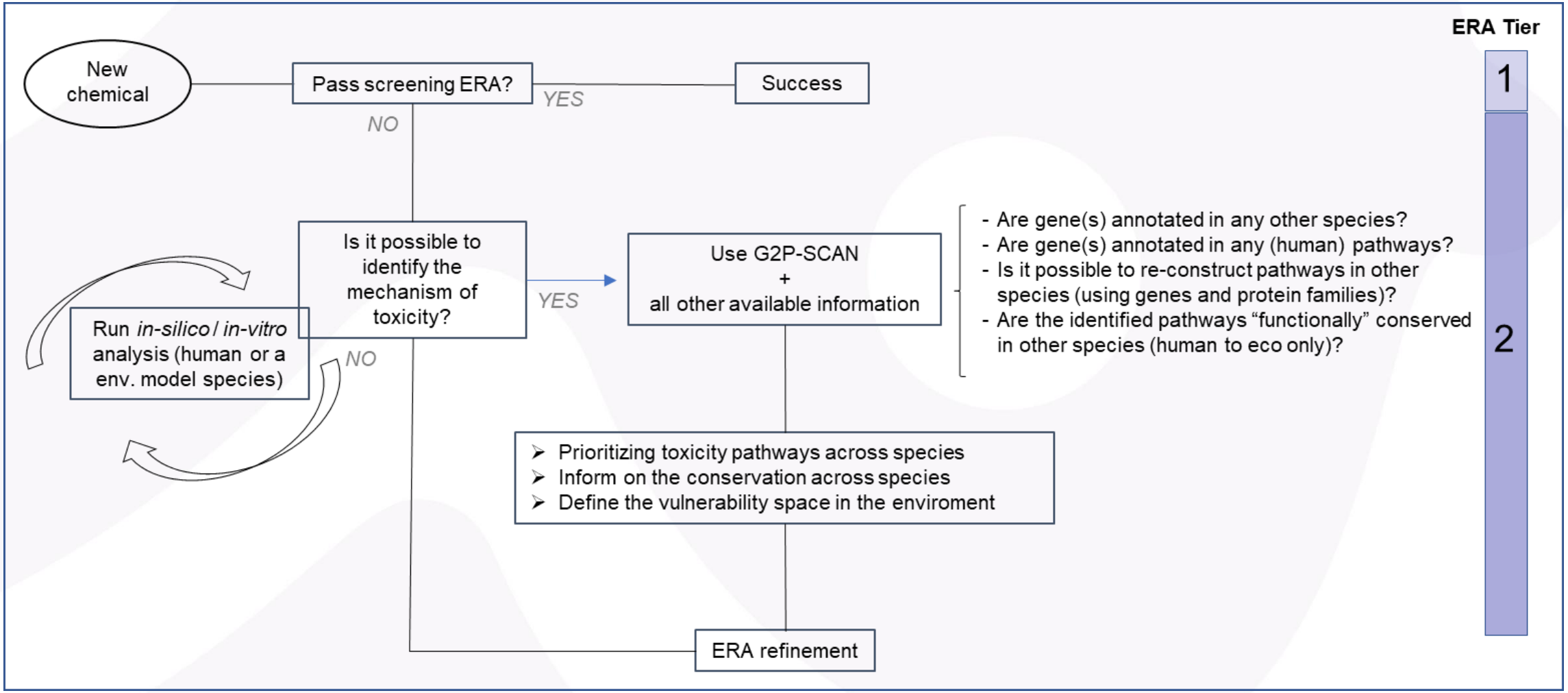
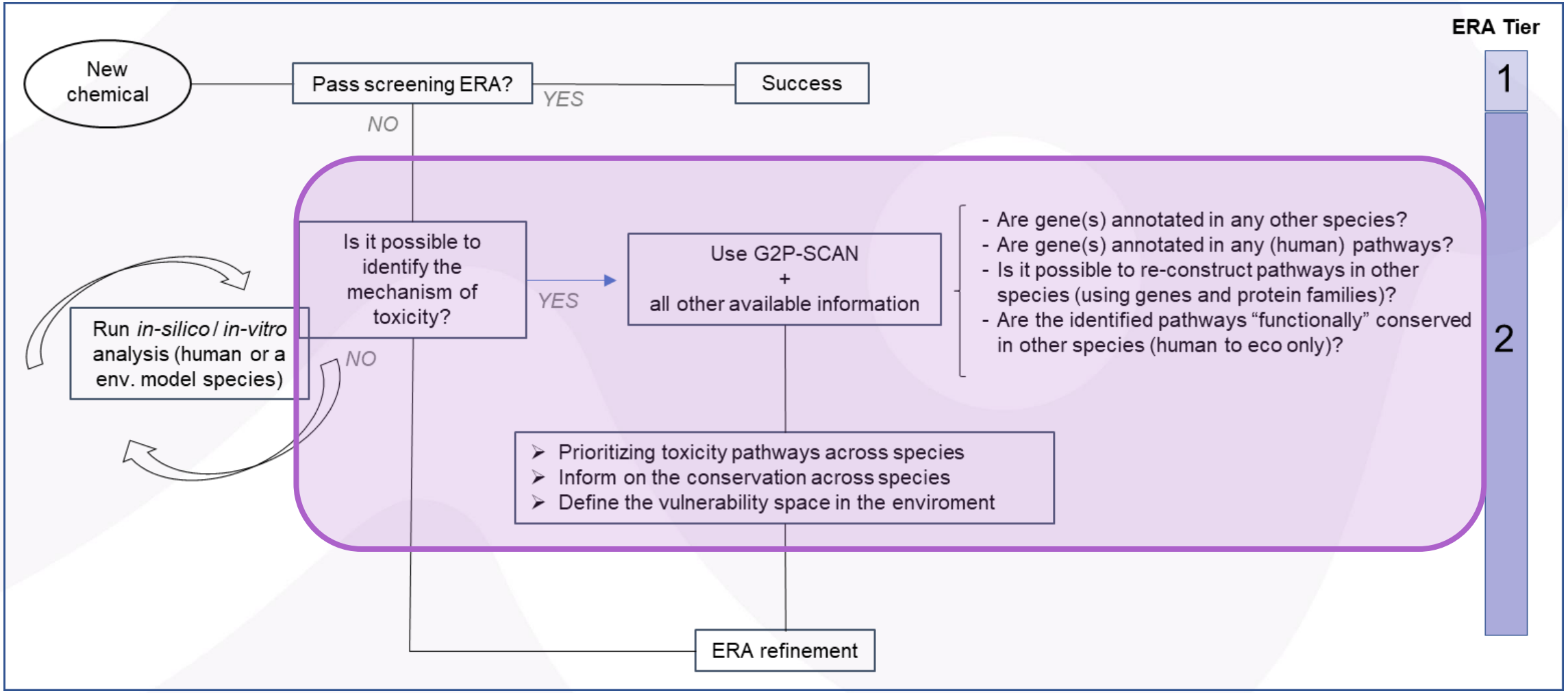
Proposed application into ERA framework

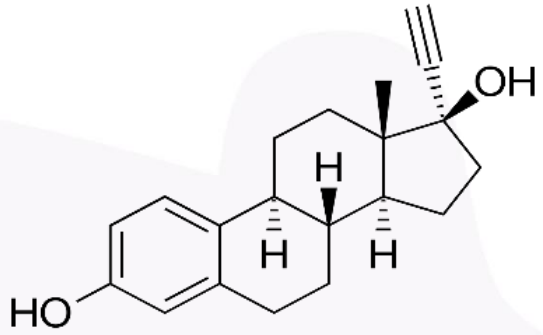


Proposed application into ERA framework



Case-study: Ethinyl Estradiol (EE2)

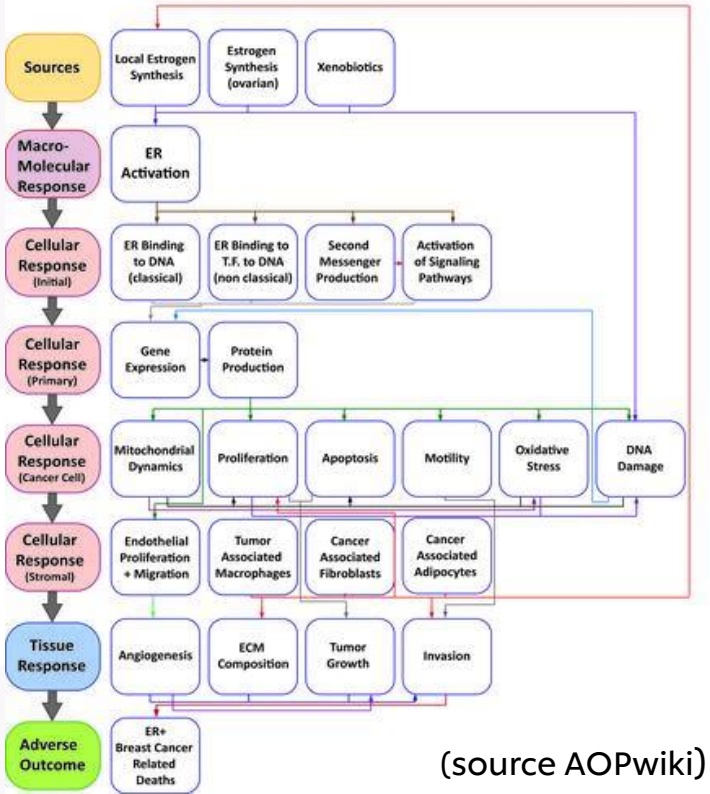
- ***Identify the mechanisms of toxicty***



Target: Estrogen Receptor 1 (ESR) - Uniprot ID: P03372

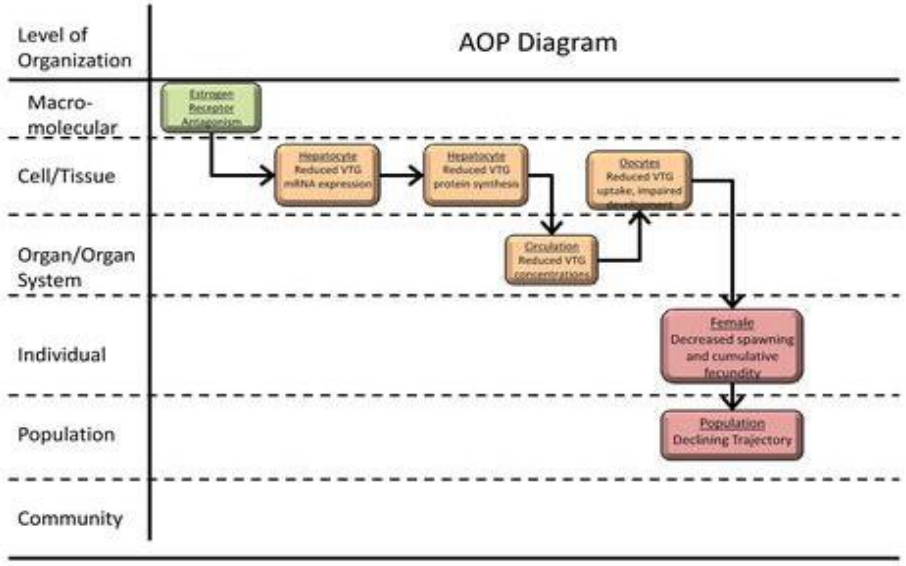
(source: Drug Bank)

AOP 200: Estrogen receptor activation leading to breast cancer



(source AOPwiki)

AOP 30: Estrogen receptor antagonism leading to reproductive dysfunction



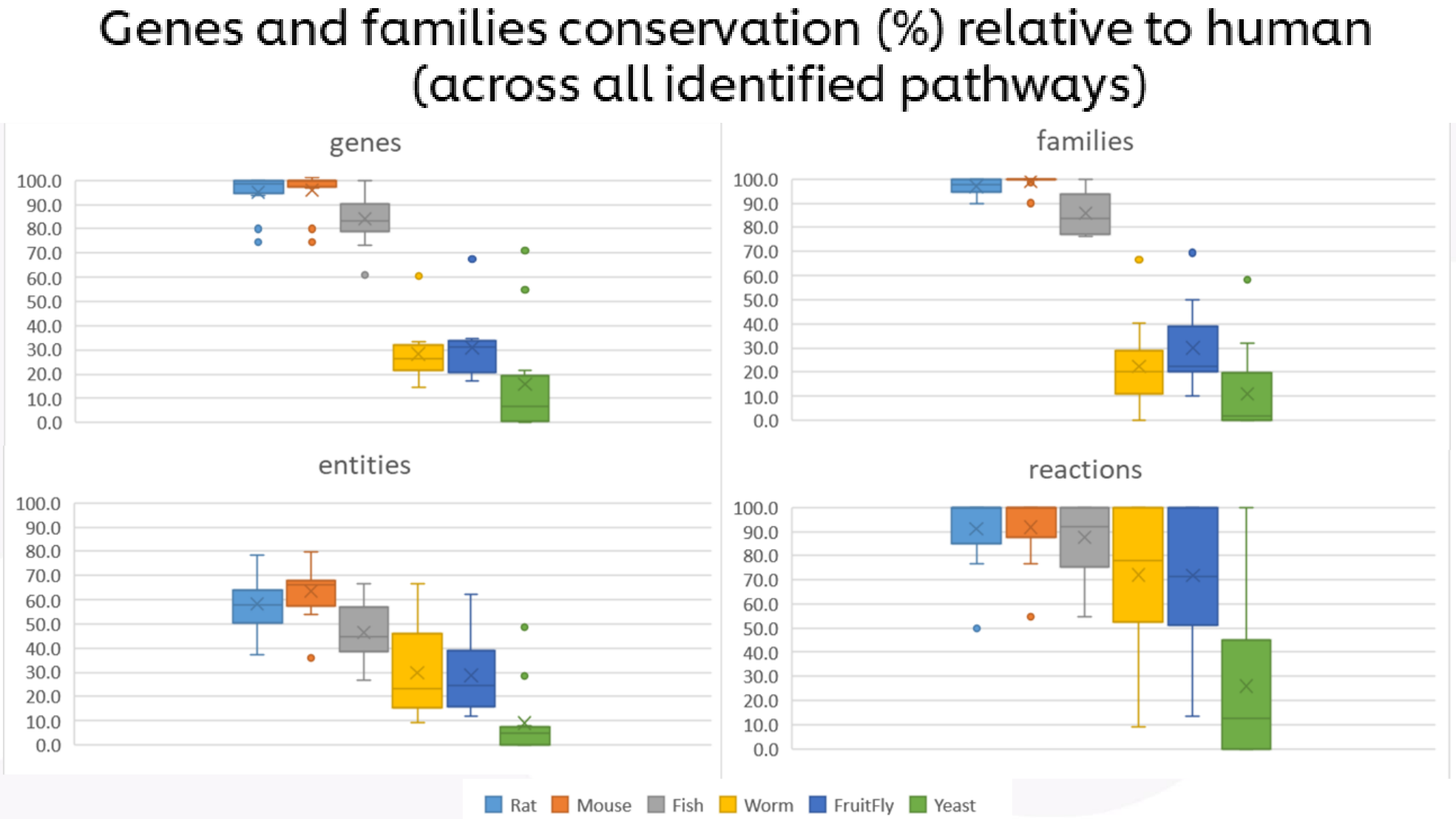
(source AOPwiki)

Case-study: Estrogen Receptor (ESR1)

➢ *Use G2P-SCAN and analyse results*

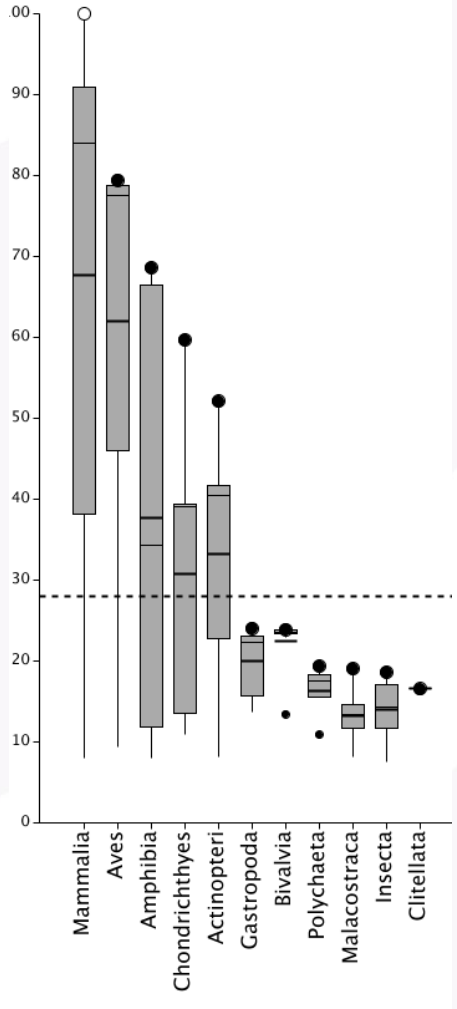
Case-study: Estrogen Receptor (ESR1)

- ***Use G2P-SCAN and analyse results***



Case-study: Estrogen Receptor (ESR1)

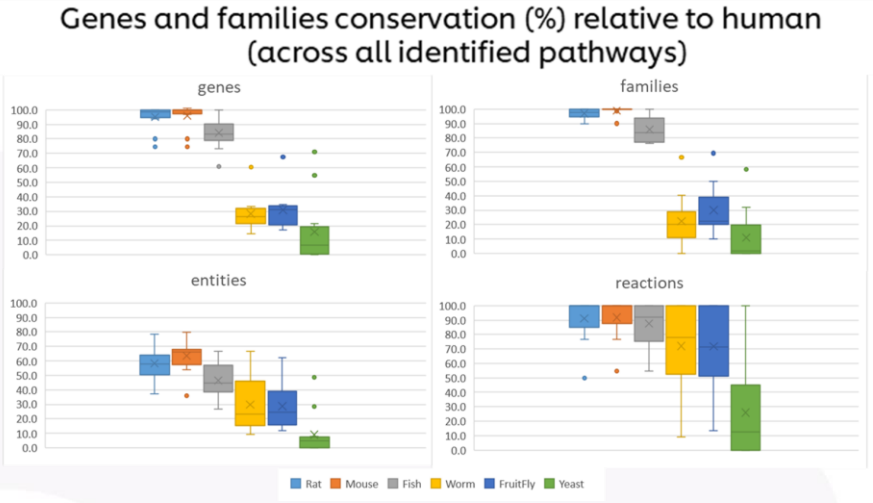
➢ *Building the Weight of Evidence*



(source SeqAPASS)

Target_name	Estrogen receptor
interaction	agonist
UniProt_ID	P03372
eukaryota	**36/62**
- fungi	0/5
viridiplantae	0/6
- chlorophyta	0/1
metazoa	**36/50**
- nematoda	0/1
- crustacea	0/1
- hexapoda	0/4
deuterostomia	**36/40**
- echinodermata	0/1
chordata	**36/39**
- tunicata	0/2
vertebrata	**36/37**
- teleostei	7/7
- amphibia	1/1
- aves	3/3
- mammalia	23/23

(source ECODrug)



NCBI Gene ID	Symbol?	Human Disease Count?	Species Name	Species specific gene ID	Species specific database	DIOPT Score?	Best Score?	Best Score reverse?	Confidence?	Publication Counts?
2099	ESR1	22 Drugbank :71 MARRVEL	Human (Homo sapiens)	3467	HGNC	NA	-	-		3237
13982	Esr1		Mouse (Mus musculus)	1352467	MGI	14/16	Yes	Yes	high	1101
24890	Esr1		Rat (Rattus norvegicus)	2581	RGD	14/14	Yes	Yes	high	459
394461	esr1		Western clawed frog (Xenopus tropicalis)	XB-GENE-482193	Xenbase	9/12	Yes	Yes	high	3
259252	esr1		Zebrafish (Danio rerio)	ZDB-GENE-020806-5	ZFIN	13/15	Yes	Yes	high	256
38912	ERR		Fly (Drosophila melanogaster)	FBgn0035849	FlyBase	2/16	Yes	No	moderate	76
4363091	nhr-286		Worm (Caenorhabditis elegans)	WBGene00044699	WormBase	1/15	Yes	Yes	low	2
179083	nhr-107		Worm (Caenorhabditis elegans)	WBGene00003697	WormBase	1/15	Yes	Yes	low	1
190264	nhr-239		Worm (Caenorhabditis elegans)	WBGene00021848	WormBase	1/15	Yes	No	low	2

(source Gene2Function)

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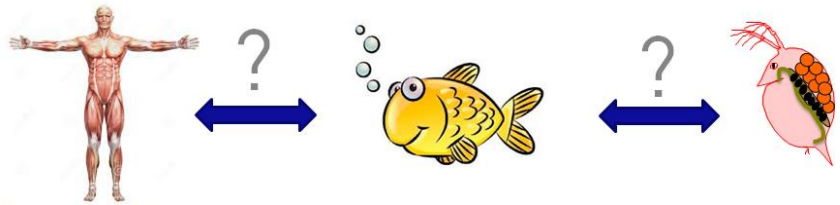
Take Home messages



No "one-size-fits-all approach"

- Considering several endpoints help supporting the WoE approach

Advantages of developing more powerful predictive methods to assess species conservation

- Allow for the identification of vulnerable species, in situations where there are not sufficient (traditional) data to run a comprehensive ERA.
- Serve as an essential driving guide in screening ERA to inform for higher tier refinement

Demonstrated utility of increased use of NAMs to inform ERA

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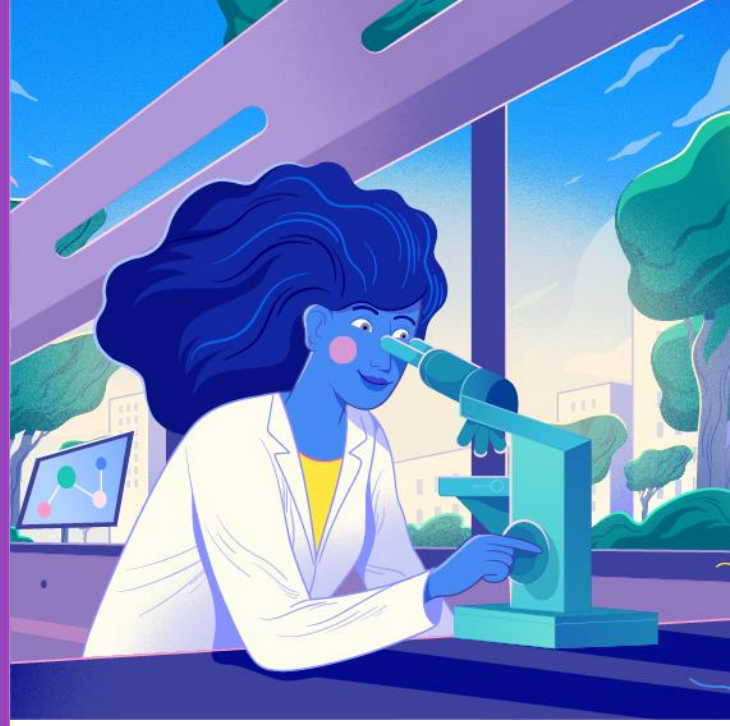
Thank you for your attention!



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