Identify chemicals potentially able to interfere with the endocrine system using a suite of complementary *in silico m*odels employing semi-automation

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TOX NAVIGATION
elena@toxnavigation.com

Elena Fioravanzo[1], Peter H.S. Russell[1]
[1]ToxNavigation, East Molesey, UK

BACKGROUND

Endocrine-disrupting chemicals are suspected to cause adverse effects in the endocrine system. In 2018 a guidance describing how to perform hazard identification for endocrine-disrupting properties by following the scientific criteria which are outlined in EU 2017/2100 and EU 2018/605 for biocidal products and plant protection products, respectively, was published by ECHA and EFSA. In this guidance computational approaches are proposed as line of evidence for endocrine activity assessment.

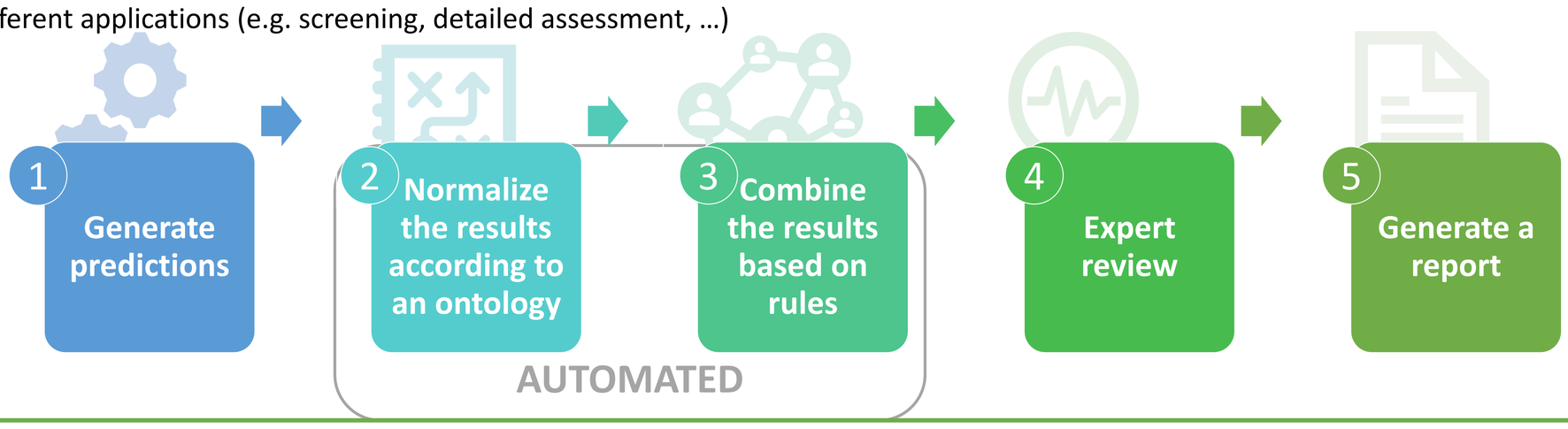
OBJECTIVES

1. To develop an *in silico* method suitable to be applied in the first step of the assessment strategy.
2. To address EATS (Estrogen, Androgen, Thyroid, Steroidogenesis) and "other" modalities.
3. To address the Mode of Action (MoA) agonist, antagonist or binding.
4. To combine predictive models developed with different methodologies.
5. To rate the predictions considering the uncertainty of the models and of the predictions.

PREDICTION WORKFLOW

Aims of the workflow

- ✓ To use existing models / existing knowledge
- ✓ To combine the different predictions into an overall assessment of the possible ED MoA (and not to generate a consensus prediction)
- ✓ To address different applications (e.g. screening, detailed assessment, …)



1 Generate predictions with 113 models

Number of models		Modalities				
		E	A	T	S	O
MoA	Agonism	9	5	--	1	6
	Antagonism	5	5	--	1	--
	Binding	19	9	20	1	20
	Multiple	2	1	2	2	5
Methods	Docking	4	2	2	2	6
	Profilers	5	2	2	2	6
	QSAR	23	13	18	1	19
	ToxCast	3	3	--	--	--

Modality	Receptor name
A	Androgen Receptor
E	Estrogen Receptor (α, β)
T	Thyroperoxidase
	Thyroid (hormone) Receptor (α, β)
S	Mineralocorticoid Receptor
	Glucocorticoid Receptor
	Aromatase

2

- Translate the original prediction (e.g. inactive, very weak, weak, …) values into the normalized values: Positive / Negative / Equivocal
- Translate the original applicability domain assessment into the normalized values: In / Out / Borderline

Applicability Domain		Normalized AD
AD	1	IN
AD_Index	> 0.6	
Conf_index	> 0.6	
AD	1	borderline
AD_Index	> 0.6	
Conf_index	< 0.6	
AD	1	IN
AD_Index	> 0.4 < 0.6	
Conf_index	> 6	

3

Elements of the combination

- Accuracy of the models: Sensitivity of the model; Type of model
- Validity of the prediction: Applicability domain
- Robustness of the prediction: % of n. predictions in the AD; Total n. of models available

4 5

15 negative calls
Grouped per mode of action
Antagonism (5), Agonism (5), Binding (5)
Grouped per methodology
Statistical (10), Rule (4), Docking (1)

1 equivocal call
Grouped per mode of action
Antagonism (1)
Grouped per methodology
Docking (1)

1 positive call
Grouped per mode of action
Antagonism (1)
Grouped per methodology
Statistical (1)

→

Experimental
Not available

Statistical-based
Final call = negative
Reliability = high

Rule-based
Final call = negative
Reliability = high

Docking
Final call = negative
Reliability = moderate

ToxCast
Not available

→

E modality
Final assessment = negative
Reliability = high/moderate

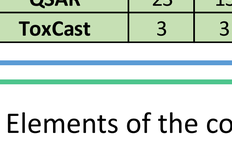
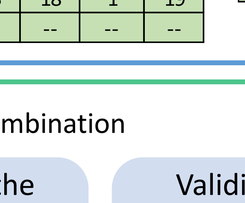
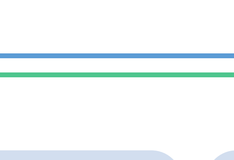
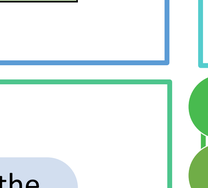
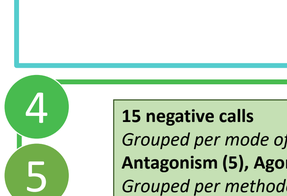
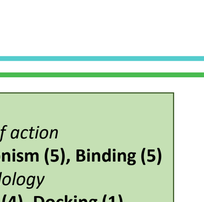
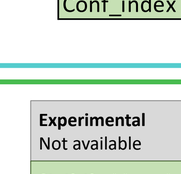
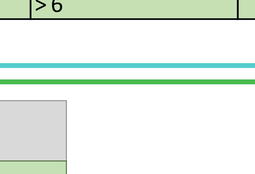
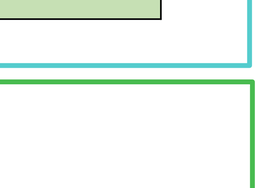
4 5

As different methodologies have different pros and cons, a final call per methodology is given together with an expert assessment of its reliability as follows:

- If all the predictions are positive or negative, the final call is respectively positive or negative and the reliability is evaluated on the basis of the sensitivity of the best model (the model with the highest sensitivity).
- If the predictions are a mix of positive and negative, an expert reliability assessment of the positive predictions is carried out and if at least one positive prediction is rated as highly reliable, the final call is positive and the reliability is evaluated on the basis of the highest sensitivity among the models resulting in a positive prediction.
- In all the other cases an expert judgment is applied and the reasoning is described.
- In general the equivocal predictions are considered only when necessary to refine the result of the final call.

Finally, a final assessment on the modality is given and a reasoning provided.

RESULTS

	From the ECHA Endocrine disruptor assessment list				*From the database EDKB (FDA)*					
	Molecule 1	Molecule 2	Molecule 3	Molecule 4	Molecule 5	Molecule 6	Molecule 7	Molecule 8	Molecule 9	Molecule 10
2D structure							Reviewed as A active (1) 			
CAS	100-21-0	84-61-7	98-54-4	106-44-5	938-16-9	n/a	438-22-2	108-43-0	10161-33-8	83792-61-4
Name	Terephthalic acid	Dicyclohexyl phthalate	4-tert-butylphenol	p-cresol	2,2-dimethylpropiophenone	4,4'-(1-fluorobutane-1,2-diyl)diphenol	androstane	3-chlorophenol	17-hydroxyestra-4,9,11-trien-3-one	3',5'-Dichloro-2-hydroxy-2-methylbut-3-enanilide
Assessed as	not ED	ED	ED	not ED	not E active	E active	not A active	not A active	A active	A active
In silico	not ED / high accuracy	ED, EA modalities, most probable MoA is E-binding/Agonist	ED, ET modalities, not clear MoA	not ED / medium accuracy	not ED	ED, EA modalities, E more probable	ED, EA modalities, E more probable	not ED	ED, EA modalities	ED, EA modalities, A more probable

SUMMARY AND FUTURE

- This *in silico* screening protocol combines the results of 113 models can predict the potential of a chemical to initiate an ED pathway with EATS modalities providing an hypothesis on the active modality and the MoA
- 10 case studies (5 ED and 5 not ED) were employed, all were correctly predicted as ED or not ED
- The *in silico* prediction provides strong evidence for prioritisation for further testing
- The protocol will be implemented in KNIME to avoid possible transcription errors
- A database containing the public training sets of the models will be built to facilitate the expert review

(1) 5α-ANDROSTANE—AN ANDROGENIC HYDROCARBON, Endocrinology, Volume 67, Issue 6, 1 December 1960, Pages 887–889, https://doi.org/10.1210/endo-67-6-887