Development of an Ontology-Driven *In Silico* Profiler for the Evaluation of Potential Endocrine Disruptors

Mark T.D. Cronin[1], Homa Basiri[1], Franklin J. Bauer[2], Bruno Campos[3], James W. Firman[1], Steve Gutsell[3], Geoff Hodges[3], Gaspard Levet[2], Jayne Roberts[3], and Paul C. Thomas[2]

1. School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK
2. KREATiS SAS, L'Isle d'Abeau, France
3. Safety and Environmental Assurance Centre, Unilever, Sharnbrook, UK

Introduction

- **A mechanism-based, *in silico* structural profiling scheme, for application within environmental chemical risk assessment, has been developed [1,2].**
- **This profiler [1,2] has a focus upon assigning compounds to mechanisms of acute toxicity, allowing primarily for facilitation of grouping and read-across.**
- **Other endpoints, e.g., endocrine activity, are poorly represented within its rules.**
- **The scheme is being expanded to account for effects related to chronic toxicity, through the identification of relevant molecular initiating events (MIEs).**

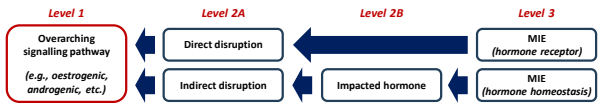
Aims

- **To develop further the present profiling tool, in order to capture knowledge of MIEs relating to endocrine activity. Specifically:**
  - **To organise existing knowledge of endocrine pathophysiology into a versatile, formalised ontology framework.**
  - **To identify putative MIE target sites related to endocrine activity.**
  - **To develop robust, MIE-derived structural alerts founded upon understanding of key structure-activity relationships (SAR).**

Methods

Creation of endocrine ontology and identification of MIEs

- **54 relevant Adverse Outcome Pathways (AOPs) from https://aopwiki.org/.**
- **AOPs organised into tiered framework, according to effect and mode of action.**
- **MIEs identified from pathways, and relevant substance activity data gathered.**



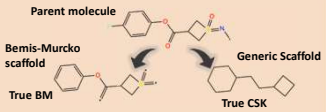
Computational Workflow for Molecular Scaffolds

Data Construction
- **Tox21** www.epa.gov/comptox-tools
- **ChEMBL** www.ebi.ac.uk/chembl/

Preprocessing Data
- **Dataset (chemical ID) curation** Structural representation (i.e., SMILES)
- **Molecular feature characterisation:** Mordred descriptors and ToxPrints
- **Feature pre-processing** Variance and multicollinearity analysis

Model Architecture
- **Machine Learning model:** Hierarchical clustering
- **Aim:** Clustering similar scaffolds based on molecular features

Scaffold Analysis
- **Murcko Scaffold generation:**



- Scaffold analysis of inactive compounds

Alert Refinement
- **Compounds grouped by scaffold**
- **Identifying key structural features associated with target affinity**
- **Building alerts upon inferred SAR**
- **Evaluation against known binders**

Known Binders
- **Inxight Drugs** https://drugs.ncats.io
- **Endocrine Disruptor Lists** https://edlists.org
- **DrugBank** https://www.drugbank.com

Molecular Modelling
- **Crystal structures of targets:** e.g., Oestrogen, androgen, thyroid
- **Software:** Chimera, Autodock Vina, Ligplot+, BIOVIA Discovery Studio

Integration within profiler:

Expansion of present tool, through establishment within of endocrine-associated rules and pathways.

Results

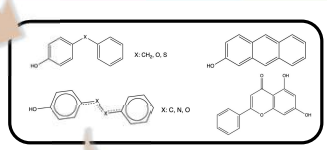
A total of 28 endocrine-relevant were MIEs identified. Three examples of scaffold analysis, informing structural alert development, are given below:

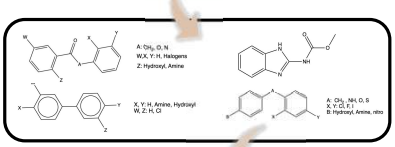
MIE list

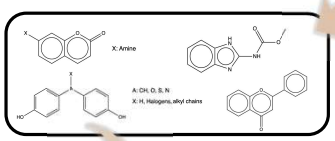
Coloured according to signalling pathway

PPAR-γ	Glucocorticoid receptor	Octopamine receptor	Thyroid peroxidase	Thyrotropin-releasing hormone rec.	Aryl hydrocarbon receptor	Thyrotropin receptor	Dual oxidase	Iodothyronine deiodinase	Juvenile hormone receptor	Progesterone receptor	Steroid 17α-mono-oxygenase	Steroid 5α-reductase	11β-Hydroxy steroid de-hydrogenase
Oestrogen receptor	Aromatase	Luteinising hormone receptor	Iodotyrosine deiodinase	Pregnane X receptor	Na+/I- symporter	Thyroid receptor	Succinate dehydrogenase	Transthyretin	Pendrin	Ecdysone receptor	Steroid 11β-hydroxylase	PPAR-α	Androgen receptor

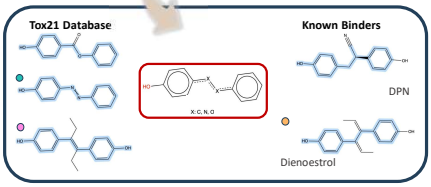
Molecular scaffolds for MIEs

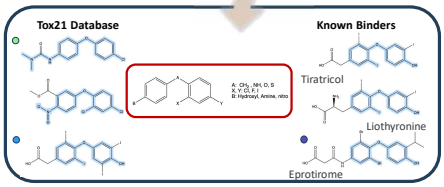


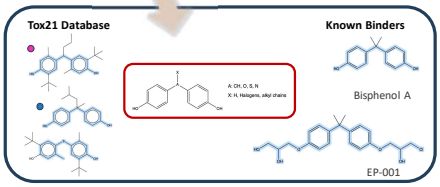




Case study molecules

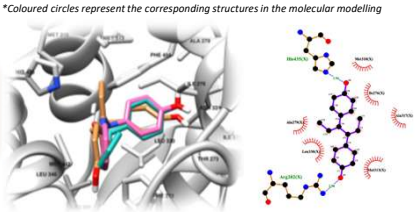


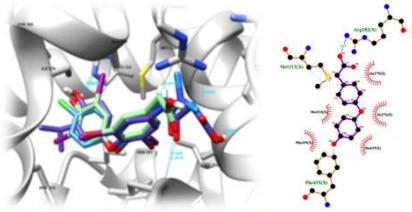


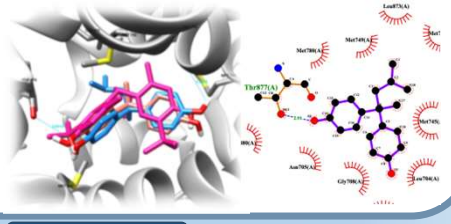


*Coloured circles represent the corresponding structures in the molecular modelling

Molecular modelling verification







Conclusions

- **A mechanistically-derived ontology for endocrine activity has been developed.**
- **MIEs, influencing a variety of endocrine pathways, have been identified.**
- **Structural alerts for these have been developed using relevant molecular scaffolds.**
- **Scaffolds are verified both with data and with modelling of ligand-receptor binding.**
- **The scheme, updated to include these endocrine-associated MIEs, will be integrated both into MechoA+ software [3] and as a profiler within the OECD QSAR Toolbox.**

References

[1] Sapounidou M *et al.* (2021) *Environ. Sci. Technol.* 55: 1897– 1907.
[2] Firman JW *et al.* (2022) *Environ. Sci. Technol.* 56: 17805-17814.
[3] Bauer FJ *et al.* (2018) *Comput. Toxicol.* 7, 36-45.

Further information → Homa Basiri: H.Basiri@ljmu.ac.uk