# Evaluation of an Ontology-Driven *In Silico* Profiler Representing Mechanisms of Action Related to Endocrine Disruption

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## Introduction and Aims

- Mechanism-based, *in silico* structural profiling tools, anchored in adverse outcome pathways (AOPs), are now well established for ecotoxicological risk assessment.
- *In silico* structural profiling tools facilitate the grouping of compounds for purposes such as read-across and the guidance of broader testing strategies. They, themselves, are not intended to be predictive.
- This study aimed to expand the scope of existing schemes [1-4] beyond acute toxic effects, to account for those mechanisms, impacting upon reproductive and developmental outcomes, collectively termed endocrine disruption (ED).
- Having constructed a broad assortment of ED-associated structural alerts, their coverage in relation to an inventory of putative endocrine-active substances (EAS) was evaluated.

## Materials and Methods

### Creation of Mechanistic Profiling Scheme Incorporating Endocrine-Related MIEs

- Development of a functional profiler to identify potential EAS proceeded through four principal stages (outlined within Figure 1).
- Relevant molecular initiating event (MIE) sites were those acknowledged as serving functions exclusive to endocrine signalling, either as key hormone receptors, or as mediators of hormone synthesis/ADME.
- Accordingly, those such as AhR, PPAR, etc., which hold potential to influence aspects of physiology beyond the endocrine system, were considered beyond the scope of the present exercise.

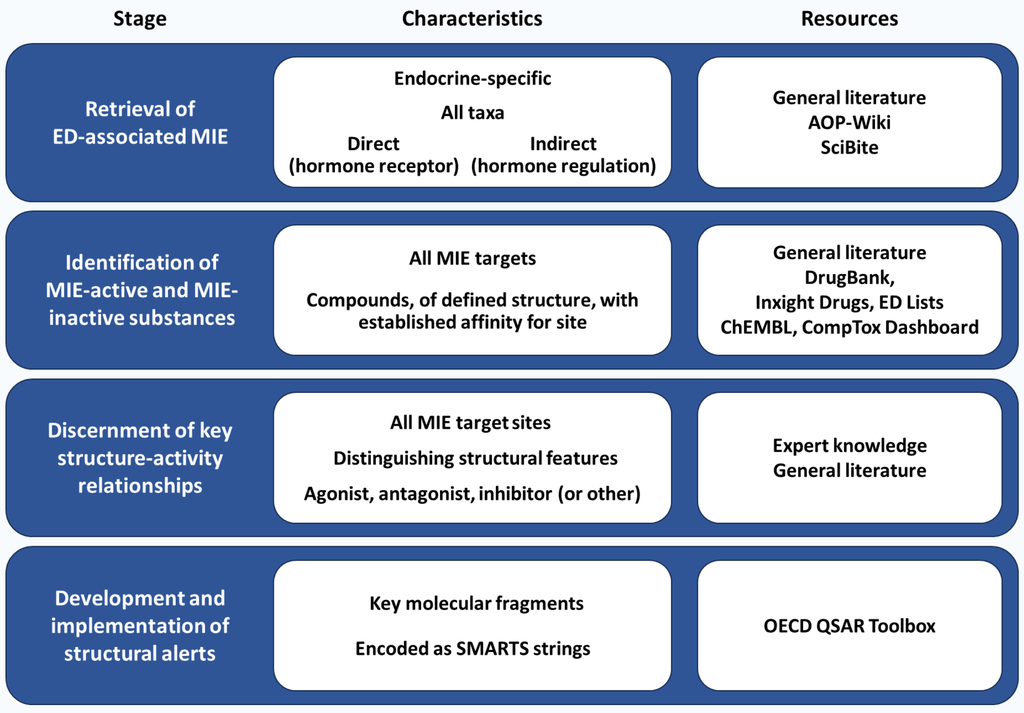



**Figure 1.** Overview of processes underlying development and implementation of ED mechanistic profiling scheme

### Evaluation of *In Silico* Profiler Coverage

- A list of 1,011 chemicals was obtained from a broader collection of potentially endocrine-active compounds, collated through the EU PARC project [5].
- Corresponding SMILES strings were passed through the implemented, SMARTS-based scheme.
- Frequency of matches, at both structural alert and MIE level, were subsequently recorded.

## Results: Coverage of the EA *In Silico* Profiler

- 477 (47.2%) of the 1,011 compounds within the EU PARC list were assigned a plausible EA mechanism.
- Associations were noted between these substances and 22, from the 28, MIE target sites.
- Frequencies of target matches are illustrated within Figure 3a (relating to the Key receptor domain) and Figure 3b (Hormone-mediated domain).
- Alerts for oestrogen receptor (n = 385) and transthyretin (n = 92) were, respectively, most commonly fired.
- Amongst those substances not aligning with alerts, there were found assortments of commercial pesticides and simple, industrial chemicals – many lacking in terms of defined MIE.

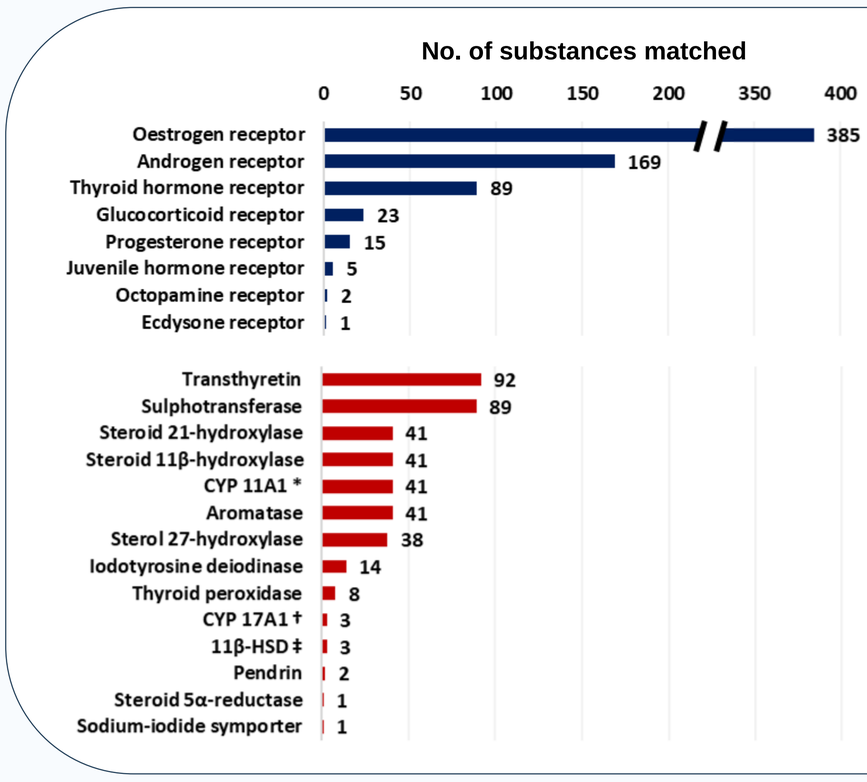



**Figure 3a.** MIE target sites, (Key receptor) ordered in accordance with frequency of matches against members of PARC-derived dataset.

**Figure 3b.** MIE target sites, (hormone-mediated) ordered in accordance with frequency of matches against members of PARC-derived dataset.

- Of the 58 alerts structural alerts present, 45 were matched by at least a single substance.
- Within Figure 4 is presented a list of all alerts fired by ten compounds or more.
- Phenolic classes, typically oestrogenic in effect (e.g., bisphenol, alkylphenol, flavonoid and paraben) are heavily represented.

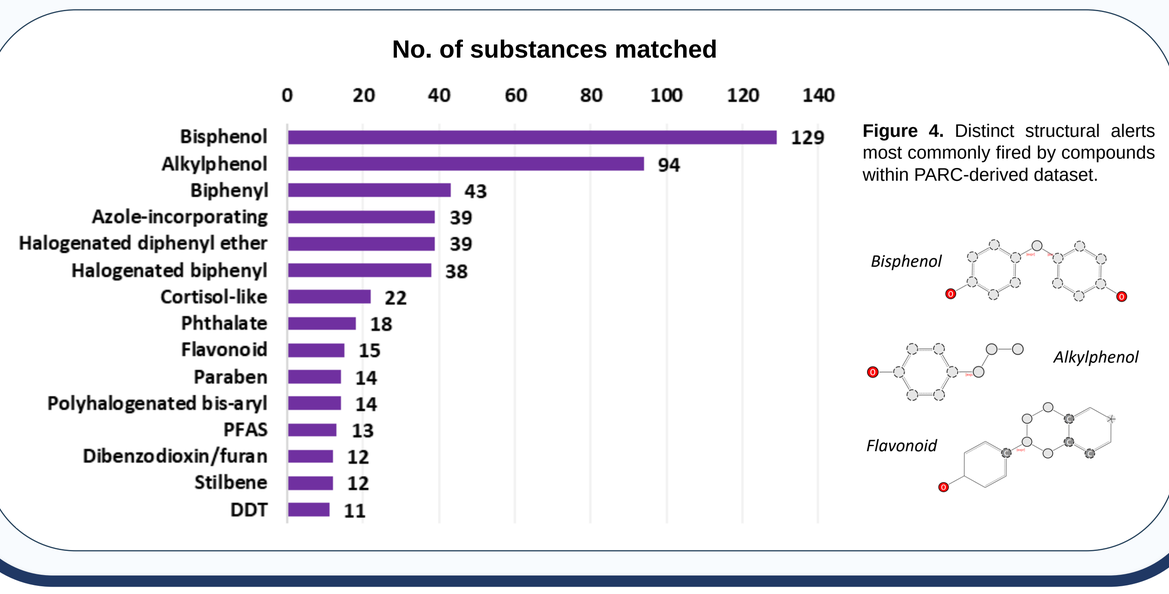
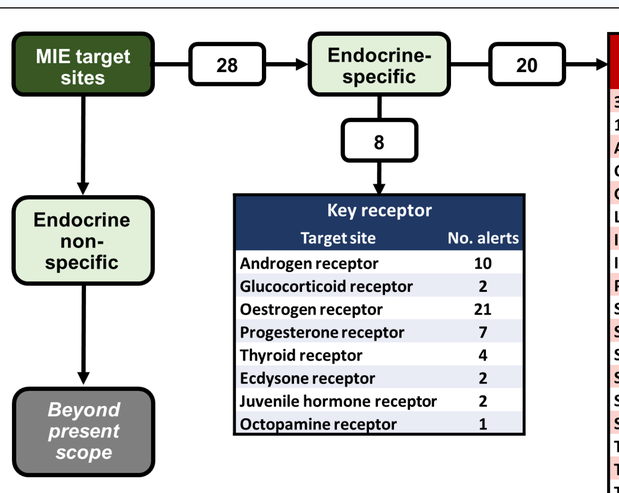



**Figure 4.** Distinct structural alerts most commonly fired by compounds within PARC-derived dataset.

## Mechanistic Ontology for Endocrine Disruption

- In all, 28 relevant endocrine-associated MIE target sites and 58 structural alerts were identified.
- Eight signalling systems are represented: Androgenic, oestrogenic, progestogenic, glucocorticoid, thyroid, ecdysteroid*, juvenoid* and octopaminergic*
- Mechanisms were defined in terms of their characteristic features, from MIE to signalling system.
- As Figure 2 depicts, distinction was drawn between "Key receptor" (i.e., direct) and "Hormone-mediated" (indirect) Endocrine Active (EA) effects, with the latter (accounting for 20 from 28 MIE targets) more common.

** Ecdysteroid, juvenoid and octopaminergic each exclusive to invertebrates*




| Key receptor | |
|---|---|
| Target site | No. alerts |
| Androgen receptor | 10 |
| Glucocorticoid receptor | 2 |
| Oestrogen receptor | 21 |
| Progesterone receptor | 7 |
| Thyroid receptor | 4 |
| Ecdysone receptor | 2 |
| Juvenile hormone receptor | 2 |
| Octopamine receptor | 1 |

| Hormone-mediated | |
|---|---|
| Target site | No. alerts |
| 3β-Hydroxysteroid dehydrogenase | 2 |
| 11β-Hydroxysteroid dehydrogenase | 4 |
| Aromatase | 3 |
| Cholesterol side-chain cleavage enzyme | 4 |
| Gonadotropin releasing-hormone receptor | 1 |
| Luteinising hormone receptor | 1 |
| Iodothyronine deiodinase | 1 |
| Iodotyrosine deiodinase | 1 |
| Pendrin | 2 |
| Sodium-iodide symporter | 3 |
| Steroid 5α-reductase | 1 |
| Steroid 11β-hydroxylase | 4 |
| Steroid 17α-monooxygenase | 2 |
| Steroid 21-hydroxylase | 4 |
| Sterol 27-hydroxylase | 2 |
| Thyroid peroxidase | 1 |
| Thyrotropin receptor | 2 |
| Thyrotropin-releasing hormone receptor | 1 |
| Transthyretin | 4 |
| Sulphotransferase | 3 |

**Figure 2.** Overview of MIE organisation, in line with devised ontology.

## Discussion and Conclusion

- A novel, mechanistically-grounded *in silico* profiler, spanning a range of endocrine-exclusive MIEs, (relevant to vertebrate and invertebrate taxa) has been developed.
- In its current form, the profiler attributed putative mechanisms of action to ~50% of substances present within an externally-sourced list of plausible EAS.
- Amongst the unassigned compounds are those which either lack in an established MIE, or else influence endocrine signalling through means which are non-specific to such pathways.
- Future scheme expansions shall seek to take account of the latter, thus further expanding upon its range.
- The profiler is intended to assist grouping, read-across and the selection of QSARs. It is NOT intended as a predictive tool, or else as indicative of whether a compound is a definitive endocrine disruptor.
- The profiler will be made freely available alongside existing schemes, and is further intended for incorporation within KREATiS MechoA+ software.

## References

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