An Extended Mechanistically-Based *In Silico* Profiler for Liver Toxicity

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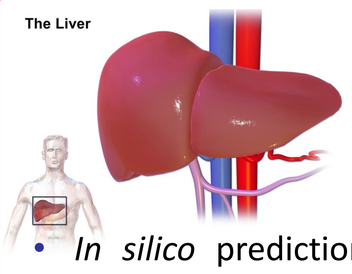
The Problem: *In Silico* Repeated Dose Toxicity Assessment



- *In silico* methods ((Quantitative) Structure-Activity Relationships ((Q)SARs) and read-across) can fill data gaps where toxicological information is missing.
- Toxicity from low and repeated dose exposure is acknowledged as being difficult to predict *in silico*.
- *In silico* prediction of organ level toxicity is one means of deriving No Observed (Adverse) Effect Levels (NO(A)ELs; adverse effects to the liver drive many NO(A)ELs
- This study aimed to extend an *in silico* profiler for liver toxicity by compiling existing knowledge and placing it in a mechanistic framework.

Methods and Resources Utilised

- Information on mechanisms of liver toxicity was retrieved through literature searches and organised in terms of chemistry relating to MIEs.
- COSMOS DB – Where possible, mechanistic information was supported by *in vivo* data relating to liver toxicity which were extracted from the COSMOS database (go to **cosmosdb.eu**).
- AOP Wiki Anchorage to available AOPs and MIEs.
- The ChemoTyper (**chemotyper.org**), as well as use of clustering and grouping, was used to identify relevant molecular fragments (chemotypes).
- Relevant chemotypes were designed and implemented as CSRML with imbedded physicochemical properties or as SMARTS.
- Physicochemical Properties calculated: hydrogen bond acceptors, hydrogen bond donors, - molecular weight, log P, total polar surface area, number of rotatable bonds, eccentric connectivity index, vertex adjacency matrix.

In Silico Models of Liver Toxicity

- Liver toxicity is commonly associated with exposure to various chemicals including pharmaceuticals and cosmetics ingredients.
- *In silico* models for liver toxicity, whilst increasing in number, are still limited.[1]
- Key limiting factors to modelling liver toxicity *in silico* are the paucity of relevant *in vivo*/ *in vitro* / human data, lack of coherent mechanistic knowledge and the complication of metabolism (de-)activation.

A Mechanistic Approach to Modelling Liver Toxicity

- A mechanistic basis to predicting toxicity *in silico* provides justification, plausibility and allows for transparency of the approach.
- Deciphering and organising mechanisms of toxic action is a key priority for the modelling of liver toxicity.
- Knowledge of mechanisms can be derived from Adverse Outcome Pathways which in turn provide inspiration for modelling from the Molecular Initiating Event (MIE).[2]
- *In silico* profilers, i.e. compilations of relevant structural alerts, provide a means to identify compounds that fall within mechanistic boundaries of toxic mechanisms.
- Table 1 summarises the relevant *in silico* profilers for liver toxicity.

Chemotype Development:

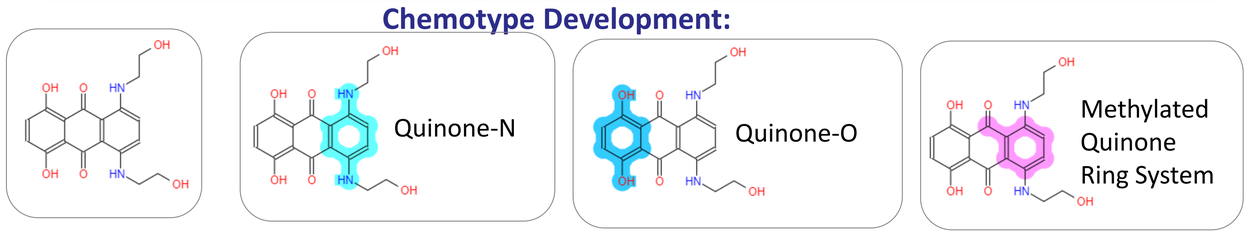
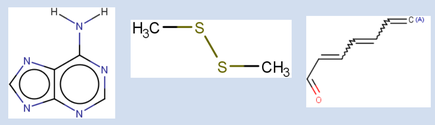
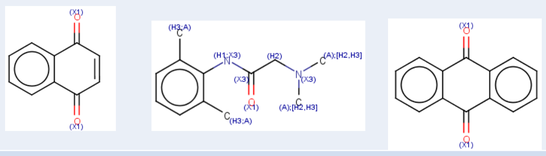
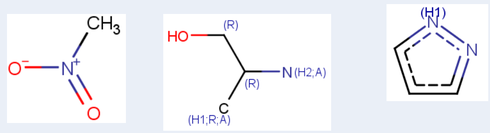
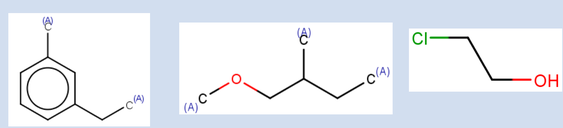


Table 1. *In Silico* Profilers for Liver Toxicity

Mechanism	Molecular Initiating Event(s)	Adverse Effects	Number and Types of Alerts	Indicative AOPs
Reactive Hepatotoxicity	Covalent binding	Fibrosis	> 100 Structural Alerts (SAs) 	#38 Protein alkylation leading to liver fibrosis
Mitochondrial Dysfunction	Disruption of proton gradient	Liver inflammation	> 20 SAs **26 csrml alerts** 	#144 Lysosomal damage leading to liver inflammation
Nuclear Receptor Disruption	Binding to Nuclear Receptors e.g. LXR, PPAR	Steatosis	> 100 SAs **756 csrml alerts** 	#34 LXR activation leading to hepatic steatosis
Phospholipidosis	Trapping of molecules within lysosomes	Excess accumulation of phospholipids	> 30 SAs **45 csrml alerts** 	None available
General Liver Toxicity	Multiple, often uncharacterised	Often undifferentiated	> 20 **16 csrml alerts** 	None available

Needs: Mapping of Mechanisms and Data

- The profiler shows strong performance in the identification of liver toxicants, although there is evidence of over-prediction.
- Profilers can be refined if and when further data e.g. from New Approach Methodologies (NAMs) become available.
- The mechanistic profiler requires further mapping of mechanisms and adverse effects.
- Further high quality *in vivo* data, as well as human data, will assist in the anchoring of profilers on relevant response and aids the definition of chemical domains.

Conclusions and Recommendations

- Over 100 alerts are provided for liver toxicity that form an *in silico* profiler.
- The profiler can be used to group chemicals according to mechanism of action.
- Such AOPs that exist for (non-carcinogenic) liver toxicity proved to be valuable resources to develop profilers.
- AOPs assisted in the understanding of MIEs; future work could use NAMs to develop the profilers further.
- Further development of profilers will be attempted as mechanisms (and AOPs) are described.
- Tools such as COSMOS DB / TOXGPS are a valuable resource to pull together toxicological information and knowledge.

References

1. Przybylak KR, Cronin MTD (2012) *Exp.Opin. Drug Metab. Toxicol*. 8: 201
2. Cronin MTD, Richarz A-N (2017) *Appl. In Vitro Toxicol.* 3: 287

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