Development of an Adverse Outcome Pathway (AOP) Network for Carcinogenicity Using Expert-Derived (Q)SAR Knowledge

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Introduction

The prediction of carcinogenicity and related toxicity endpoints has always been a principal research area for *in silico* (Q)SAR systems and, as a result, software relating to these endpoints is well developed. In recent years, predictions provided by these systems have become embedded in regulatory guidance, where they may be used to replace or augment other testing methods. Consequently, it is important that the predictions are as accurate as possible and are provided in such a way that they can be easily integrated with other sources of evidence. For example, suggested revisions to the ICH S1 guidance are under consideration which would negate the need to conduct a two year rodent carcinogenicity assay if there is knowledge of pharmacologic targets and pathways, alongside toxicological and other data, which could provide sufficient information to anticipate the outcome of a carcinogenicity bioassay [1]. This review highlights the work undertaken so far to rearrange knowledge contained within Derek Nexus into an AOP network for carcinogenicity (Figure 1). This will allow for 1) easier interpretation of the evidence available that relates to a prediction, 2) integration of existing and emerging *in vitro* and *in vivo* assays, and 3) expansion of the scope of predictions which can be made for this endpoint.

1&2: Using Derek Nexus to derive an AOP network

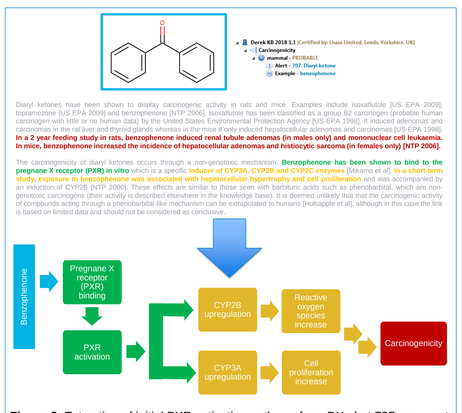

Figure 2: Extraction of initial PXR activation pathway from DX alert 707 comments

Derek Nexus (DX) [2] contains knowledge for potential toxicophores within alerts. These alerts detail data, scope and most importantly proposed toxicity modes of action. This information was extracted and rearranged to form a skeleton AOP network.

- Information pertaining to the mechanism of action was extracted from structural alerts associated with carcinogenicity, these being: carcinogenicity, chromosome damage, mutagenicity, non-specific genotoxicity, oestrogenicity, peroxisome proliferation.
- Description comments were used to assign an molecular initiating event (MIE) to each structural alert with consideration of the requirement for metabolic or photo activation.
- 85 MIEs or key events (KEs) linking a compound class to carcinogenicity were assigned to 310 DX alerts (Figure 1).
- All MIEs and KEs were used to build a network of 26 AOPs in Cytoscape [3] which delineate various mechanisms which lead to carcinogenicity.

For example, benzophenone activates alert 707 for carcinogenicity. The alert comments define the MIEs (in green) and KEs (in yellow) which lead to the adverse outcome (in red). These can be arranged into an AOP, initiated by pregnane X receptor (PXR) binding and activation, which upregulates various CYP450 enzymes (Figure 2).

3: Review

With a skeleton network in place, each of the pathways was subjected to a detailed review. This involved using literature references to provide biological plausibility, and in some cases, empirical evidence to support key event relationships. A controlled terminology was also established to 1) ensure consistency within the network and 2) facilitate interaction with external data sources. While no additional MIEs or AOPs were identified within this comprehensive review, the complexity of the network increased (Table 1). This is illustrated by the expansion of the CAR AOP, which increased the number of pathways to four (Figure 3). The KEs have been grouped where appropriate in order to simplify the network. This approach holds the advantage of aiding in the viewing of the network, while retaining knowledge in a structured format for interrogation.

Information	Number
MIEs	26
AOPs	26
Pathways	>70
Non-genotoxic AOPs	13
Genotoxic AOPs	13

Table 1: Numbers relating to the Carcinogenicity AOP network after review

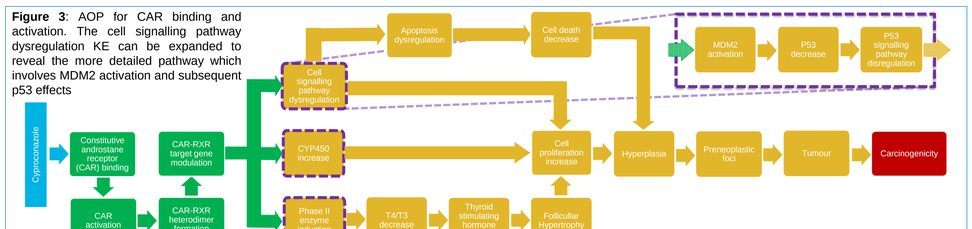

Figure 3: AOP for CAR binding and activation. The cell signalling pathway dysregulation KE can be expanded to reveal the more detailed pathway which involves MDM2 activation and subsequent p53 effects

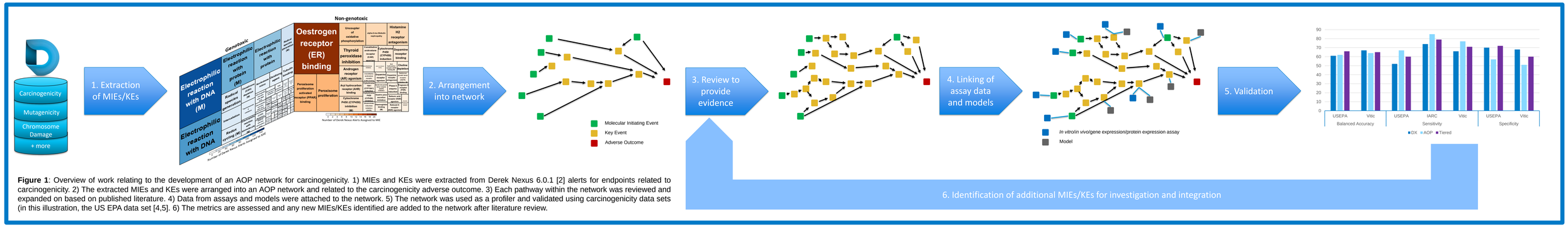

Figure 1: Overview of work relating to the development of an AOP network for carcinogenicity. 1) MIEs and KEs were extracted from Derek Nexus 6.0.1 [2] alerts for endpoints related to carcinogenicity. 2) The extracted MIEs and KEs were arranged into an AOP network and related to the carcinogenicity adverse outcome. 3) Each pathway within the network was reviewed and expanded on based on published literature. 4) Data from assays and models were attached to the network. 5) The network was used as a profiler and validated using carcinogenicity data sets (in this illustration, the US EPA data set [4,5]. 6) The metrics are assessed and any new MIEs/KEs identified are added to the network after literature review.

4: Assay Data and Models

Using this AOP framework, it is easy to associate toxicological data and models to KEs. As well as data from regulatory *in vitro* and *in vivo* assays (such as Ames or chromosomal aberration tests), emerging high-throughput screening assays or gene/protein expression assays can be associated to the network. Appropriate models can also be integrated with the network. Organizing evidence within this framework can help the user make more informed decisions on the balance of available information. There are a number of different factors which can be considered and used to reason between the different evidence (Figure 4):

- Associations between evidence (is it on the same pathway or not)
- Proximity of evidence on the pathway to the adverse outcome
- Biological complexity of system used to generate evidence (*in vitro*, *in vivo*)
- Type of evidence (prediction, assay, regulatory assay)
- The applicability domain of the assay or evidence used to support a pathway

With this in mind, a initial tiered approach to reason between models and genotoxicity data was used, taking these considerations into account.

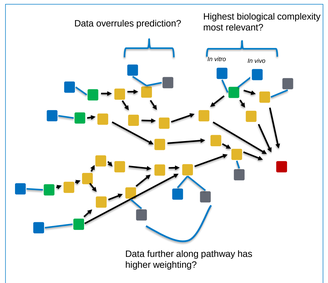

Figure 4: Illustration of factors to consider when evidence is attached to an AOP network.

5&6: Validation and Further Investigation

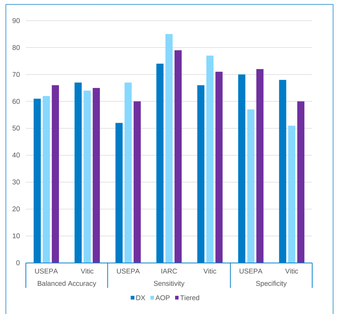

Figure 5: Metrics for validation of Derek Nexus (DX), the carcinogenicity AOP network and AOP network with the tiered approach to reasoning applied, using data sets from the US EPA, IARC and Vitic 3.0.3

Using the AOP network as a rudimentary profiler, the approaches with and without tiered reasoning were validated and compared with Derek Nexus using data sets compiled from the US EPA [4,5], IARC (using only the compounds classified as 1, 2A or 2B) [6] and Vitic 3.0.3 [7] (Figure 5).

- The AOP-based approaches increase sensitivity when compared with traditional structural alerts as other sources of data that can indicate KEs on the path to carcinogenicity are taken into account.
- This increase in sensitivity comes at the cost of specificity as not all positive results on the pathway are indicative of a positive adverse outcome
- Using a tiered reasoning approach can improve specificity as the most relevant data is taken into account first

Initial analysis of the substances which are “false negatives” revealed classes for which MIEs and KEs had not previously been identified in DX e.g. radionucleotides, which produce ionizing radiation to cause DNA damage, or a nanomaterial such as MWCNT-7, which causes fibrosis [6]. These newly identified MIEs and KEs can be investigated and pathways added to the network as necessary.

Conclusions

This work highlights how expert-derived knowledge in Derek Nexus can be rearranged into an AOP network. This allows for 1) more detailed knowledge and understanding of MOAs, 2) easier interpretation of evidence and information contained within the network, and 3) integration of *in vivo* and *in vitro* data. This methodology also allows expansion of the scope of predictions which can be made when compared to DX, as this approach can allow for substances other than small molecules to be considered. In the future, it is hoped that further data, models and information related to e.g. ADME or histopathological observations can be linked into the network.

References

[1] ICH S1(R1) Guideline, https://www.ich.org/products/guidelines/safety/article/safety-guidelines.html
[2] Derek Nexus v6.0.1, https://www.lhasalimited.org/products/derek-nexus.htm
[3] Cytoscape 3.5.1, https://cytoscape.org/
[4] Becker et al., Regul. Toxicol. Pharmacol., 2017, 90, 185-196, DOI: 10.1016/j.yrtph.2017.08.021
[5] United States Environmental Protection Agency, http://npic.orst.edu/chemicals_evaluated.pdf
[6] International Agency for Research on Cancer (IARC), https://monographs.iarc.fr/agents-classified-by-the-iarc/
[7] Vitic 3.0.3, https://www.lhasalimited.org/products/vitic.htm