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 alco (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 guide are under consideration which would regulate 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 assay (1). This review highlights the work undertaken so far to reframe 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 particular endpoint, (2) prediction of existing and novel 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

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 a molecular initiating event (MIE) to each structural alert with consideration of the requirement for metabolic or photo activation.

- 65 MEAs or key events (KEs) linking a compound class to carcinogenicity were assigned to 310 DX alerts (Figure 1).

- All MEAs and KEs were used to build a network of 38 AOPs in Cytoscape [3] which delineate various mechanisms which lead to carcinogenicity.

For example, benzeneophene activates alert 770 for carcinogenicity. The alert comments define the MEAs (e.g. 2,4,6-trinitrotoluene) and KEs (e.g. endonuclease) which lead to the adverse outcome (in this case). These can be arranged into an AOP network, initiated by pregnane X receptor (PXR) binding and activation, which upregulates various CYP450 enzymes (Figure 2).

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. Organzing 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 (e.g on the same pathway or not).
- Priority 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 reasoning between models and genotoxicity data was used, taking these considerations into account.

5&6: Validation and Further Investigation

Using the AOP network as a rudimentary assay, the approaches with and without tiered reasoning were validated and compared with Derek Nexus using data sets compiled from the US EPA [4,5], AHRN (using only the compounds classified as 1, 2A or 2B) [6] and Vito v3.0.3.7 (Figure 5).

- The AOP-based approach increases 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 MEAs and KEs had not previously been identified in DX e.g. radiomimetics, which produce ionizing radiation to cause DNA damage, or a ramastereous such as K/WNT7, which causes fibrosis [6]. These newly identified MEAs and KEs can be investigated and pathways added to the network as necessary.

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 in 1) ensure consistency within the network and 2) facilitate interaction with external data sources. While no additional MEAs or KEs were identified within this comprehensive review, the connectivity 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.

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 vitro and in vivo 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