Using Data Associated with a Developmental and Reproductive Toxicity Adverse Outcome Network to Aid Safety Assessments

5077/ P185

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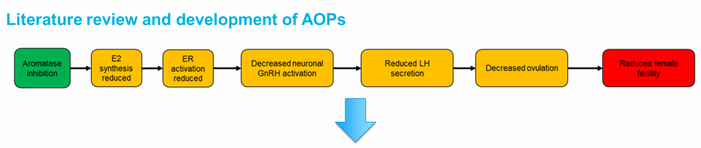
DART, alternative assays and AOPs

Developmental and reproductive toxicity (DART) is an important safety assessment endpoint. Traditional (*in vivo*) methods used to assess DART endpoints involve large numbers of animals and are time consuming and costly [1]. With the drive towards animal-free toxicity testing, increasing volumes of alternative assays, models and data are being developed. To conclusively undertake a risk assessment, all available relevant data should be utilised. However, as alternative assays often measure discrete biological steps in a mechanism of toxicity (and not traditional toxicity endpoints), it can be difficult to understand the context and significance of their results in isolation. Adverse outcome pathways (AOPs), a formalised approach to documenting mechanisms of toxicity, provide this contextualisation [2]. AOPs comprise key events (KEs) linked to each other through key event relationships (KERs). Each KE must be measurable – as a result, it should be possible to link an assay to each KE. This feature can provide a mechanistic understanding for relevant assays, and allows for the review of multiple assay types within the framing of a mechanism of toxicity. Many potential uses of AOPs have been hypothesised and discussed in the literature [3,4] – however, for AOPs to become a useful tool in risk assessment, it is likely that comprehensive networks of mammalian-relevant pathways leading to AOs of regulatory significance will be needed.

Development of a DART AOP network

Mammalian-relevant AOPs for DART endpoints within the public domain are relatively limited [5]. Therefore, to address this knowledge gap, a list of targets (enzymes, proteins or biological processes) thought to be relevant to DART was curated from information found in relevant Derek Nexus alerts, a published P&G model [6] and member interactions. These targets were investigated using a literature-based approach and, where possible, AOPs were curated/developed (e.g. [7]). In-house standardisation of terminology has allowed for the integration of these AOPs into a network of DART-relevant pathways (Fig. 1). This network is contained within the AOP tool, Kaptis [8]. Data for assays relevant to KEs within the network was also curated (e.g. traditional and alterative *in vivo* assays and activity based *in vitro* assays).



AOP network metrics	MIEs	Total KEs (MIEs/KEs/AOs)	KERs	Assays	Assay data	Unique compounds
Totals	54	290	373	50	413482	191133

Figure 1: Taking a literature-based approach to develop an AOP network with assay data associations [7].

Utility of a chemically aware AOP network

There are many possible ways a chemically aware DART AOP network can be used to improve safety assessments [3]. These could span many stages in the development of a chemical (early screening/compounds selection through to aiding regulatory decisions). However, when considering the DART potential of a compound of interest it is likely that at most, only a small number of AOPs would be relevant to an individual compound of interest and therefore unguided access to the entire network may not be useful. Therefore, an approach was explored where assay data and similarity searching were used to identify potentially relevant AOPs for individual compounds to help guide safety assessments (Fig. 2). To achieve this, a proof-of-principle tool was developed which allows for Tanimoto-based similarity searching of the data associated to the AOP network. Both single and multiple chemicals can be input into the tool, and the output of the tool provides the AOPs which may be relevant to the compound of interest as well as a list of all data and compounds used to identify the pathway(s).

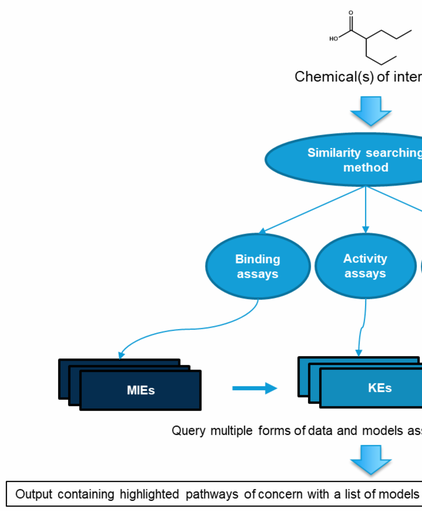


Figure 2: Similarity searching methodology to identify potentially relevant AOPs

Validation and ICH S5 (R3)

The ICH S5 (R3) guideline provides examples of instances where a positive result (i.e. an indication of malformations or embryo-foetal lethality (MEFL)) in a suitable alternative assay coupled with a plausible mechanism of action can be used in place of traditional animal studies (Fig. 3) [9]. Where a positive result in an alternative assay exists but a plausible mechanism is not known, then similarity searching of the AOP network could help provide relevant mechanisms. This use-case provided a useful means to validate the performance of our AOP network. We utilised a publicly available zebrafish assay dataset [10,11] which contained an overall call for the endpoint of DART. For the purposes of validating our AOP network, we focused on the positive compounds and after structural curation this resulted in a dataset of 199 positive compounds.

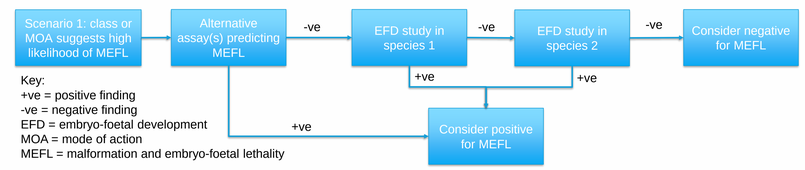


Figure 3: Recreation of the ICH S5 (R3) alternative assay and mode of action (MOA) workflow [9].

In our proof of principle tool, a fragment-based fingerprint method was utilised and several thresholds of similarity used. A true positive was assigned when similar compounds within the AOP network were identified, and AOP(s) were suggested for the compound of interest. When using our methodology (Fig. 4), we can see that:

- At 100% similarity, positive data for 89 compounds are present in the network.
- When the threshold of similarity is decreased, more compounds are associated with potentially relevant AOPs.
- At a threshold of 80%, two thirds of the positive compounds are associated with a potential AOP.

Examples of AOPs predicted for in the dataset include 20 compounds predicted as aromatase inhibitors, 11 as aryl hydrocarbon receptor binders and 36 as oestrogen receptor binders.

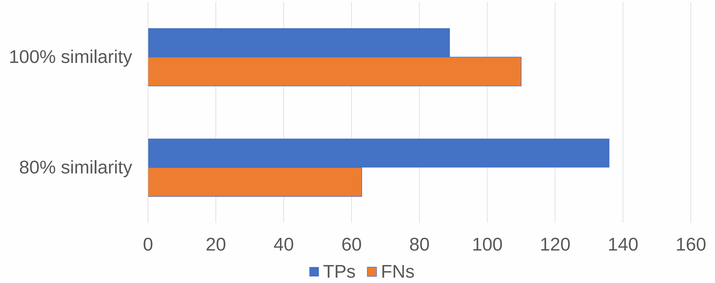


Figure 4: Performance of the similarity searching methodology on the DART AOP network at 100% and 80% similarity.

Conclusions

This network may serve as a useful resource for DART-based safety assessments. Validation of the network demonstrated a good coverage of the positive zebrafish data with predicted modes of action for two-thirds of the positive compounds in the zebrafish dataset (at 80% similarity). It also identified potential gaps within our network. These could be either knowledge or data gaps. As the AOP network develops, these gaps will likely be filled.

References

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