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Using an AOP network to make hazard predictions for developmental and reproductive toxicity

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Challenges of DART testing

Developmental and reproductive toxicity (DART) is an important regulatory toxicity endpoint which is currently primarily assessed using *in vivo* mammalian models. These assessments are often run at a relatively late stage in a compound's development and involve large numbers of animals [1]. As a result, the assessments are expensive and raise ethical concerns – for drug development, a DART finding could also result in a late-stage halt of a compound's development. Therefore, early identification of DART hazards is useful to enable better prioritisation of candidate compounds. Many different new approach methodologies (NAMs) are being developed to enable animal-free safety assessments. However, confident use of these NAMs requires an understanding of the mechanistic relevance of their outputs.

(Quantitative) structure-activity relationships ((Q)SARs) represents a class of *in silico* NAMs which allow for hazard detection without the requirement of a test substance. Both statistical models and expert-derived structure-activity relationships (SARs) have been developed to predict for DART endpoints, including Derek Nexus which contains expert-derived SAR alerts (Fig. 1) [2]. These models are often trained on available historic *in vivo* toxicity data and, although they can be relatively accurate, their coverage of chemical space in which confident predictions can be made is somewhat limited [3,4].

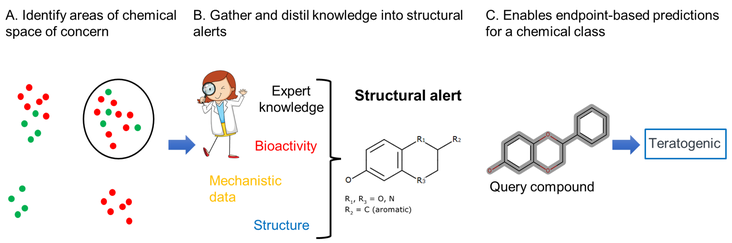

Figure 1: Depiction of the development and use of an expert-derived structure-activity relationship. Image taken from [7].

AOP-based solutions

Adverse outcome pathways (AOPs) represent a method of documenting mechanisms of toxicity [5]. These mechanisms are documented through key events (KEs): the initial KE represents the molecular initiating event (MIE), whilst the terminal KEs describe adverse outcomes (AOs). We have demonstrated that a framework can be built around AOPs to incorporate relevant QSAR models, structural alerts and NAMs around specific KEs. This enables additional relevant information to be presented to assessors, including providing mechanistic rationale for query compounds (Fig. 2) [6, 7]. As demonstrated in the following sections, the scaling of these methods to an entire DART AOP network could provide a valuable DART hazard screening tool, and a framework upon which to contextualise NAMs and aid weight-of-evidence assessments.

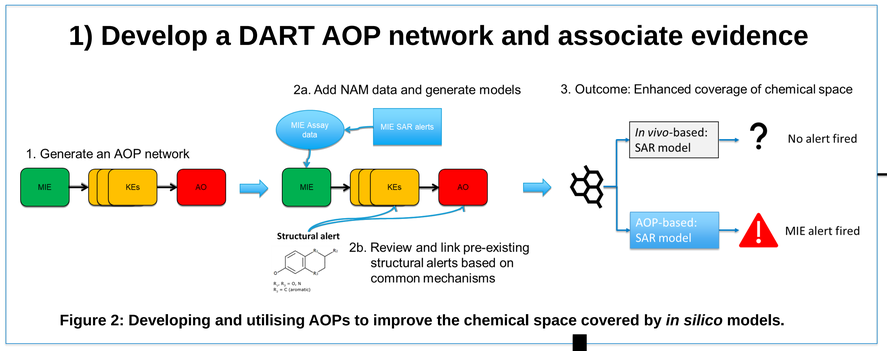

Figure 2: Developing and utilising AOPs to improve the chemical space covered by *in silico* models.

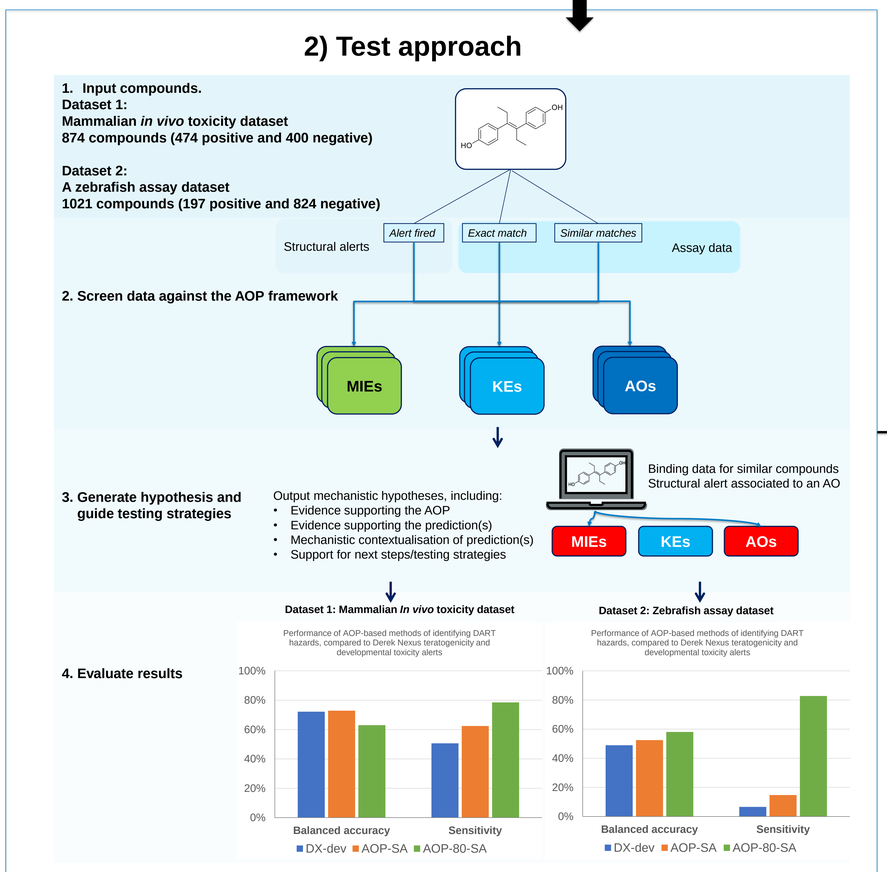

Figure 4: Hazard screening workflow based on the DART AOP network

A DART AOP network

A DART AOP network has been developed using a literature-based approach. The network is predominantly composed of developmental toxicity pathways. However, a proportion of the network relates to fertility toxicity and neurodevelopmental toxicity pathways. Assay data (both NAM and traditional) and Derek Nexus SAR alerts have been associated to the network. The current structure of the network is described in Fig. 3 and Table 1.

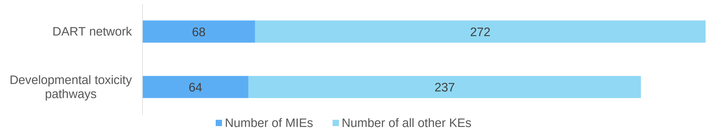

Figure 3: Composition of the DART AOP network

Table 1: Assays, data and models associated to the DART AOP network

	MIE	KE	AO	Network total
Assays	46	2	5	**53**
Studies	1,194,548	2,375	3,485	**1,165,084**
Normalised compounds	288,315	281	1,794	**288,826**
Contextualised compounds	269,983	275	1,746	**270,308**
Derek alerts	115	101	61	**240**

A DART AOP predictive framework

An evaluation of the performance of the AOP network as a DART hazard identification tool was conducted (workflow described in Fig. 4). Two datasets were used in this evaluation: an *in vivo* mammalian toxicity dataset predominantly comprising pharmaceutical compounds, and a zebrafish dataset from the ToxCast database [8] (Fig. 4, step 1). The AOP predictive framework (Fig. 4, step 2) utilises both the SAR alerts associated to the network, and a Tanimoto similarity method to enhance predictivity through the identification of data for structurally similar compounds.

The outputs from the alerts and similarity searching method can be used in isolation or combined (Fig. 4, step 3). The performance of the network when only using the structural alerts associated to the network (AOP-SA) and also using the structural alerts in combination with study findings for similar compounds – using an internal fingerprint method and a similarity threshold of 80% (AOP-80-SA) – were evaluated (Fig. 4, step 4). These results were compared to DART-relevant Derek Nexus alerts (DX-dev).

Conclusions

Our method shows vast improvement in sensitivity when compared to the currently available SAR models. Therefore, in its current form, the DART AOP network is a very useful hazard screening tool – allowing for the early identification of developmental toxicants. Mechanistic hypotheses generated by the AOP framework provides additional confidence in the predictions, and guidance on further testing strategies. Future research will include evaluating how to combine these outputs with those from promising NAM technologies to further enable weight-of-evidence assessments.

References

[1] Chapman et al, Regulatory Toxicology and Pharmacology, 66 (2013), 88-103 (10.1016/j.yrtph.2013.03.001). [2] Marchant et al, Toxicology Mechanisms and Methods, 18 (2008), 177-187 (10.1080/15376510701857320). [3] Weyrich et al, Birth Defects Research, 114 (2022), 812-842 (10.1002/bdr2.2062). [4] Marzo et al, Toxicology, 370 (2016), 127-137 (10.1016/j.tox.2016.09.015). [5] Ankley et al, Environmental Toxicology and Chemistry, 29 (2010), 730-741 (10.1002/etc.34). [6] Myden et al, Reproductive Toxicology, 108 (2022), 43-55 (10.1016/j.reprotox.2022.01.004). [7] Myden et al, Current Research in Toxicology, 5 (2023), 100124 (10.1016/j.crtox.2023.100124). [8] EPA dashboard, https://comptox.epa.gov/dashboard/assay_endpoints/Tanguay_ZF_120hpf_ActivityScore.