Improving *In Silico* Predictions in Developmental and Reproductive Toxicology

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Abstract # 2404

1. Challenges facing *in silico* approaches for DART

Developmental and reproductive toxicity (DART) continues to be a challenging area for developing in silico models, partially due to a lack of data availability across a broad chemical space. In addition, DART encompasses a wide range of complex endpoints, of which there is likely to exist multiple mechanisms leading to each adverse outcome [1]. Therefore, for users to have confidence in using *in silico* approaches, predictive systems will have to cover large areas of chemical and biological space. While there is a large amount of publicly available DART data, a significant amount of detailed information from DART studies, including those conducted by pharmaceutical companies, remain with the sponsor. A collaboration between Bristol-Myers Squibb (BMS) and Lhasa Limited was established to highlight the benefits of data sharing for predicting DART (Figure 1).

Collaboration goals:

1. **Broaden the chemical space available for evaluating *in silico* approaches for predicting DART**
2. **Improve *in silico* models to cover newly identified regions of chemical and biological space of concern**
3. **Share knowledge to support the development of alternative approaches**

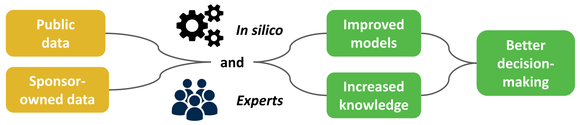


Figure 1: Sponsor-owned data can be integrated with data from public sources to improve *in silico* models and increase domain knowledge, which together can facilitate better risk assessments

2. QSAR approaches for predicting DART

Current off-the-shelf methods for predicting DART use (quantitative) structure-activity relationships (QSARs) and rely heavily on data derived from toxicity studies (Figure 2). Two general approaches exist:

1. **Expert rule-based systems** use a knowledge base containing SARs and mechanistic knowledge curated by experts.
2. **Statistical approaches** are trained directly on curated data and algorithms identify relationships between chemical descriptors and toxicity outcomes.

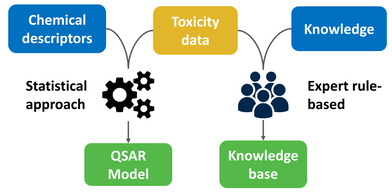


Figure 2: Current QSAR approaches for predicting DART

Adverse outcome pathways (AOPs) describe sequences of measurable and essential events which can lead to an adverse outcome (Figure 3) [2]. These pathways provide a framework to centralise knowledge, data and predictive models relevant to toxicity endpoints.

AOP frameworks are beneficial to both modellers and risk assessors. For example, QSAR modellers can take advantage of the large volume of data in bioactivity databases and build models which predict for the interaction between a query molecule and a target of interest. Risk assessors working with outputs from such models can then utilise the knowledge within the AOP and run appropriate assays to progress a risk assessment.

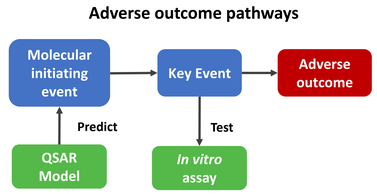


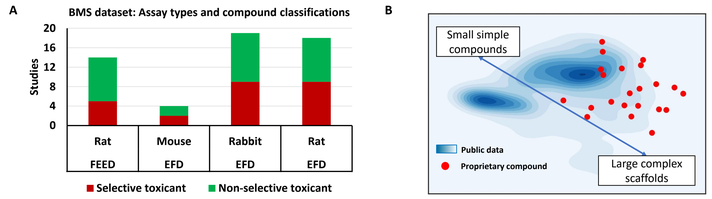
Figure 3: Using AOPs to facilitate DART risk assessments

3. Data curation and model validation

- From a BMS database, 55 *in vivo* embryo-fetal development (EFD) and fertility and early embryonic development (FEED) studies for 22 unique pharmaceuticals were extracted and curated (Figure 2A).
- A public dataset was curated consisting of 60 compounds described as either positive or negative for developmental or reproductive toxicity in the literature.
- The compounds in the curated BMS dataset were not present in any DART dataset held by Lhasa Limited and occupy areas of chemical space where less is known about the DART liabilities of molecules (Figure 2B).
- The BMS dataset adds value to existing knowledge, both in terms of coverage of chemical space and in depth due to the range of studies available for each compound.

Figure 2A: Types of studies present in the BMS dataset. Selective toxicants were those that demonstrated developmental toxicity at doses with no maternal toxicity.

Figure 2B: Illustration of chemical space occupied by the BMS dataset (red dots) in comparison to other DART datasets [3,4] (blue density plot). Compound coordinates are based on structural fingerprints which are represented in two dimensions.



The expert rule-based system, Derek Nexus [5], and an AOP-based approach [6] were evaluated using the curated datasets (Figure 3). The AOP-based approach had an increased sensitivity compared to Derek Nexus, both against the BMS and public dataset. This result has been attributed to two factors. Firstly, the AOP model describes a greater number of mechanisms and therefore has greater coverage of relevant biological space. Secondly, the AOP-based method uses QSARs trained on large bioactivity databases enabling greater coverage of chemical space. However, both approaches had limited coverage of the reproductive toxicants in the BMS dataset.

BMS dataset

	TP	FP	TN	FN
Derek Nexus	0	0	9	13
AOP model	2	4	5	11

Public dataset

	TP	FP	TN	FN
Derek Nexus	6	5	25	24
AOP model	13	10	20	17

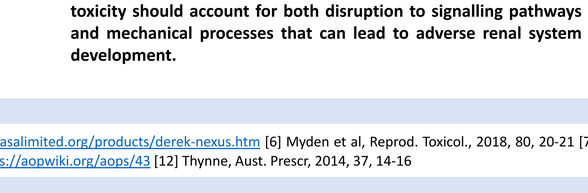


Figure 3: Performance of *in silico* models against the datasets curated in this study. TP = True positives, FP = False positives, TN = True negatives, FN = False negatives.

4. Adverse outcome pathway development

The compounds in the BMS dataset were reviewed alongside their prediction results, in order to identify areas of chemical and biological space not adequately covered by the *in silico* models. Evaluating developmental toxicants from the BMS dataset alongside data from the public domain failed to identify any suitable structural alerts, due to the sparseness of chemical space occupied by the reproductive toxicants. However, two pathways were identified that were not currently described in the AOP-based model. The pathways and key supporting evidence for these two AOPs are given below:

AOP: RET inhibition leading to malformations of the kidney and urinary tract

Species: Mammal, Sex: Male and Female, Life stage: Embryo-fetal

- Compounds binding to the RET receptor tyrosine kinase (RET) can antagonise RET-mediated signalling pathways [7].
- Mouse knockout models with suppressed RET-mediated signalling exhibit inhibited ureteric bud growth [8, 9].
- Interactions between the ureteric bud and metanephric mesenchyme enable formation of the kidney [8].
- Rat and rabbit embryo-foetal studies involving RET inhibitors can result in urinary system malformations[10]. However, not all compounds in this chemical class cause urinary malformations:

RET inhibitors causing urinary malformations		RET inhibitors not causing urinary malformations	
R-788	Urinary, cardiac and reproductive malformations.	Vandetanib	Embryo-fetal loss and cardiac malformations
Sorafenib	Urinary malformations and embryo-foetal loss	Sunitinib	Embryo-fetal loss and craniofacial malformations
Regorafenib	Urinary malformations	Alectinib	Embryo-fetal loss and skeletal variations

Organising evidence in this way highlights the concordance between the observed toxicity and mechanistic knowledge. The spectrum of malformations observed for RET inhibitors could be due to effects on multiple targets. RET inhibitors can also inhibit VEGFR, which is believed to cause developmental toxicity via the disruption to angiogenesis [11].

AOP: SGLT-2 inhibition leading to adverse renal system development

Species: Mammal, Sex: Male and Female, Life stage: Renal development

- Sodium-glucose cotransporter-2 (SGLT-2) is primarily expressed in the proximal tubules of the kidneys and is responsible for glucose reabsorption into the bloodstream [12]. Inhibition of the transporter increases the concentration of glucose in the filtrate, which results in an increase in the volume of urine produced.
- Pharmaceuticals targeting SGLT-2 tested in post-natal developmental toxicity studies, produced renal pelvic dilations in juvenile rats [10]:

Compound	Post-natal developmental toxicity
Dapagliflozin	Renal pelvic and tubular dilations
Canagliflozin	Renal pelvic dilations
Ertugliflozin	Renal pelvic dilations

The adverse outcomes observed have been attributed to the inability of the juvenile rat to handle larger volumes of urine during development [10]. These findings highlight a potential risk for use of these compounds during pregnancy, due to the functional maturation of the human kidney occurring *in utero*. Further research is needed to establish a greater understanding of the human relevance and quantitative nature of these findings.

5. Collaboration perspectives

Conclusions:

1. **Increasing the availability of DART data through data sharing supports the development of *in silico* models and domain knowledge through the construction of AOPs.**
2. **Pharmaceuticals can occupy unique regions of chemical space, which can hinder the generation of structural alerts due to the lack of supporting analogues.**
3. **AOP frameworks can organise both knowledge and data to enable models to cover relevant areas of chemical and biological space. Thus risk assessors are provided with relevant information to base decisions on.**
4. **Two DART MIEs were highlighted during this collaboration: RET inhibition and SGLT2 inhibition. The supporting evidence for these mechanisms were curated into an AOP framework.**

Recommendations:

1. **Data sharing should aim to support decision-making for risk assessments and therefore should include toxicity, mechanistic, exposure and alternative assay data for both toxicants and non-toxicants**
2. **Developers of alternative approaches for predicting developmental toxicity should account for both disruption to signalling pathways and mechanical processes that can lead to adverse renal system development.**

6. References: [1] Scialli et al, Birth Defects Res., 2018, 110, 840-850 [2] Ankley et al, Environ. Toxicol. Chem., 2010, 29, 730-741 [3] Matthews et al, Regul. Toxicol. Pharmacol., 2007, 47, 115-135 [4] ToxRefDB, https://doi.org/10.23645/epacomptox.6062545.v1 [5] Derek Nexus 6.0.1, https://www.lhasalimited.org/products/derek-nexus.htm [6] Myden et al, Reprod. Toxicol., 2018, 80, 20-21 [7] Gotink et al, Angiogenesis, 2010, 13, 1-14 [8] Manié et al, Trends in genetics, 2001, 17, 580-589 [9] Arighi et al, Cytokine. Growth. Factor. Rev., 2005, 16, 441-467 [10] Toxicity study summaries for pharmaceuticals were curated from product information documents [11] Developmental Vascular Toxicity, https://aopwiki.org/aops/43 [12] Thynne, Aust. Prescr, 2014, 37, 14-16