Can the Second Species be Replaced by an Adverse Outcome Pathway-based In-silico Model?

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Background

- Developmental toxicity testing for pharmaceuticals usually involves one rodent (e.g., rat) and one non-rodent species (e.g., rabbit) [1]. This process requires many animals, making it costly, time-consuming, and raising ethical concerns [2].
- Applying the 3Rs principles (Replacement, Reduction, and Refinement) to developmental toxicity testing is challenging due to potential risks of misclassifying teratogenic compounds. High confidence in alternative approaches is needed to move away from animal testing for this endpoint.
- Although non-animal methods for DART testing are challenging, incremental progress can be made by reducing the number of species used. Determining the appropriate species to protect human health requires a thorough understanding of toxicity mechanisms.
- Adverse outcome pathways (AOPs) are pragmatic collections of biological evidence outlining the mechanisms leading to an adverse outcome, starting with a molecular initiating event and progressing through a sequence of key events. Lhasa Limited has developed a comprehensive DART AOP network associated with various data types including in vitro data and expert rule-based alerts, used to develop a framework for DART screening [2] (Figure 1).
- To build confidence in non-traditional DART assessments, we evaluated whether our AOP framework, combined with a NAM and a rodent study, could identify all teratogens using a dataset of 226 compounds. If successful, this approach could potentially limit the need for a second in vivo species.

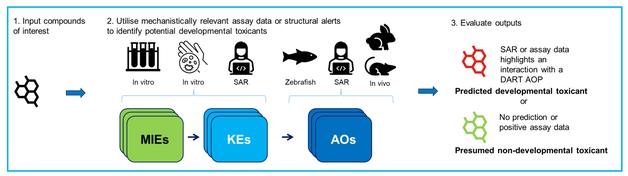

Figure 1: A DART AOP framework which utilises KE relevant assay data and structure activity relationship models to identify potential developmental toxicants [2]. The network contains 68 MIEs and 340 KEs in total. However, we focused on the developmental toxicity pathways, which are a subset of these 340 KEs.

References

1) ICH S5 (R3) (2020). https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s5-r3-guideline-detection-reproductive-and-developmental-toxicity-human-pharmaceuticals-step-5-revision-4_en.pdf (accessed January 29, 2025). 2) Myden, A. et al. (2024). Computational Toxicology, 31, p.100325. 3) Truong L, et al. Toxicological sciences. 2014 Jan 1;137(1):212-33. 4) EPA, Tanguay et al. zebrafish activity score, (n.d.). https://comptox.epa.gov/ dashboard/assay-endpoints/Tanguay_ZF_120hpf_ActivityScore (accessed December 2, 2020).

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Methods

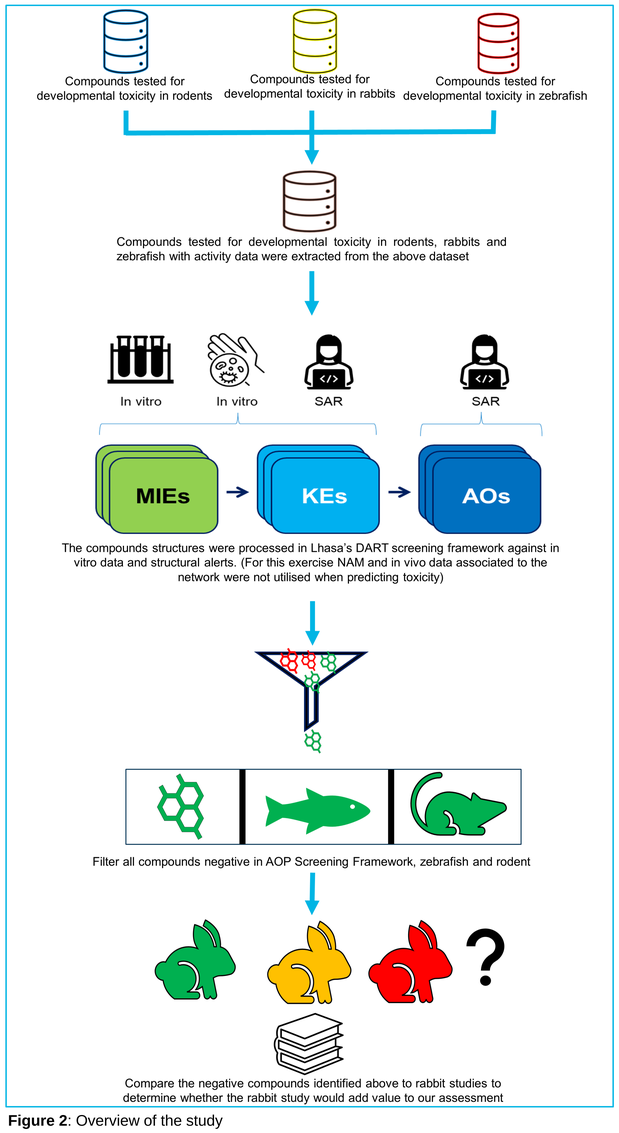

Figure 2: Overview of the study

Results: Data curation

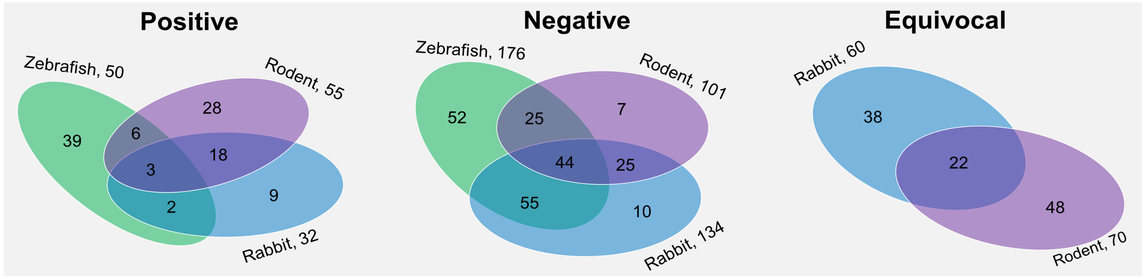

Figure 3: Overview of the dataset used for the study

- A result is considered positive when developmental toxicity is observed at a lower dose than maternal toxicity. It is deemed equivocal when developmental toxicity occurs at the same dose as maternal toxicity. A negative result is assigned when developmental toxicity is observed at a higher dose than maternal toxicity or when no toxicity has been observed.
- Within our database, the number of compounds studied in rodents, rabbits and zebrafish were 226.
- The results of the toxicity tests varied significantly across the three test systems. Three compounds were positive in all the three test systems whereas 44 compounds were negative in all the three test systems (Figure 3).
- Out of 101 compounds negative in rodent, 69 are negative in rabbit indicating some level of uncertainty in the current traditional in vivo study (Figure 3).

Results: Method performance

- After processing the 226 compounds tested in rodents, rabbits, and zebrafish using the AOP screening framework, 19 compounds were found to be negative. Upon assessing the zebrafish data for these 19 compounds, 17 were negative. In rodent data, 13 out of the 17 compounds were negative (Figure 4).
- Further examination of the rabbit data for these 13 compounds revealed that 7 were negative and 6 were equivocal (Figure 4). An equivocal call is made when developmental and maternal toxicity are observed at the same dose necessitating a comprehensive review before drawing a conclusion as stated in the ICH S5 guideline [2].
- Upon reviewing these 6 compounds, experts concluded based on published reviews that they are non-teratogenic. This indicates that our approach could potentially eliminate the requirement for a second species in developmental toxicity testing (Figure 4).

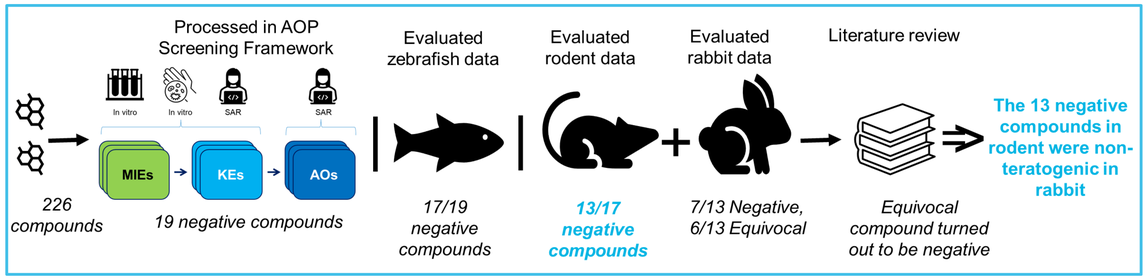

Figure 4: The results from our proposed approach to limit the need for a second in vivo species assessment

Conclusions

- Our approach successfully identified all teratogens within the dataset and in this instance conducting the rabbit study would provide no-additional benefit for hazard identification.
- This research highlights the potential for alternative assays in combination with an in silico approaches to remove one species from the developmental toxicity assessments and remain protective for human health.
- This is a first investigation into this approach which has yielded promising results. However, further research, including the use of a larger dataset is necessary to evaluate this methodology.
- To further increase the confidence in the uptake of new approach methodologies, it is essential for stakeholders to collaborate, share knowledge and identify methods to reduce uncertainties.