Enabling Next Generation Carcinogenicity Assessment

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Introduction

There are currently many initiatives underway across the globe aiming to improve the quality of human carcinogenicity safety assessment by utilising existing data from established studies and new approach methods (NAMs) to reduce our reliance on animal testing. Although the context of decisions made using these new paradigms may vary significantly according to use case, there are many commonalities between the concepts used to build them. Development of a universal methodology that enables weight-of-evidence (WoE), integrated approaches to testing and assessment (IATA), or other approaches for carcinogenicity assessments will allow translation between paradigms and facilitate better re-use of science and evidence. AOPs have been shown to provide a framework to organise, contextualise and rationalise evidence for ICH S1B(R1) WoE assessments [1]. Thus, in this work we analyse the utility of AOPs as a framework for carcinogenicity assessment across different domains.

Methods

Three different areas of industry, or toxicity spaces (from here on classified as domains) and examples of developed/developing WoE assessments or IATAs were evaluated:

- Pharmaceuticals – ICH S1B(R1) WoE assessment framework (in regulation) [2]
- Agrochemicals – ReCAAP WoE assessment framework (developed and published) [3]
- Non-genotoxic carcinogenicity – OECD Expert working group IATA framework (in development, progress published) [4]

For each of these domains, the concepts described in each example approach were extracted and compared to find commonalities and these concepts were harmonised (where possible). The adverse outcome pathway (AOP) concept (as used in the *in silico* solution Kaptis [5]) was then examined to identify how concepts map to approaches which capture evidence and how decisions can be supported

Conclusions

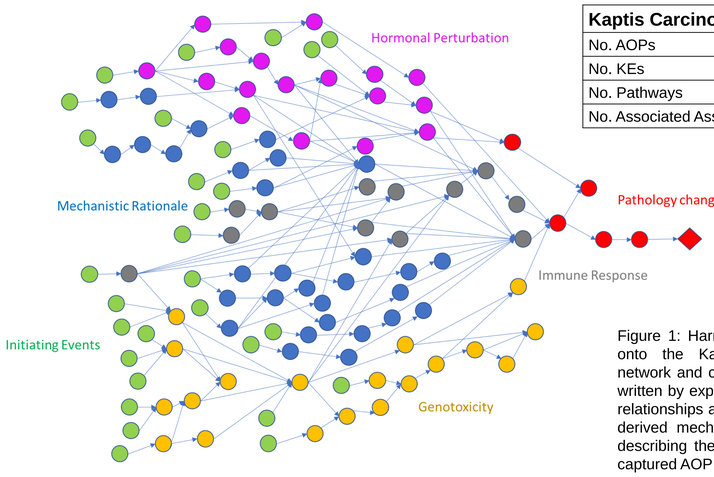
While there will necessarily be multiple ways of improving on the historical paradigm of animal testing for carcinogenic potential of a substance within different domains, there are key concepts and knowledge which can be shared. Capture and digitisation of the data and evidence relating to these concepts in a way that is flexible enough to allow translation between approaches will not only allow different approaches and evidence to be used in a consistent and interoperable way between use cases, but also facilitate the evolution of carcinogenicity safety assessments as new paradigms continue to be developed.

Commonalities in Domain WoE/IATA Examples and How AOPs can enable Decision Making

	Industry/Domain			
Harmonised Concepts	**Pharmaceutical (ICH S1B(R1)) [2]**	**Agrochemical (ReCAAP Framework) [3]**	**Non-Genotoxicity (OECD IATA EWG) [4]**	**Key Differences**
Initiating Events	On-target safety; Secondary Pharmacology		MIEs	ReCAAP does not take into account target(s)
Pathology Changes	Histopathology from chronic studies	Findings from acute and sub-chronic findings	Cell proliferation; Changes in morphology	OECD IATA takes more overall view, whereas others look for tissue changes at different time periods
Hormonal Perturbation	Hormonal perturbation	Hormonal perturbation		OECD IATA does not look specifically at hormonal changes, but looks for more general changes
Genotoxicity	Genotoxicity	Genotoxicity		OECD IATA has genotoxicity as prerequisite before applying IATA
Immune Response	Immune modulation	Immune suppression	Immune response	ReCAAP specifies immune suppression
Exposure		Use patterns and exposure scenarios	Exposure	ICH S1B(R1) is a hazard-based approach, but some rationale can be applied in the WoE based on rat exposure margin
Mechanistic Rationale	Additional investigative studies	Mechanistic support	Building common mechanisms into IATA	ICH S1B(R1) asks for additional studies to determine mechanisms findings cannot be rationalised.

Table 1: Harmonisation of concepts utilised in the assessment of carcinogenicity in different domains. Note that only concepts shared between some/all domains are shown

Map harmonised concepts to AOP framework



Kaptis Carcinogenicity AOP Network	
No. AOPs	32
No. KEs	120
No. Pathways	289
No. Associated Assays	60

Figure 1: Harmonised concepts mapped onto the Kaptis Carcinogenicity AOP network and colour coded. The AOPs are written by experts and therefore key event relationships are all supported with expert-derived mechanistic rationale. Statistics describing the current level of knowledge captured AOP knowledgebase [5,6]

After the concepts were extracted for each domain example, it was clear there were commonalities in concepts used across the frameworks (Table 1). While these have been described in different ways, they can be harmonised, thus showing that a common framework to help in decision making could be used for each domain.

The harmonised concepts can be mapped onto the AOP framework (Figure 1), demonstrating that this framework is applicable for organising evidence for example domains. Using AOPs which are annotated with evidence can bring clarity to these types of assessments, giving consistent, scientifically robust and transparent answers to questions that can be asked during the assessment process [6] (Figure 2). It has already shown how AOPs can be used to help with WoE assessments for ICH S1B(R1) [1] (Figure 3). At each stage of the assessment, the relevant questions can be asked to direct testing and rationalise findings.

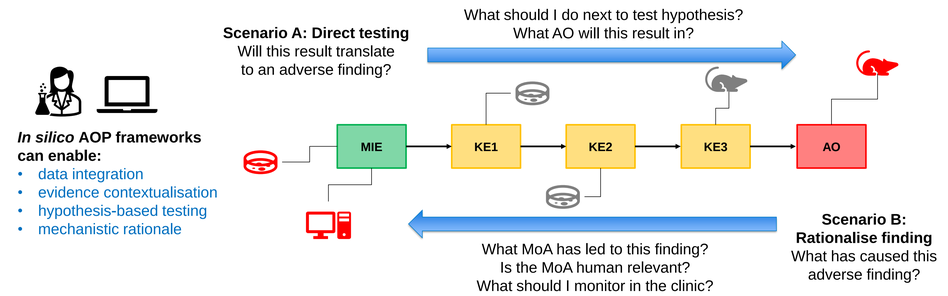
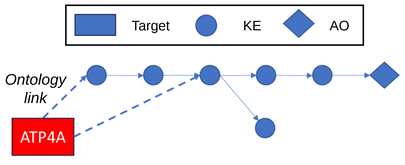


Figure 2: illustration of how AOPs can be used to direct testing of mechanistic hypotheses (Direct) or help give context to adverse findings coming from assays measuring AOs (Rationalise).

Example: ICH S1B(R1) WoE assessment for Lansoprazole

Factor	*Kaptis Outcome*	*Reasoning*
On-target safety	Positive	ATP4A linked to KEs in a carcinogenicity AOP via ontologies
Secondary Pharmacology	Positive	AhR binding and agonism observed, potential carcinogenic MoA
Genotoxicity	Negative	Positive *in vitro* CA and MN findings overruled by negative *in vivo* CA and MN finding; all other studies negative
Histopathology	Positive	Stomach, hyperplasia, organ weight increase observed, human relevant
Hormonal Perturbation	Negative	Some findings in rat positive, dog *in vivo* chronic studies negative. Likely human irrelevant
Immune Modulation	Negative	Rat *in vivo* chronic toxicity study showed no immune relevant findings in tissues
Overall: compound is likely to be carcinogenic – no rat study required		

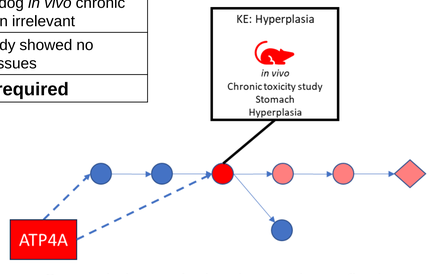
Figure 3: Summary of ICH S1B(R1) WOE assessment for Lansoprazole. The outcome of assessment for each factor was determined by framing the totality of data and evidence in Kaptis. By defining the potential link of the target to an AOP for carcinogenicity, the downstream KEs can be tested to determine this mechanism is relevant. The confirmation of stomach hyperplasia confirms the on-target finding



Lansoprazole's primary target, ATP4A, is expressed in stomach tissues. No known AOP but ontologies can link target to KEs in an AOP which results in stomach tumours.

What should I do next to test this hypothesis?

Test compound for hyperplasia in appropriate assay



Stomach hyperplasia observed, confirming targeting ATP4A can result in findings which could lead to carcinogenicity

References

[1] Stalford et al, Regul Toxicol Pharmacol, 2021; [2] ICH S1B(R1); [3] Hilton et al, Regul Toxicol Pharmacol, 2022; [4] Jacobs et al, Arch Toxicol, 2020; [5] Kaptis v2.0.0 prerelease; [6] Cayley et al, ALTEX, 2023