Utilising Adverse Outcome Pathways as a Framework to Organise Evidence and Support Carcinogenicity Risk Assessment

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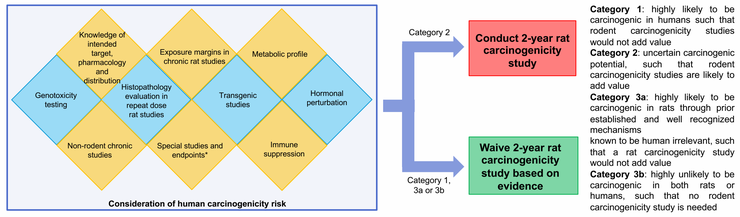
ICH S1 and a Weight of Evidence Approach

The ICH S1 guidelines relate to the assessment of human carcinogenicity risk for new pharmaceuticals [1]. To satisfy this guidance, rodent carcinogenicity studies are routinely carried out. However, these tests are time consuming, expensive and use a lot of animals. Moreover, they do not always produce results of human relevance. In order to alleviate these shortcomings, recent proposals for changes to the guidance suggest using a weight of evidence (WOE) approach [2,3]. This method involves collating evidence from other relevant sources, such as genotoxicity assays, chronic repeat dose studies and knowledge of pharmacology, in order to categorise human carcinogenic risk. The results may then negate the need for a rodent carcinogenicity study (Figure 1).



Category 1: highly likely to be carcinogenic in humans such that rodent carcinogenicity studies would not add value
Category 2: uncertain carcinogenic potential, such that rodent carcinogenicity studies are likely to add value
Category 3a: highly likely to be carcinogenic in rats through prior established and well recognized mechanisms known to be human irrelevant, such that a rat carcinogenicity study would not add value
Category 3b: highly unlikely to be carcinogenic in both rats or humans, such that no rodent carcinogenicity study is needed

Figure 1: Illustration of ICH S1(R1) proposed approach to assessment for the need for rodent carcinogenicity testing. Blue diamonds indicate evidence that should be considered, originally proposed by Sistare et al [2] and yellow diamonds the suggested additional evidence considered as part of the ICH S1(R1) approach [3]. * this includes *in silico* models and receptor binding assays.

One challenge in using WOE is the organisation and interpretation of this disparate information in a consistent way to reach a meaningful conclusion. A potential solution is to use an adverse outcome pathway (AOP) framework. In previous work, we took knowledge captured in Derek Nexus, rearranged it into an AOP network and expanded upon the initial findings through literature review (Figure 2) [4]. Since then, we have associated models, assays and data to the network (Figure 3).

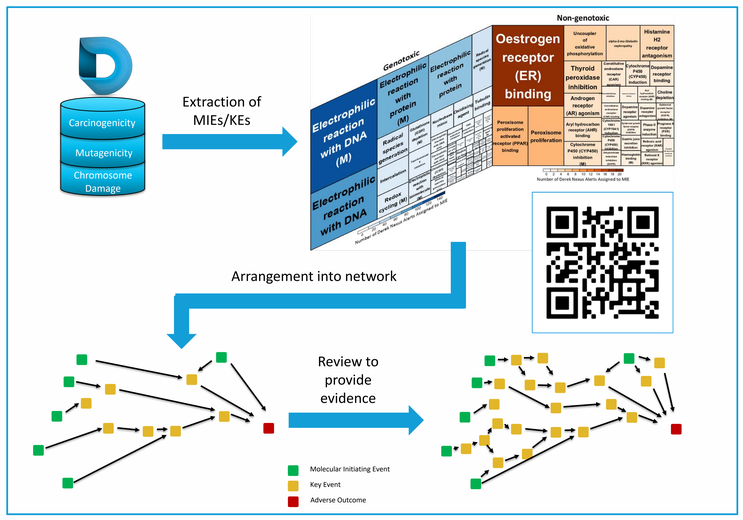
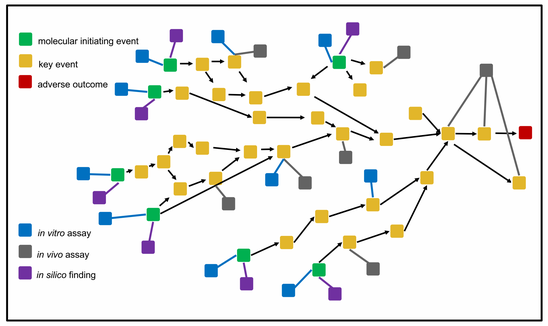


Figure 2: Approach to building an AOP framework, based on knowledge stored within Derek Nexus [4,5]. For more detail, please scan the QR code.

Reasoning between data using an AOP framework



Current AOP Network Statistics	
AOPs	39
MIEs	39
Pathways	>350
Assays Associations	73
Measurement Associations	69
Derek Nexus Alert Associations	291
Compound Associations	>12000
Study Associations	>2000

Figure 3: Illustration of how the current carcinogenicity AOP network fits together with assays and measurements, and the current figures indicating the size of the network.

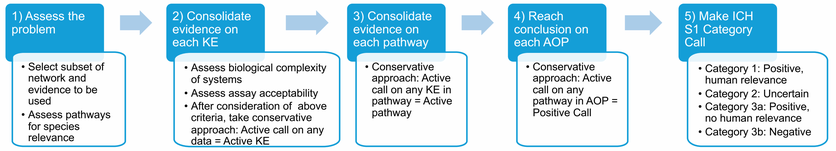


Figure 4: Proposed reasoning workflow to assess carcinogenic potential based on ICH S1(R1) guidelines

For ICH S1, the current methodology being investigated at Lhasa Limited to reason between data takes a conservative approach using the AOP network previously developed (Figure 3) to assess carcinogenicity (Figure 4). However, determining the species relevance of each pathway being assessed (step 1) and the biological complexity and acceptability of each assay in the problem space we are assessing (step 2) is paramount to determine the final category call. At the key event (KE) level (step 2), if there is data from *in vivo* assays available, then the data from these is always used over *in vitro* assay data. As well as this, if it is determined that an assay does not test adequately for the mode of action (MOA) of interest (e.g. in a relevant tissue), then negative results from these assays are rejected. This determination is currently made by using Derek Nexus to check for hypotheses about potential MOAs.

AOP Framework Reasoning Application: Clofibrate

Figure 5 shows how the current reasoning methodology can be applied to determine the carcinogenic potential of Clofibrate. Evidence was obtained from Vitic [6], IARC [7] and Derek Nexus [5]. The model and assay associations led to identification of the KEs to study, which in turn, identified the subset of AOPs to assess. The reasoning workflow (Figure 4) was then applied to determine KE outcomes (step 2), pathway outcomes (step 3) and AOP activity conclusions (step 4). This led to a classification of the compound as **Category 3A**, given that the positive outcomes observed are all from AOPs which are unlikely to be human relevant. While organ weight increase could be indicative of hyperplasia, the same study does not report an increase in cell proliferation in the 13-week time period, and longer-term studies give unclear results, indicating this increase was likely because of hypertrophy, and these gross findings are consistent with the rodent specific mechanisms suggested from *in silico* profiling. In step 2, some *in vitro* positive evidence was found for chromosome structure damage, however as there was also *in vivo* negative data, this overruled the positive results.

1) Assess the Problem						2) Consolidate Evidence for KE			3) Consolidate Evidence for Pathway	4) Consolidate Evidence to Reach Conclusion for AOP
AOP	Human Relevant	Pathway	KE	Evidence	Result	Complexity of System	Acceptability	KE Outcome	Pathway Outcome	AOP Outcome
Genotoxicity*	Likely	A	Inherited DNA Mutation	Ames Test	Negative	*In vitro*	Accepted	Negative	Negative	Negative
				TGR Mutation Assay	Negative	***In vivo***	Accepted			
		B	Chromosome Structural Damage	*In vitro* CA Test	Conflicted	*In vitro*	Accepted	Negative	Negative	
				In vitro CA Test	Equivocal	*In vitro*	Accepted			
				In vitro CA Test	Negative	*In vitro*	Accepted			
				In vitro CA Test	Positive	*In vitro*	Accepted			
				In vivo CA Test	Negative	***In vivo***	Accepted			
			Micronucleus Formation	*In vitro* MN Test	Negative	*In vitro*	Accepted	Negative		
H2HR Binding	Unlikely	C	Gastric Acid Secretion Inhibition	Derek Nexus Carc Alert	PLAUSIBLE	*In silico*	Accepted	Positive	Positive	Positive
PPAR Binding	Unlikely	D	PPAR Binding, Peroxisome Proliferation	Derek Nexus Carc Alert	PLAUSIBLE	*In silico*	Accepted	Positive	Positive	Positive
All	Unlikely	-	Hypertrophy, Hyperplasia, Peroxisome Proliferation	13-week subchronic study	Increase in organ weight	***In vivo***	Accepted	Positive	-	Positive

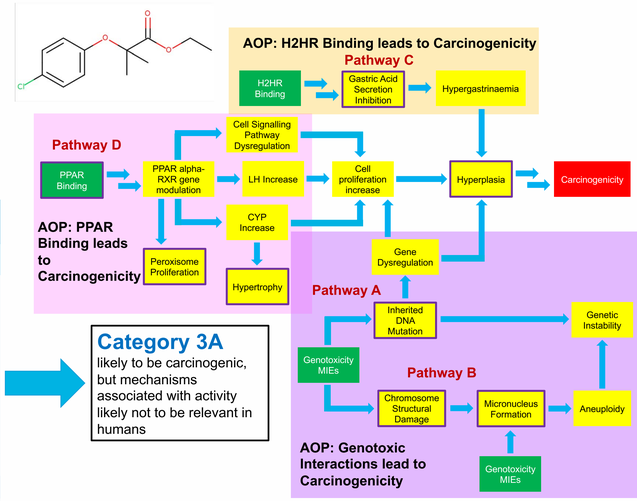


Figure 5: Assessing the carcinogenic potential of Clofibrate using and AOP framework to reason between evidence. H2HR - histamine H2 receptor; PPAR – peroxisome proliferator-activated receptor; TGR – transgenic rodent; CA – chromosomal aberration; MN – micronucleus. *: There were 15 genotoxicity AOPs identified as part of this assessment, however as the genotoxicity evidence was common for all these pathways, the AOPs have been combined. Purple outlines on KEs highlight where there is evidence associated with the pathways.

Conclusions

This work shows that evidence relating to carcinogenic risk can be organised into an AOP framework, making it easier to interpret and reason between data, models and knowledge. Structuring and reasoning between different sources of evidence in this way allows for a transparent and logical conclusion to be reached, which has the potential to negate the need for rodent carcinogenicity studies in more situations than if using an unstructured strategy for assessment. The approach also allows for additional knowledge beyond assay results to be captured, which may put findings into context and save time, money and animals.

References

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