Application of AOPs for Weight-of-Evidence Assessments: Rationalising Evidence for the ICH S1B(R1) Guidance Addendum

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Concept

The recent publication of the ICH S1B(R1) draft addendum [1] reflects the paradigm shift occurring across industry with respect to moving away from long-term animal studies for carcinogenicity. Using evidence gathered throughout the pharmaceutical development process, the guidance suggests that the totality of this data should be sufficient, using a weight-of-evidence (WoE) approach based around six factors, to indicate if conducting a rodent carcinogenicity study would be of any additional value. Adverse outcome pathway (AOP) networks have been advocated as a way of organising and contextualising evidence in a framework upon which an integrated approach to testing and assessment (IATA) can be built [2]. Using the structure this concept provides, an approach to assess the evidence for ICH S1B(R1) and make a relevant decision has been developed [3], which is transparent, consistent, and robust. Practically, the results can be viewed in three different ways – as an AOP network, ICH S1B(R1) factor AOP networks, and as individual AOPs (Figure 1).

To show how the views can provide different pieces of information, and thus collectively provide a complete picture of what the outcome is and why, a previously developed set of carcinogenicity AOPs [4] were 1) combined as an AOP network, and 2) related to the factors through the key events to derive the ICH S1B(R1) factor networks. The given evidence from a case studies in the ICH S1(R1) guidance [1] was associated to each view and the approach described by Stalford et al [3] applied to give predictions for the adverse outcome.

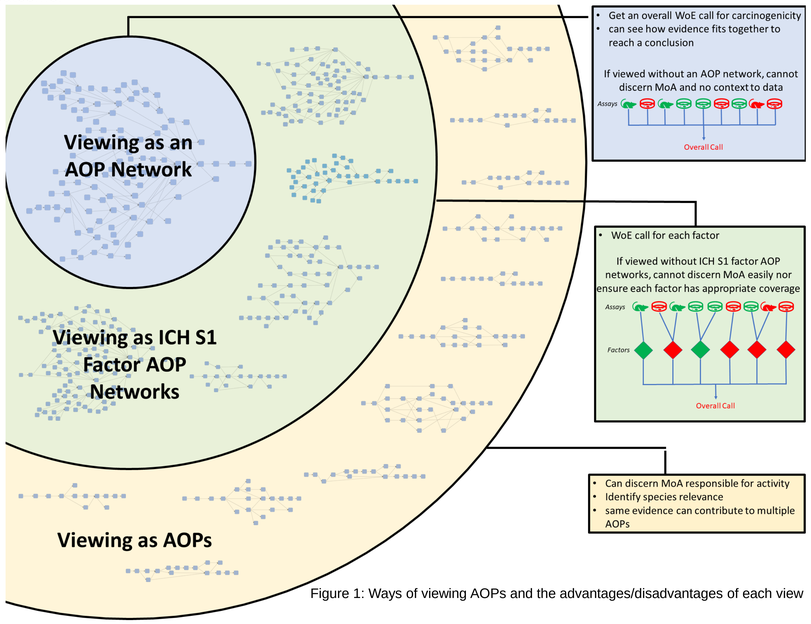


Figure 1: Ways of viewing AOPs and the advantages/disadvantages of each view

Combining these results showed that the same conclusion was reached using the AOPs as in the ICH S1B(R1) example, however:

- The evidence is structured in a way such that it is easy to see how the data fits together and is contextualised,
- An overall WoE call and a WoE call for each factor is derived, which are transparent, robust and can be consistently applied,
- The mode-of-action (MoA) occurring, and identification of their human relevance can be deduced. While the case study did not define the target or MoAs involved, it is shown that AOPs are a powerful tool to work out these critical pieces of information,
- The framing of evidence in this way can reduce uncertainty in conclusions, or direct further testing which could negate the need for rodent carcinogenicity studies.

This work shows that organising evidence on different views of AOPs can give a transparent, robust and consistent method to give an answer to the question "is my compound likely to be carcinogenic or non-carcinogenic in humans?" which can form part of the final decision for ICH S1B(R1) regulatory submission.

ICH S1B(R1) Case 2: A small molecule antagonist of a neuronal G-protein coupled receptor

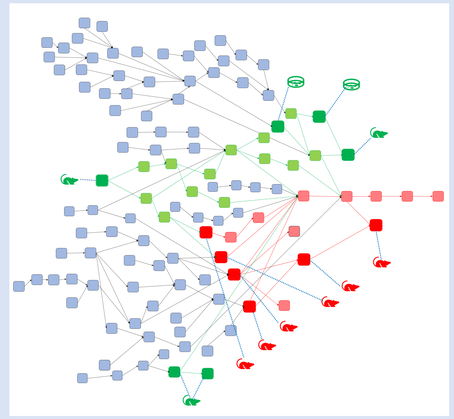
Weight-of-Evidence information given [1]:

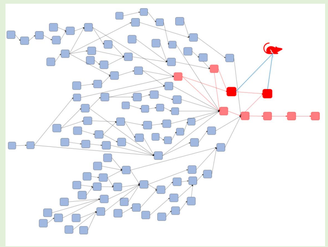
Evidence type	Findings
Knowledge of intended target and pathway pharmacology	-Target knock-out study shows no findings related to carcinogenicity -Long-term studies with other compound with same pharmacological target associated with thyroid follicular cell adenoma/carcinoma in rats
General Toxicology from Chronic Rat Study	-Increased liver hypertrophy and organ weight at 50x to 74x margin to human exposure. -Increased thyroid follicular hypertrophy at 170x to 670x margin to human exposure. -No evidence of human specific metabolites. -An active major human metabolite in humans was also present in rats
Hormonal Perturbation	-Reduced adrenal weight without histopathological correlates and reduced ACTH level at >74x human exposure in the chronic rat study, consistent with inhibition of drug target. Response noted to be growth suppressive. -Irregular estrous cycles and decreased pregnancy rate were observed at 60-fold human exposure, and decreased numbers of corpora lutea, implantations, and live embryos were observed at >500-fold human exposure in a fertility study in rats. Considered consistent with inhibition of drug target. -No treatment-related changes observed in reproductive organ weight or histopathology in chronic rat study.
Genetic Toxicology	-No evidence of genotoxic potential of parent or major human metabolite based on criteria from ICH S2(R1) Guidance
Immune Toxicology	-No treatment-related changes in clinical pathology, lymphocyte subsets, or histopathology of immune tissues
Additional Special Investigations	-Increased induction of CYP1A2 and CYP3A1 demonstrated -Bone and teeth fluorosis related to defluorination of compound, demonstrated not to occur in humans

AOP Network view:
this compound is **likely to be carcinogenic**

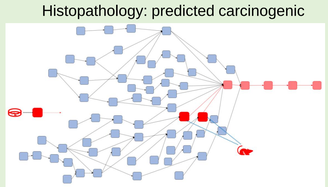
Arranging the evidence on the whole network clearly shows how it fits together and relates to each other in the context of the adverse outcome. For this example it can be seen that evidence for hypertrophy in the liver and thyroid are responsible for the carcinogenic call. Here we assume:

- Positive histopathology finding in any tissue/organ gives positive call for associated KE
- Lack of complete reporting for no histopathology findings in all tissues/organs gives unknown calls for other histopathology-related KEs overall

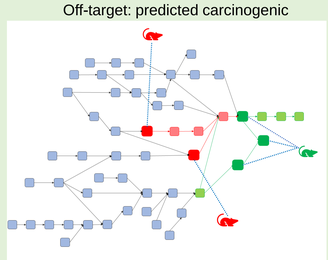




Histopathology: predicted carcinogenic



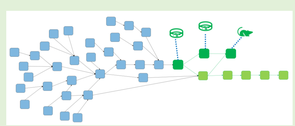
Off-target: predicted carcinogenic



Hormonal Perturbation: predicted non-carcinogenic

ICH S1 Factor Network view:
this compound is likely to be carcinogenic **through an off-target effect and tissue changes**

Arranging AOPs into networks related to each factor gives an indicator of what class of effects could be responsible for overall calls. Here for this example, it is clear that associated evidence points to an off-target effect and histopathology observed (in the liver and thyroid) contributing to the carcinogenic outcome; whereas it is clear that genotoxicity and immunotoxicity do not contribute to the overall call. For hormonal perturbation, the mix of positive and negative data comes to an overall outcome of non-carcinogenic as the reasoning implemented allows for *in vivo* data down-stream in the AOP to overrule upstream positive findings i.e. effects observed earlier do not propagate into histopathology in reproductive tissues.

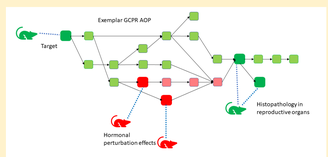


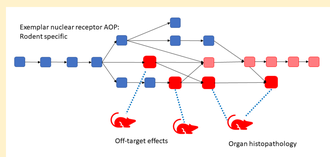
Genotoxicity: predicted non-carcinogenic

Immunotoxicity: predicted non-carcinogenic

AOP view:
this compound is likely to be carcinogenic through an off-target effect and tissue changes **caused by a rodent-specific mechanism. Therefore unlikely to be carcinogenic in humans**

While the example from the guidance does not mention what the specific target is, nor the MoA responsible for off-target effects or tissue changes, when evidence is placed on AOPs, the MoAs are discernible, showing how the evidence fits together for the target MoA and identifying that off-target effects are likely related to a nuclear receptor-mediated MoA. The target AOP predicts that the compound is non-carcinogenic by that MoA. Although the nuclear receptor AOP predicts the compound to be carcinogenic, information associated to the AOP indicates that the MoA is rodent-specific, which is consistent with histopathology findings. Therefore it can be determined that is unlikely to be carcinogenic in humans.





References: [1] ICH Harmonised Guideline S1B(R1) Draft (2021). [2] OECD, Series on Testing and Assessment, Guidance No. 260 (2016). [3] Stalford et al, Regul Toxicol Pharmacol (2021). [4] Stalford et al, SOT Poster (2019).