Using Adverse Outcome Pathways as a Framework for Carcinogenicity Assessment in ICH S1B(R1)

Susanne Stalford

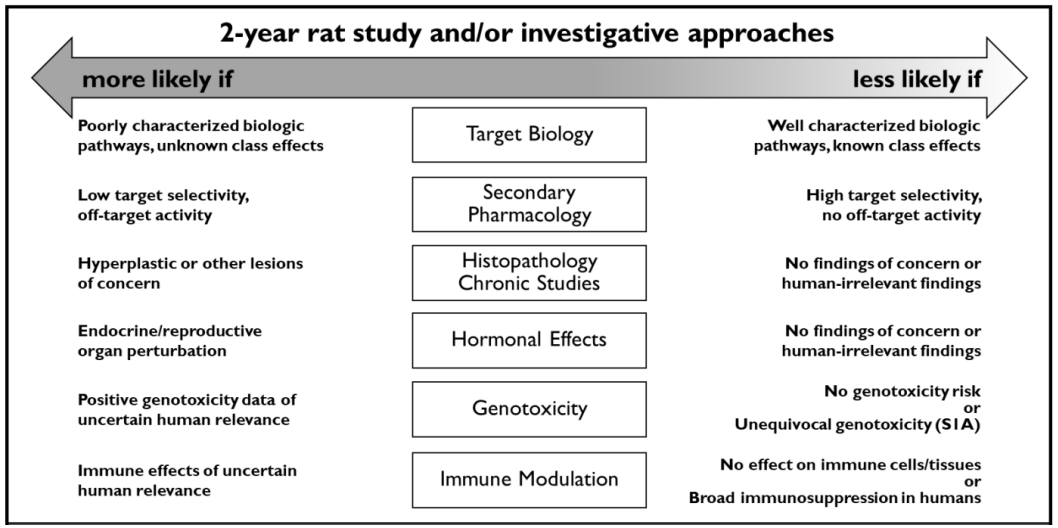
Principal Scientist

susanne.stalford@lhasalimited.org

Content

- ICH S1B(R1): Reducing rat carcinogenicity testing for pharmaceuticals
- How Adverse Outcome Pathways Can Help
- Adverse Outcome Pathway Development
- In Practice: Case Study

ICH S1B(R1) Addendum



Weight-of-Evidence approach based on six factors

+

one of

Short/medium-term in vivo rodent study

Long-term in vivo rodent carcinogenicity study

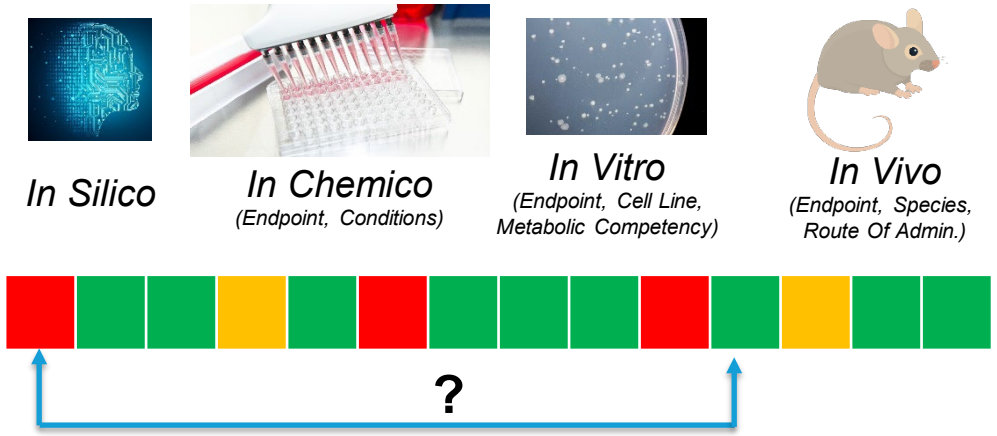
https://www.ich.org/page/safety-guidelines

Weight-of-Evidence Assessments

- The effort to move towards an integrated approach to testing and assessment (IATA) is correlated with a rise in the volume of alternative evidence (e.g. *in vitro*, *in silico*)

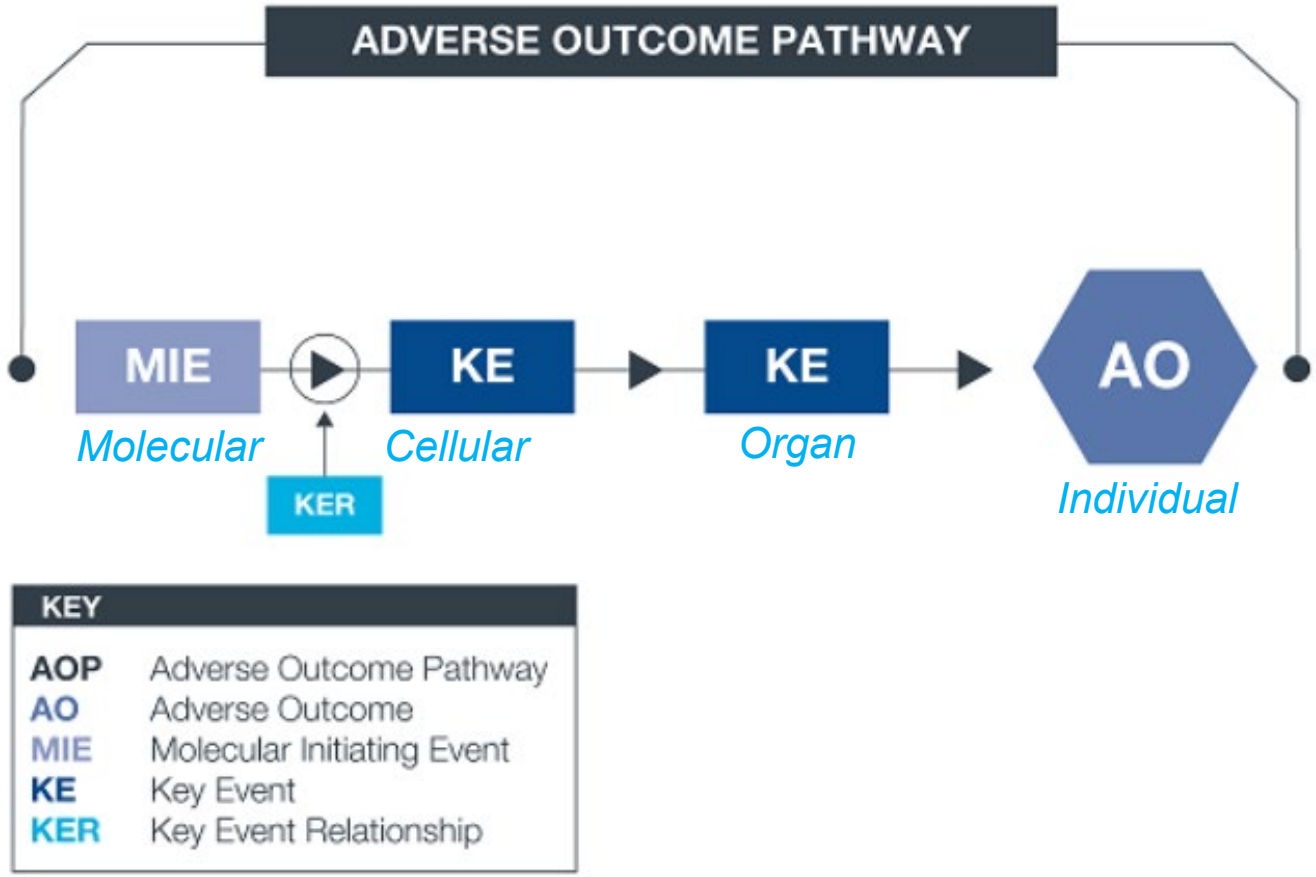
- It is likely that many different types of evidence will be needed to replace traditional animal models

- Combining evidence from different sources into an overall conclusion can be a significant challenge
  - What is the assay actually measuring?
  - How closely is this assay linked to human toxicity?
  - How does this result relate to findings from other assays/models?



https://www.flickr.com/photos/flamephoenix1991/8376271918

What Is An AOP?

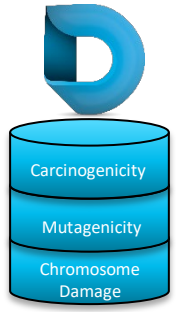


Why use AOPs?

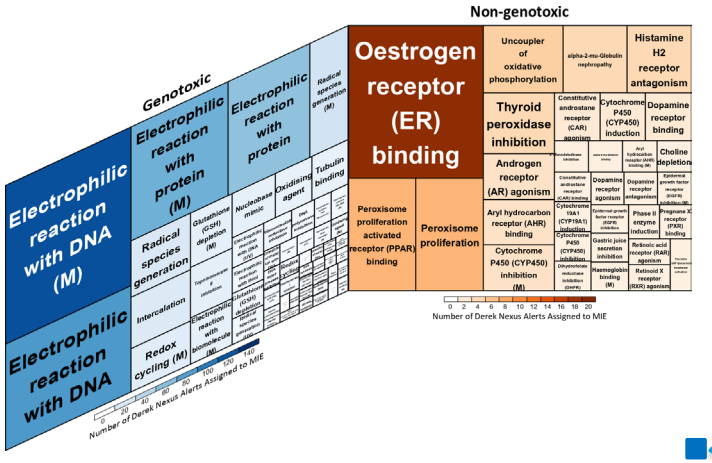
- Advocates AOPs as way to organise and contextualise data, and make conclusions
- Can identify areas to focus on to increase confidence
- Captures right level of complexity
- Accounts for human relevance
- Not categorical
- Adaptable

The AOP concept can be applied as a framework to develop IATA as it allows one to: (a) evaluate in a structured way the existing information that is available for the chemical(s) of interest (see Figure 3) and possibly conclude on the hazard based on existing information; (b) identify and generate the type of information that might be required to increase the confidence level concerning evidence of a particular hazard; and (c) iteratively suggest which information is required to make a regulatory decision (see Figure 4). By evaluating existing information, an AOP

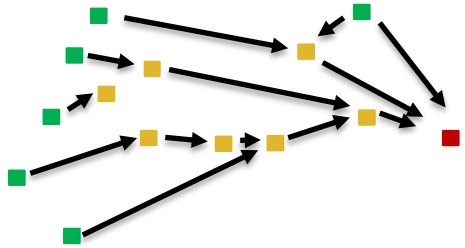
Carcinogenicity AOP Development



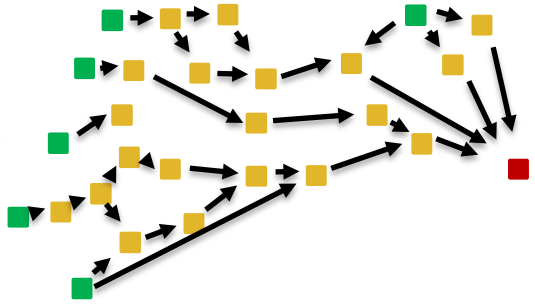
Extraction of MIEs/KEs



Arrangement into network

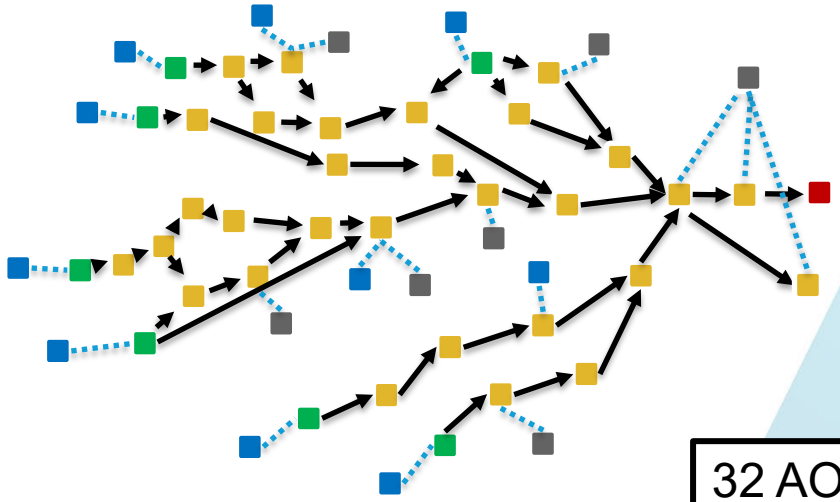


Expansion



Linking evidence

Relevant Derek alerts
Binding assays
In vitro/in vivo genotox
Repeat dose studies

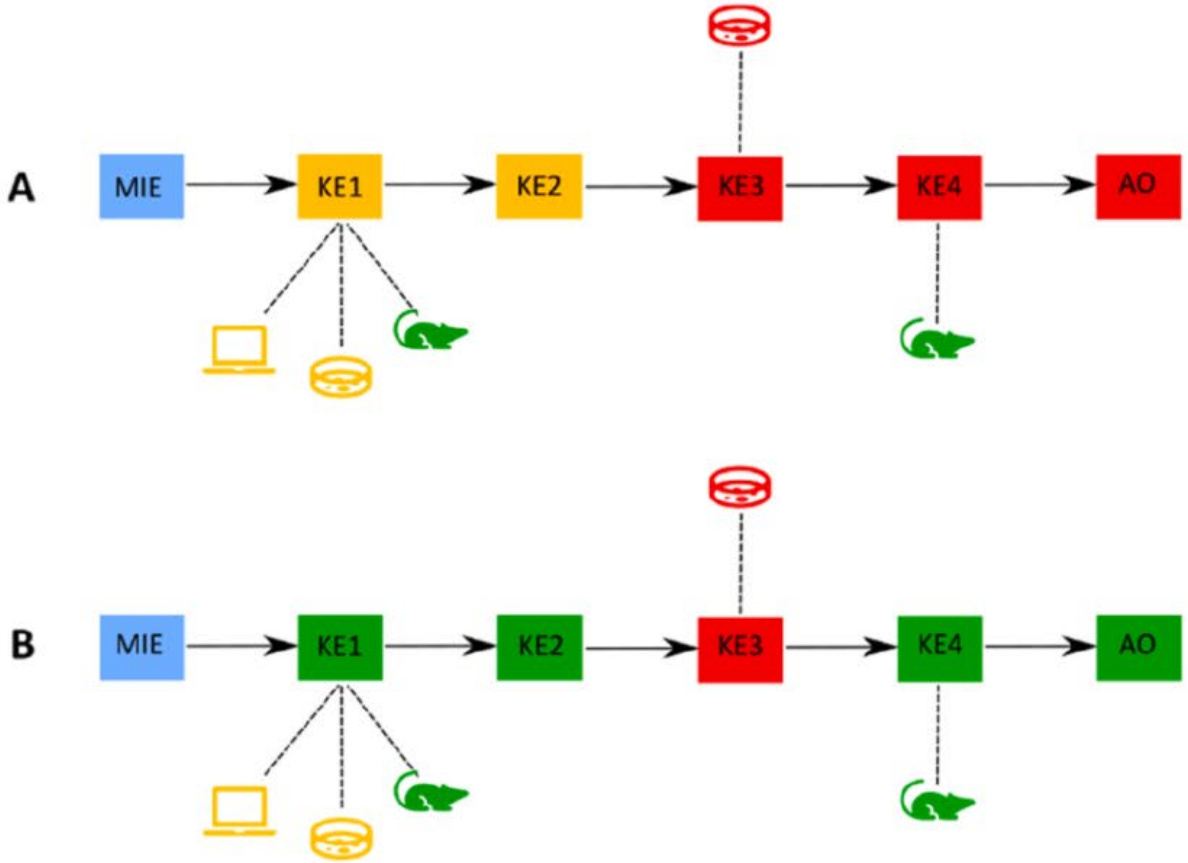


= molecular initiating event
= key event
= adverse outcome
= model
= assay

Cayley et al, ALTEX 2023 40(1) 34-52

32 AOPs
- 12 genotoxic
- 20 non-genotoxic

Reasoning between Evidence



Stalford et al, Regul Toxicol Pharmacol 2021, 127, 105071

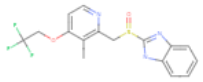
Kaptis

ICH S1

Return to home

Likely to be Carcinogenic

Compound ID: Lansoprazole **Target:** ATP4A



Last run on: 2023-02-08 16:04:28

Last run by: susanne.stalford@lhasalimited.org

Show / Hide Factors

On Target	Nothing to report
Off Target R	Positive
Histopathology R	Positive
Hormonal Perturbation R	Positive
Genotoxicity R	Positive
Immunotoxicity	Positive

Histopathology: 34 AOPs

28 | 1 | 5

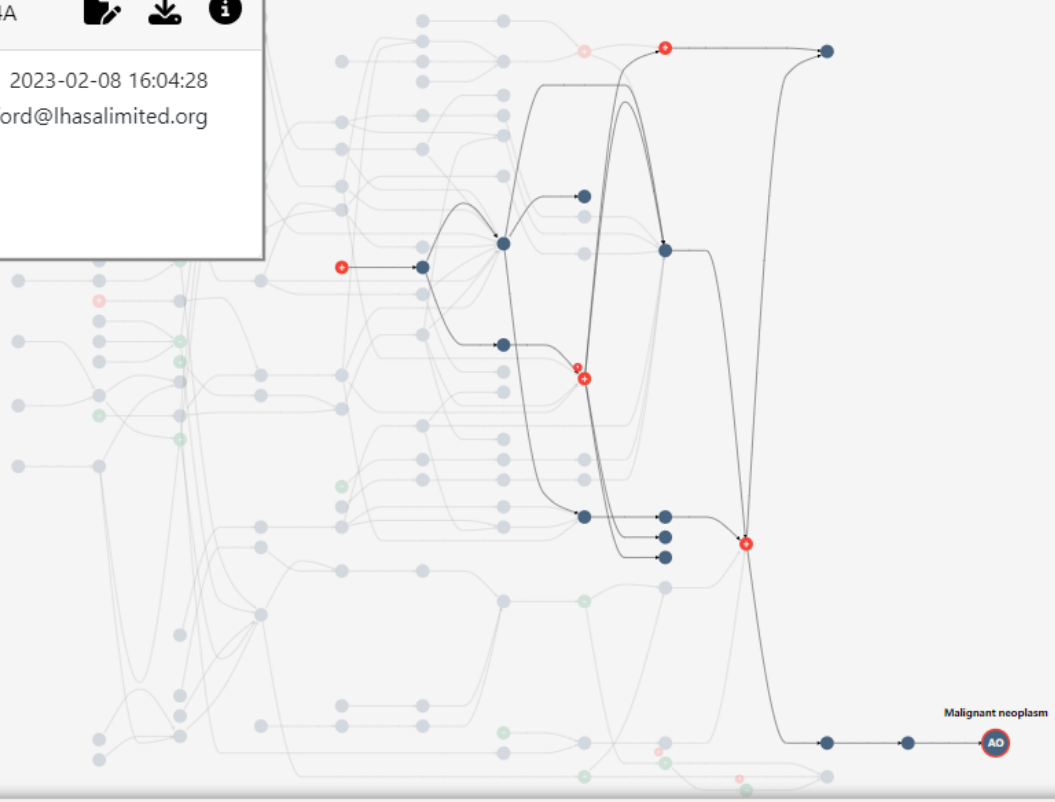
Partial factor coverage - 5 AOPs do not have enough data to make a call.

Aryl hydrocarbon receptor (AHR) activation leading to carcinogenicity
AOP Call: Positive 1

DNA incorporation of unnatural nucleotide leading to carcinogenicity
AOP Call: Positive 2

DNA nucleobase photocycloaddition leading to carcinogenicity
AOP Call: Positive 2

DNA nucleobase reaction with metal complex leading to carcinogenicity
AOP Call: Positive 2



AOP: Aryl hydrocarbon receptor (AHR) activation leading to carcinogenicity Positive

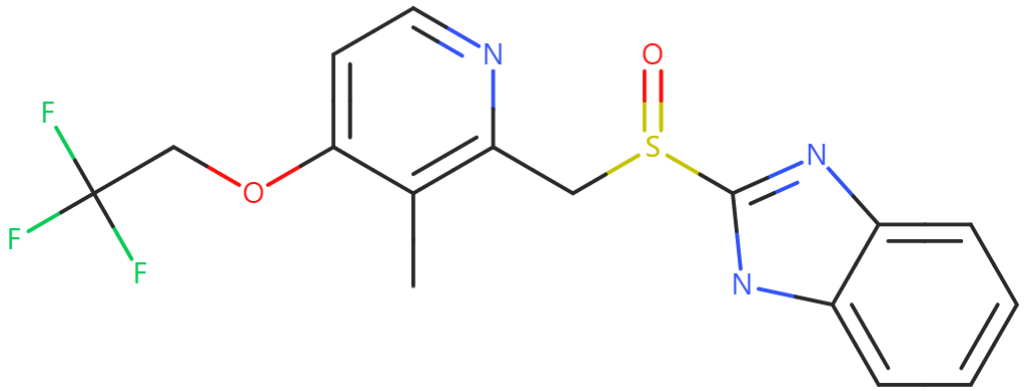
ID: AOP31

Source/Verifier: Lhasa Limited

Evidence:

	In vivo	In vitro	In silico
Positive	4	1	0
Negative	0	0	-

In Practice - Lansoprazole

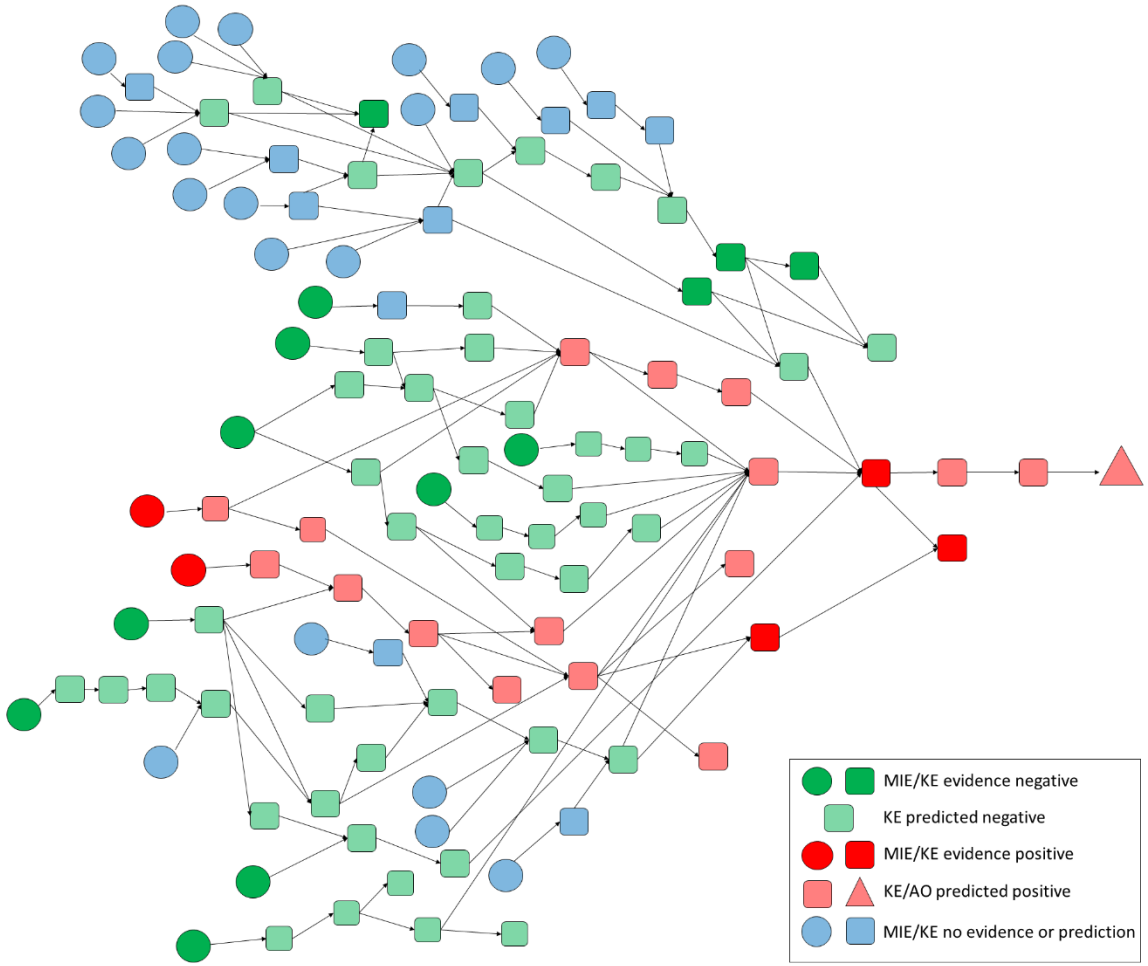


- Marketed Pharmaceutical
- Gastric Acid Inhibitor

Data from:
- Vitic
- Open Targets
- DrugBank
- ToxCast
- ChEMBL
- Drugs@FDA
- Derek Nexus

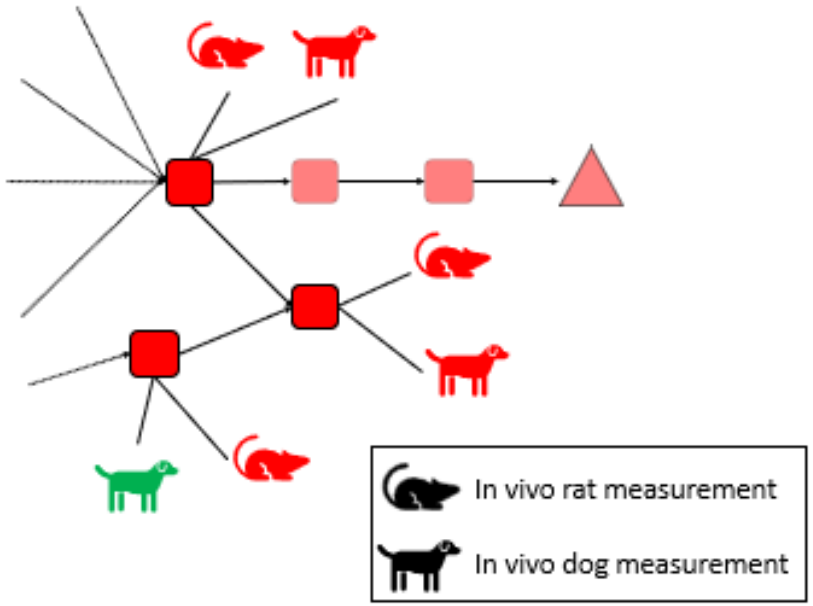
Assay	Outcome
Aryl hydrocarbon receptor binding	Positive
Pregnane X Receptor binding	Positive
Other binding or in silico predictions	Negative
Ames	In silico Negative In vitro Negative
Mouse lymphoma assay	Negative
Chromosome Damage	In vitro Positive In vivo Negative
Micronucleus	In vitro Conflicted In vivo Negative
Unscheduled DNA synthesis	In vitro Negative In vivo Negative

Initial Assessment



- All available evidence associated
- Reasoned to give a positive call for carcinogenicity
- Expert review required to elucidate what factors contribute to outcome

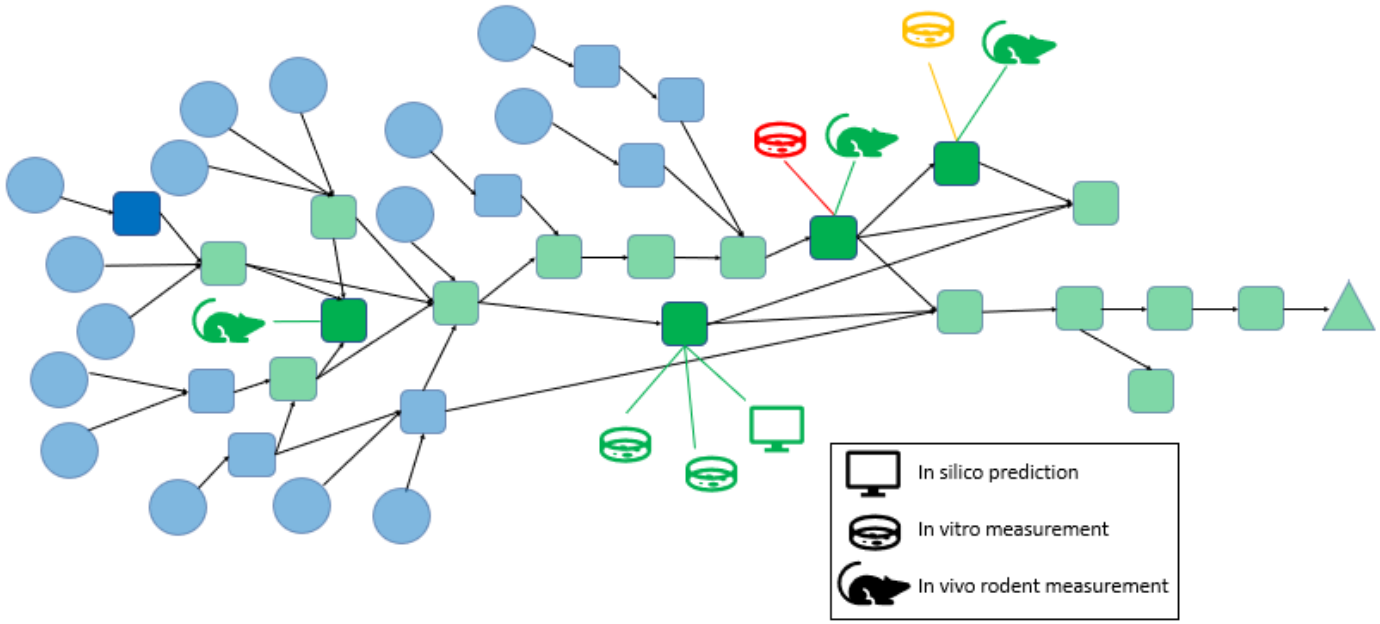
Histopathology Factor Assessment



Assay	Measure	Tissues (species)
Repeat-Dose Sub-chronic Assay	Hyperplasia	Stomach (rat, dog)
	Hypertrophy	Stomach (rat, dog), liver (rat)
	Organ Weight Increase	Heart (rat), liver (rat, dog), lungs (rat), kidney (rat, dog), ovaries (dog)
Repeat-Dose Chronic Assay	Hyperplasia	Stomach (rat, dog), testes (rat)
	Hypertrophy	Stomach (rat, dog)
	Organ Weight Increase	Stomach (rat)

- Histopathology findings indicate definite concern that compound is carcinogenic
- Findings consistent with other compounds in pharmacological class

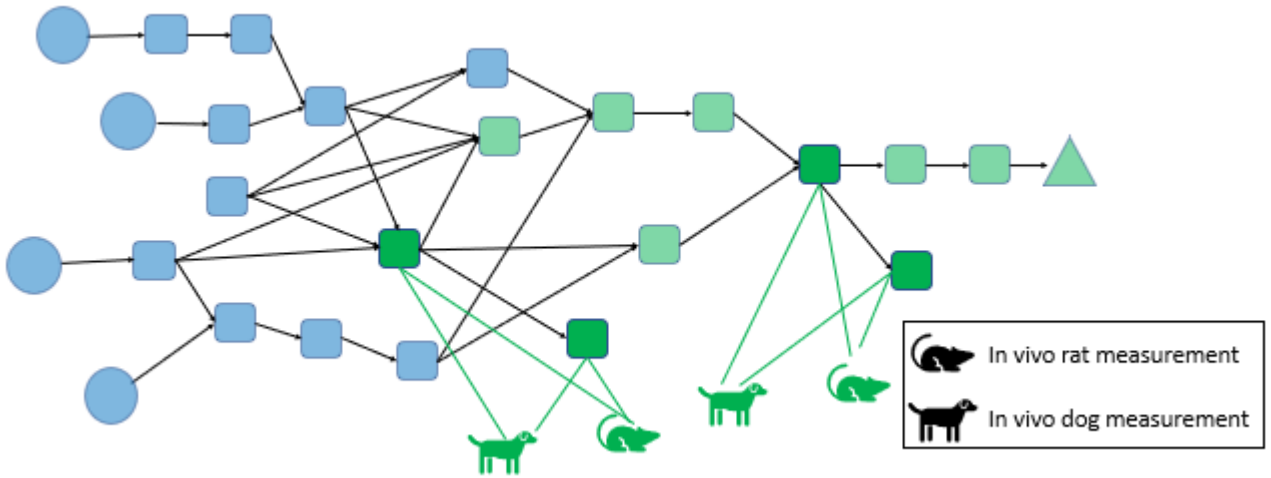
Genotoxicity Factor Assessment



Assay	Outcome
Ames	In silico Negative In vitro Negative
Mouse lymphoma assay	Negative
Chromosome Damage	In vitro Positive In vivo Negative
Micronucleus	In vitro Conflicted In vivo Negative
Unscheduled DNA synthesis	In vitro Negative In vivo Negative

- Some genotoxicity findings in vitro which could be concerning
- In vivo evidence conclusively non-concerning

Immune Modulation Factor Assessment



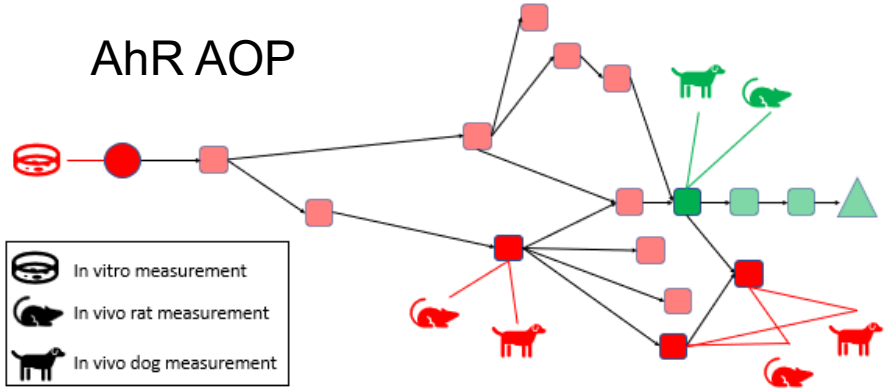
- No positive findings in immune system relevant tissues or organs
- No positive findings for blood chemistry
- Clear that immune modulation is not a concern for carcinogenicity in this case

Secondary Pharmacology Factor Assessment

- Potential off-target concern from AhR and PXR

Assay	Outcome
Aryl hydrocarbon receptor binding	Positive
Pregnane X Receptor binding	Positive
Other binding or in silico predictions	Negative

AhR AOP



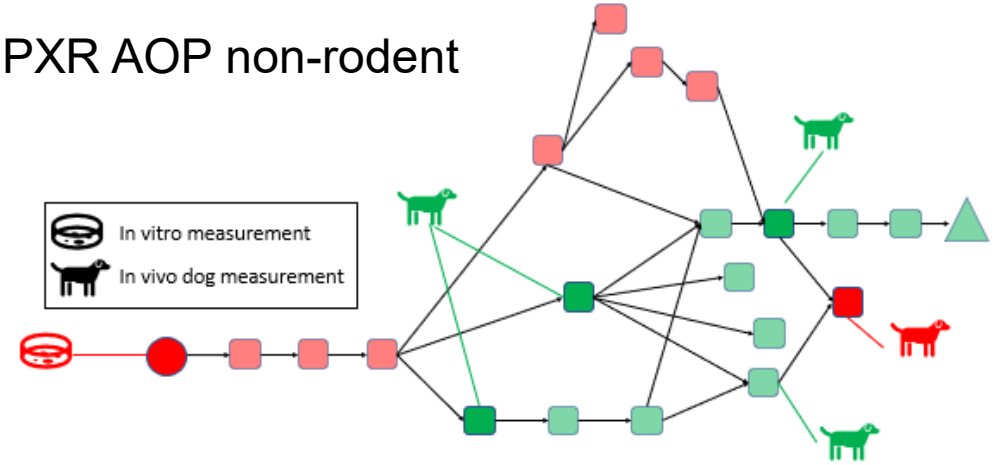
- Taken in context with additional data
  - Increase in hepatic enzymes
  - No observations of hyperplasia in relevant organs
  - Not likely to contribute to carcinogenic potential

Secondary Pharmacology Factor Assessment

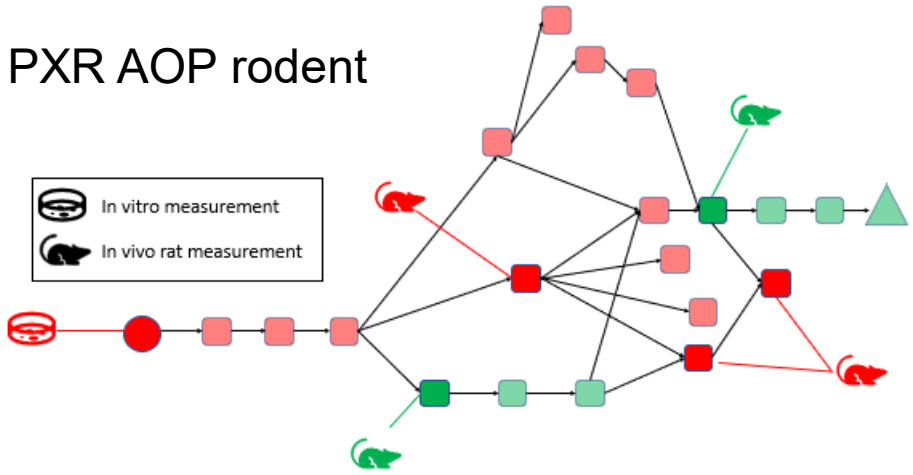
- Potential off-target concern from AhR and PXR

Assay	Outcome
Aryl hydrocarbon receptor binding	Positive
Pregnane X Receptor binding	Positive
Other binding or in silico predictions	Negative

PXR AOP non-rodent

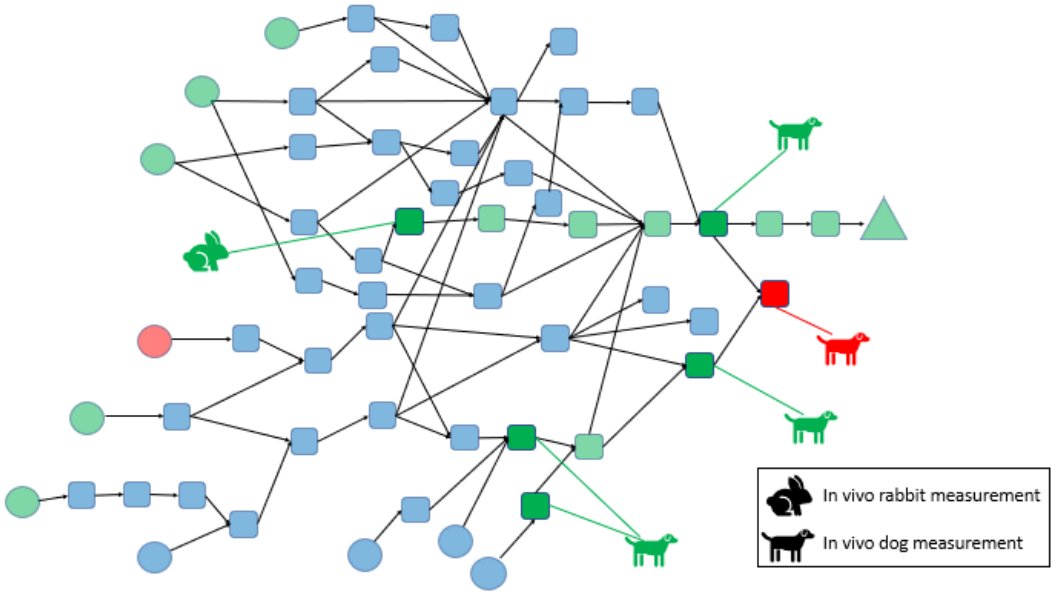


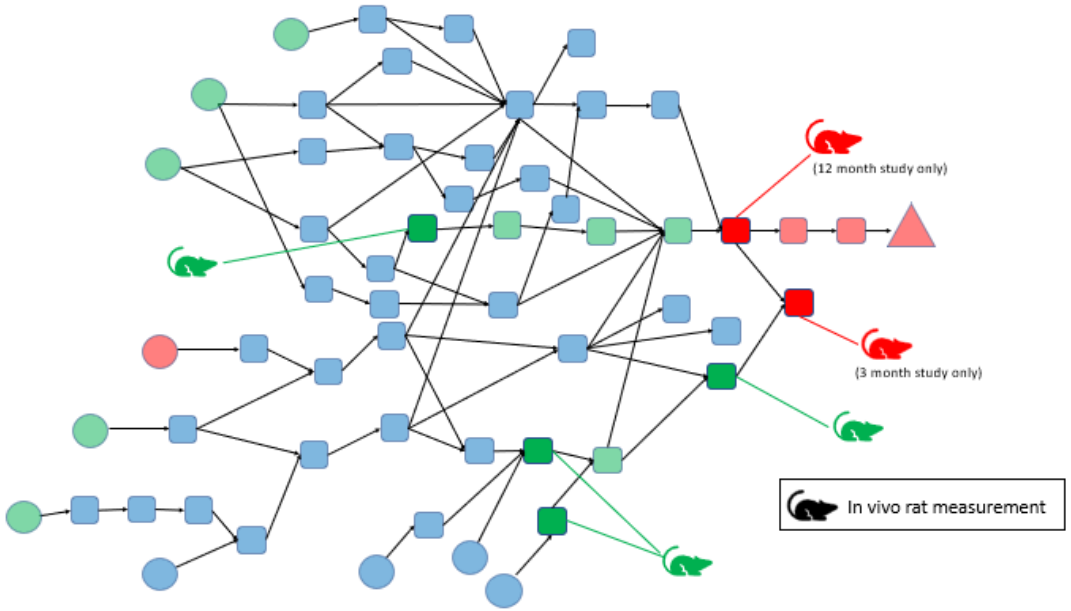
PXR AOP rodent



- No observations of hyperplasia in relevant organs
- Differences between species in hepatic enzyme activity – may only be rat relevant
- Not likely to contribute to carcinogenic potential

Hormonal Effects Factor Assessment

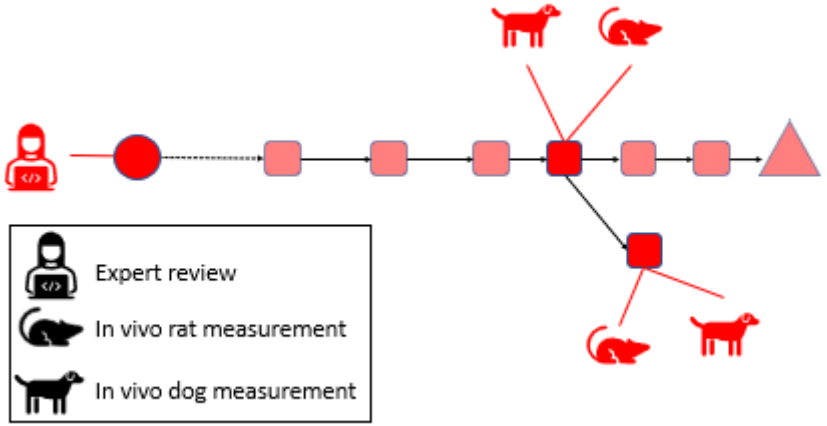




- Species differences indicate potential concern for rat, but not non-rodent
- Inconsistent findings in relevant organs for rats between subchronic and chronic studies
- Conclude no carcinogenic concern in humans, but potential concern in rats

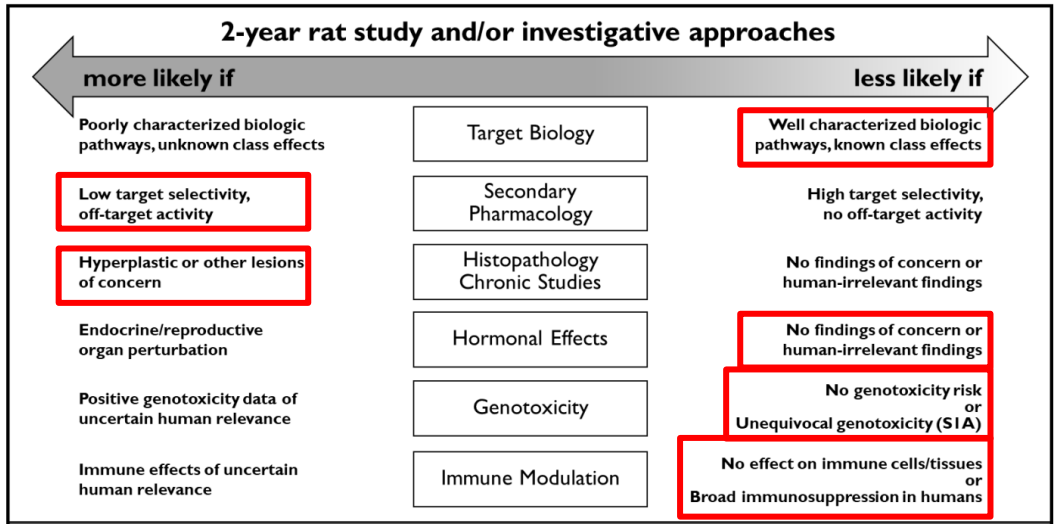
Target Biology Factor Assessment

- ATP4A not part of a known AOP so review of available literature undertaken
  - Lansoprazole primary pharmacologic MoA to act as protein pump inhibitor, supressing gastric acid production
  - ATP4A predominantly expressed in stomach and homologous across species
  - Similar compounds (pharmacologically) induce stomach tumours in rat carcinogenicity studies
  - In knockout studies, a number of adverse effects observed, including hyperplasia in the stomach
- Very likely that inhibiting ATP4A will drive mechanisms leading to carcinogenicity in humans and rats
- Can link target into AOP to show this effect



Lansoprazole Conclusion

- ICH S1B(R1) assessment
  - Carcinogenic potential in humans is likely, such that a two year rat study would not add value
  - Would likely encounter stomach tumours due to target biology
  - Possibly encounter rat specific tumours in hormone related organ
- 2-year rat study has been conducted
  - Stomach tumours observed
  - Additional testicular tumours seen



Conclusion

- We can now make better use of preclinical data to assess the risk of cancer
- AOPs are an effective framework for organising and contextualising evidence
- Kaptis provides the means to reason between evidence to aid decision-making
  - Gives transparent, scientifically robust and consistent outcomes
  - Facilitates the WoE when a lot of information needs to be combined and analysed
  - Incorporates best practices
  - A full S1 assessment can be made within the software
  - Adaptable so evidence from NAMs can be incorporated
- Expert review is important for assessment
  - Increases confidence in outcomes, provides additional information to support assessment

Lhasa Limited

Thanks to:

Alex Cayley
Steven Kane
Emma Hill
Tasha Jones
Alun Myden
Reza Zarei
Dan Newman
Jonathan Vessey

For more info:

hello@lhasalimited.org

shared **knowledge** • shared **progress**

Lhasa Limited
Granary Wharf House, 2 Canal Wharf
Leeds, LS11 5PS
Registered Charity (290866)
Company Registration Number 01765239

+44(0)113 394 6020
info@lhasalimited.org
www.lhasalimited.org

Work in progress disclaimer

This document is intended to outline our general product direction and is for information purposes only, and may not be incorporated into any contract. It is not a commitment to deliver any material, code, or functionality, and should not be relied upon. The development, release, and timing of any features or functionality described for Lhasa Limited's products remains at the sole discretion of Lhasa Limited.