Development of an Adverse Outcome Pathway Network to Support an Integrated Approach to Testing and Assessment of Carcinogenic Risk

Dr. Susanne A. Stalford

Senior Knowledge Research Scientist

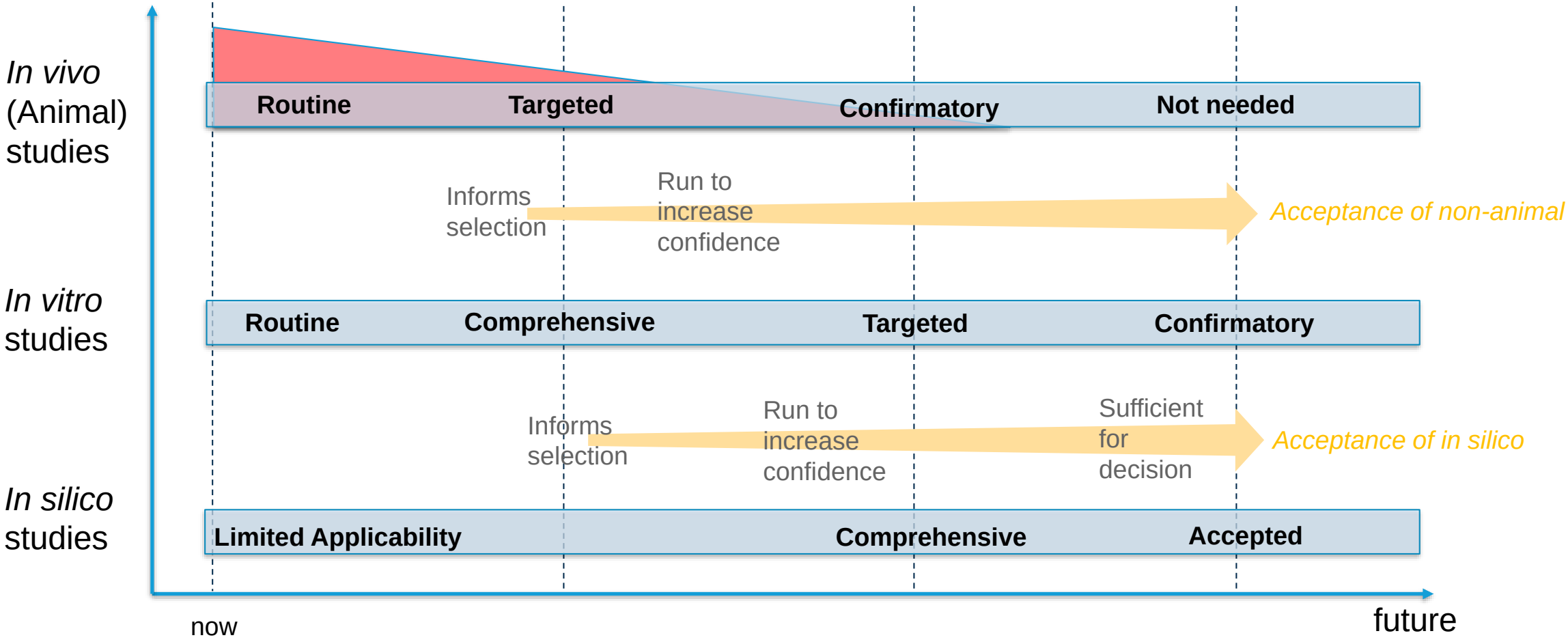
susanne.stalford@lhasalimited.org

Overview

- A View of the Future of Safety Assessment
- Introduction to AOPs
- Rearranging Current Knowledge
- Integration of Evidence with Pathways
- Reasoning
- Example application - clofibrate
- Summary

Lhasa Limited

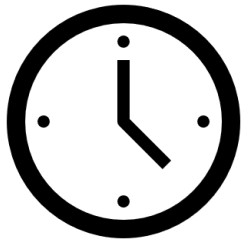
A View of the Future of Safety Assessment



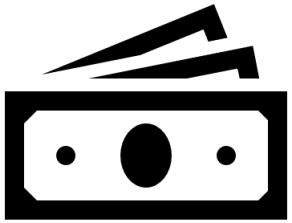
Lhasa Limited

Current Status of Carcinogenicity Testing

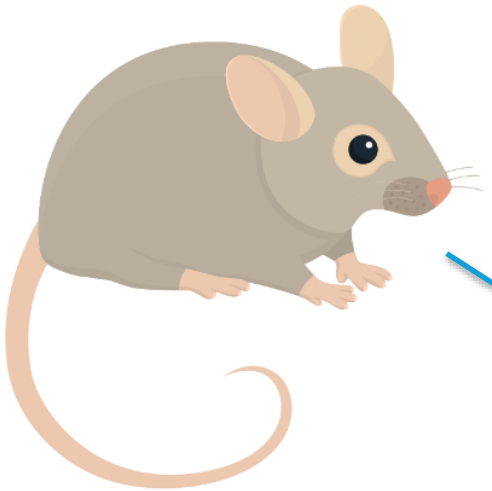
OECD Test No. 451

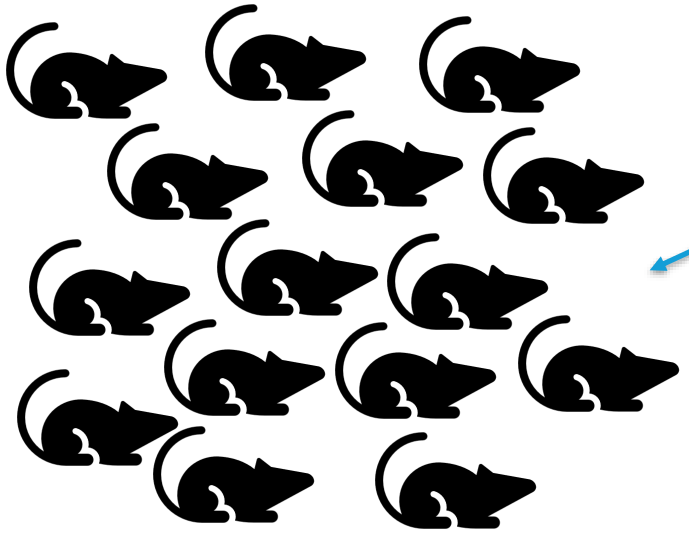


Time



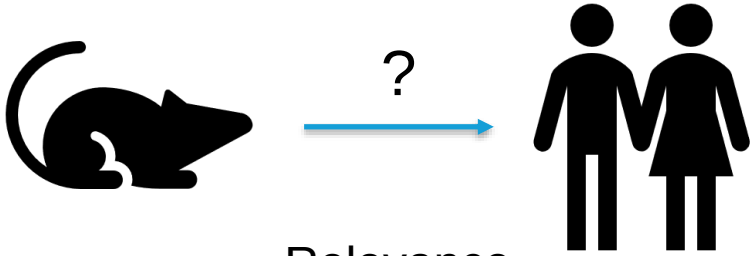
Cost





Ethical

Pesticides
Biocides
Industrial Chemicals
Pharmaceuticals
Veterinary Medicines

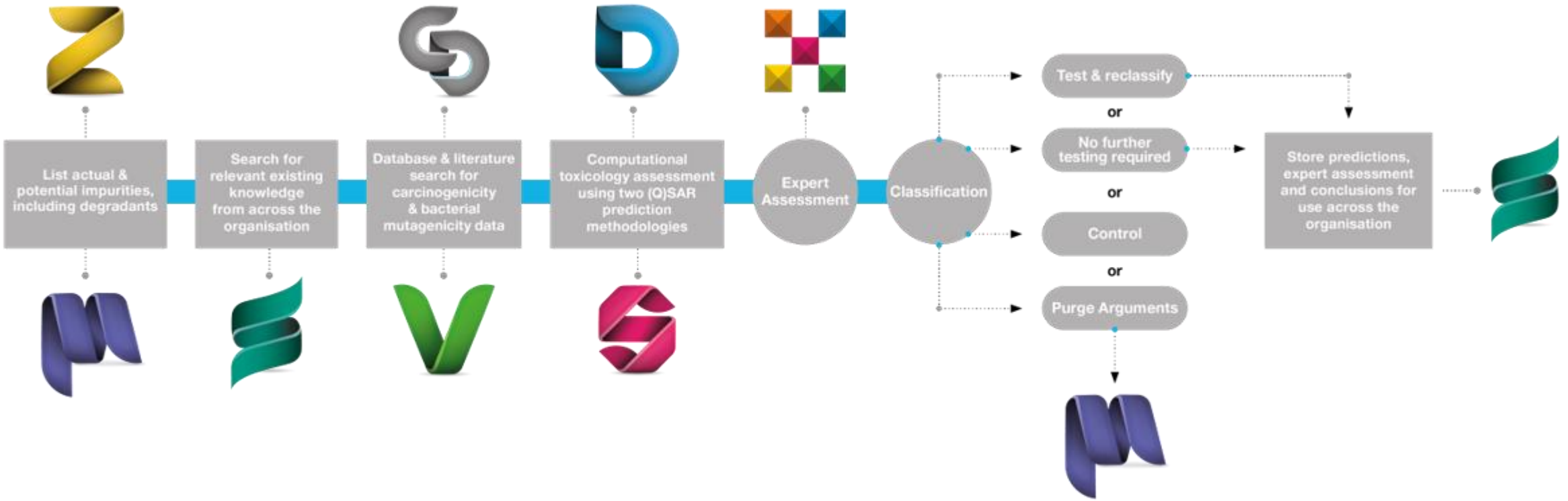


Relevance

Lhasa Limited

Alternatives

In silico models

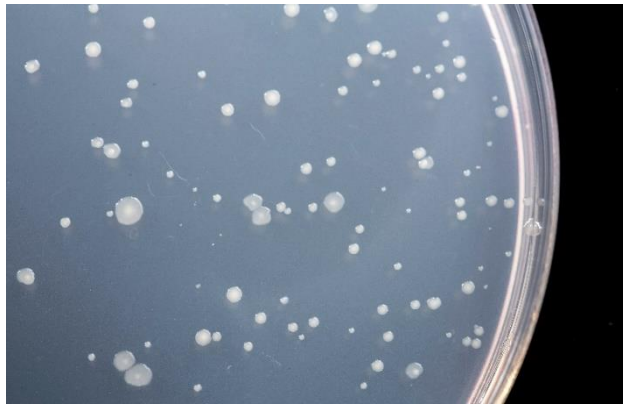


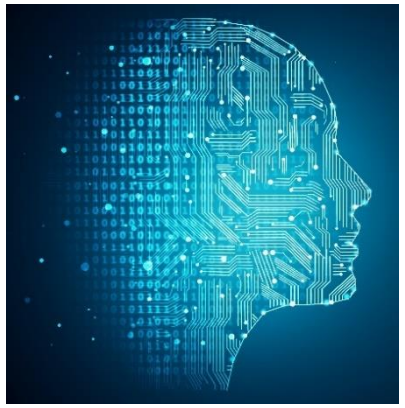
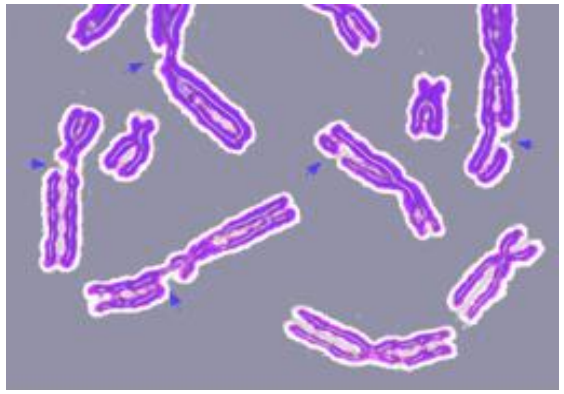
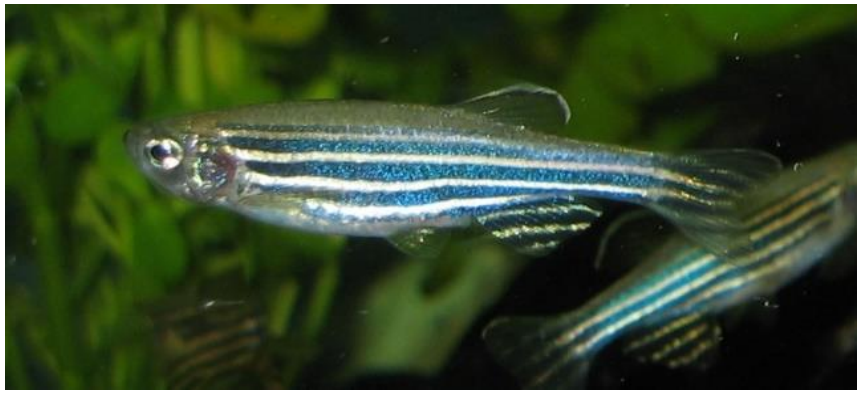
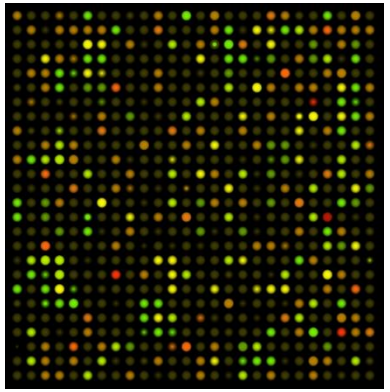
ICH M7 – Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

Lhasa Limited

Alternatives

In vitro, high-throughput and other models



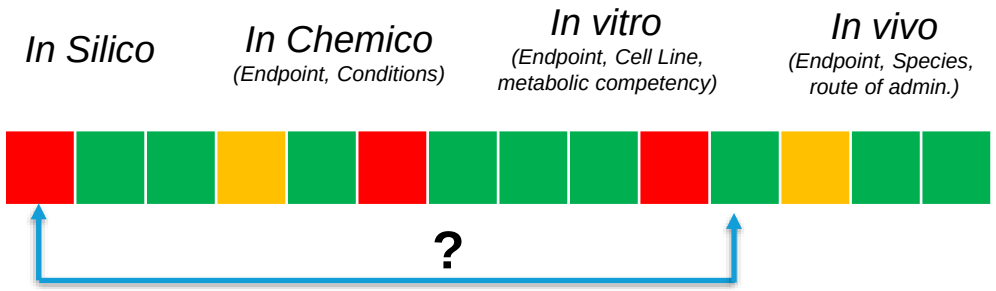
Lhasa Limited

IATA

- The effort to move toward 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*) in order to make more accurate predictions of human risk than the existing animal models

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

- Combining evidence from different sources can be a significant challenge
  - What is the assay measuring?
  - How closely is this assay linked to the adverse outcome?
  - How does this result relate to findings from other assays/models?



- Adverse outcome pathways (AOPs) provide a framework to contextualise these assays

http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

Lhasa Limited

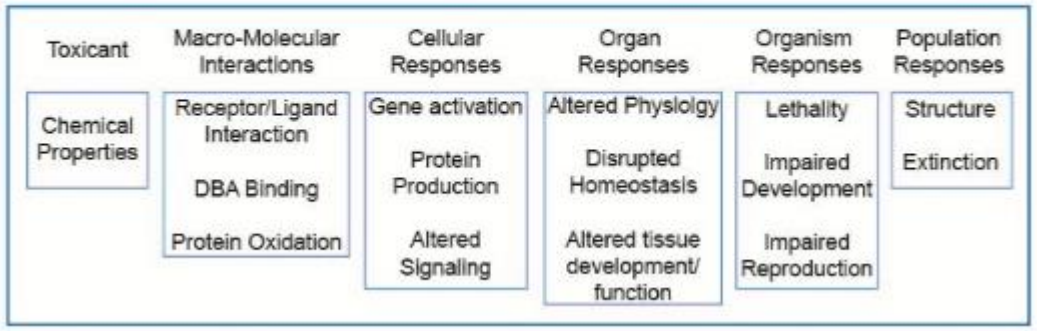
The Adverse Outcome Pathway Concept

OECD
BETTER POLICIES FOR BETTER LIVES

What is an Adverse Outcome Pathway?

The OECD launched a new programme on the development of **Adverse Outcome Pathways (AOP)** in 2012. An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (see figure below). AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

Schematic representation of the AOP illustrated with reference to a number of pathways:



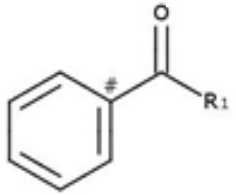
Ankley et al.; Environmental Toxicology And Chemistry; 29; 2010; 730-741

http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

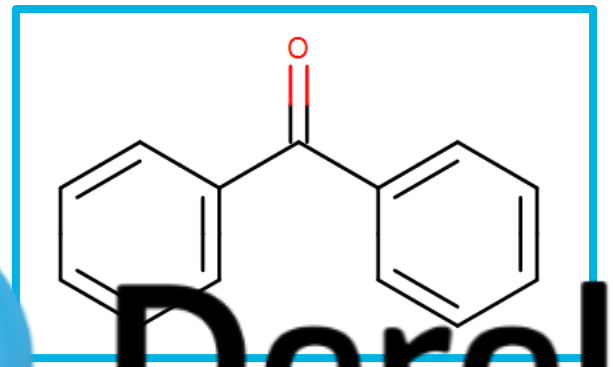
Lhasa Limited

How Derek Nexus Predicts Toxicity

707: Diaryl ketone



R1 = C (aromatic)
C# cannot be part of a ring fusion
N atoms bonded to an aromatic carbon are not allowed here



Derek nexus

- Derek KB 2018 1.1 [Certified by: Lhasa Limited, Leeds, Yorkshire, UK]
  - Carcinogenicity
    - mammal - PROBABLE
      - Alert - 707: Diaryl ketone
      - Example - benzophenone

Comments

Diaryl ketones have been shown to display carcinogenic activity in rats and mice. Examples include isoxaflutole [US EPA 2009], topramezone [US EPA 2009] and benzophenone [NTP 2006]. Isoxaflutole has been classified as a group B2 carcinogen (probable human carcinogen with little or no human data) by the United States Environmental Protection Agency [US EPA 1998]. It induced adenomas and carcinomas in the rat liver and thyroid glands whereas in the mice it only induced hepatocellular adenomas and carcinomas [US EPA 1998]. In a 2 year feeding study in rats, benzophenone induced renal tubule adenomas (in males only) and mononuclear cell leukaemia. In mice, benzophenone increased the incidence of hepatocellular adenomas and histiocytic sarcoma (in females only) [NTP 2006].

The carcinogenicity of diaryl ketones occurs through a non-genotoxic mechanism. Benzophenone has been shown to bind to the pregnane X receptor (PXR) in vitro which is a specific inducer of CYP3A, CYP2B and CYP2C enzymes [Mikamo et al]. In a short-term study, exposure to benzophenone was associated with hepatocellular hypertrophy and cell proliferation and was accompanied by an induction of CYP2B [NTP 2000]. These effects are similar to those seen with barbituric acids such as phenobarbital, which are non-genotoxic carcinogens (their activity is described elsewhere in the knowledge base). It is deemed unlikely that that the carcinogenic activity of compounds acting through a phenobarbital-like mechanism can be extrapolated to humans [Holsapple et al], although in this case the link is based on limited data and should not be considered as conclusive.

Query Compound

Adverse Outcome

Molecular Initiating Event

Key Event

Lhasa Limited

Adverse Outcome Pathway Translation

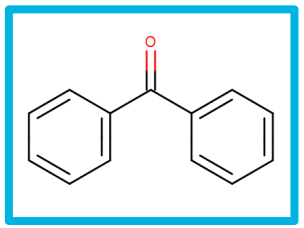
Molecular

Cellular

Organ

Individual

Benzophenone



Molecular initiating event

Benzophenone has been shown to bind to the pregnane X receptor (PXR) in vitro…

Pregnane X receptor (PXR) binding

PXR activation

Key event

…inducer of CYP3A, CYP2B and CYP2C enzymes

CYP2B upregulation

CYP3A upregulation

Key event

In a short-term study, exposure to benzophenone was associated with hepatocellular hypertrophy and cell proliferation…

Cell proliferation increase

Adverse outcome

In a 2 year feeding study in rats, benzophenone induced renal tubule adenomas (in males only) and mononuclear cell leukaemia. In mice, benzophenone increased the incidence of hepatocellular adenomas and histiocytic sarcoma (in females only).

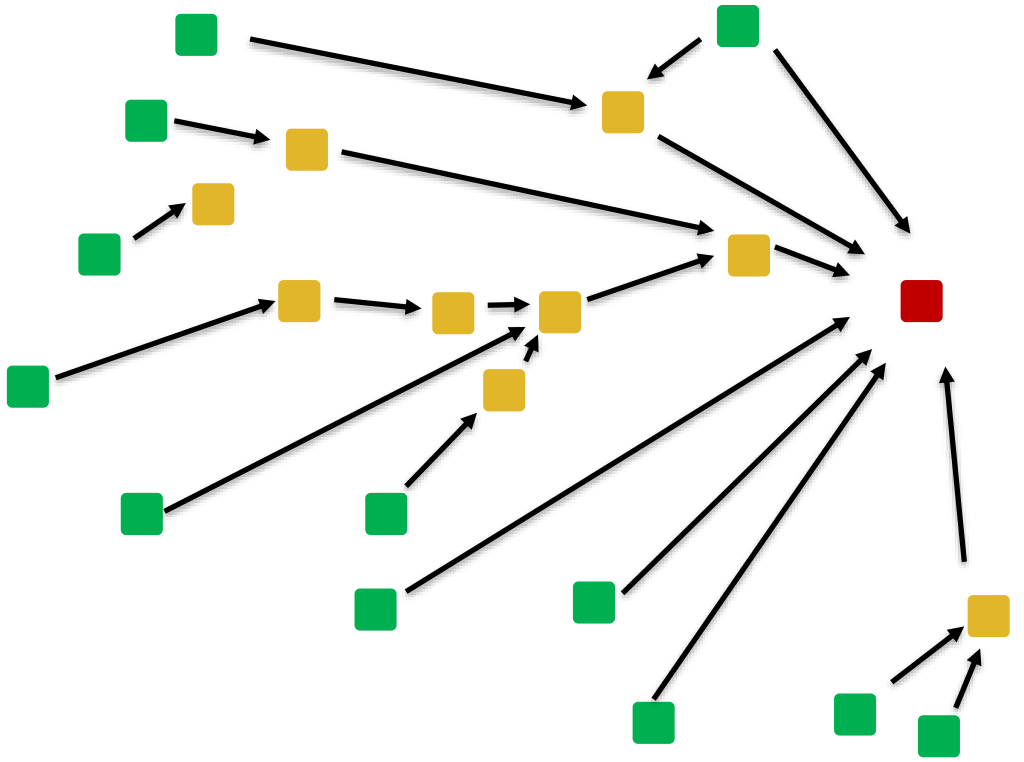
Carcinogenicity

Lhasa Limited

Rearranging Derek Nexus Knowledge

- Knowledge of MIEs/KEs contained in alerts relating to carcinogenicity within Derek Nexus v.6.0.1 was rearranged
- 85 KEs linking a compound class to carcinogenicity were assigned to 310 Derek Nexus alerts

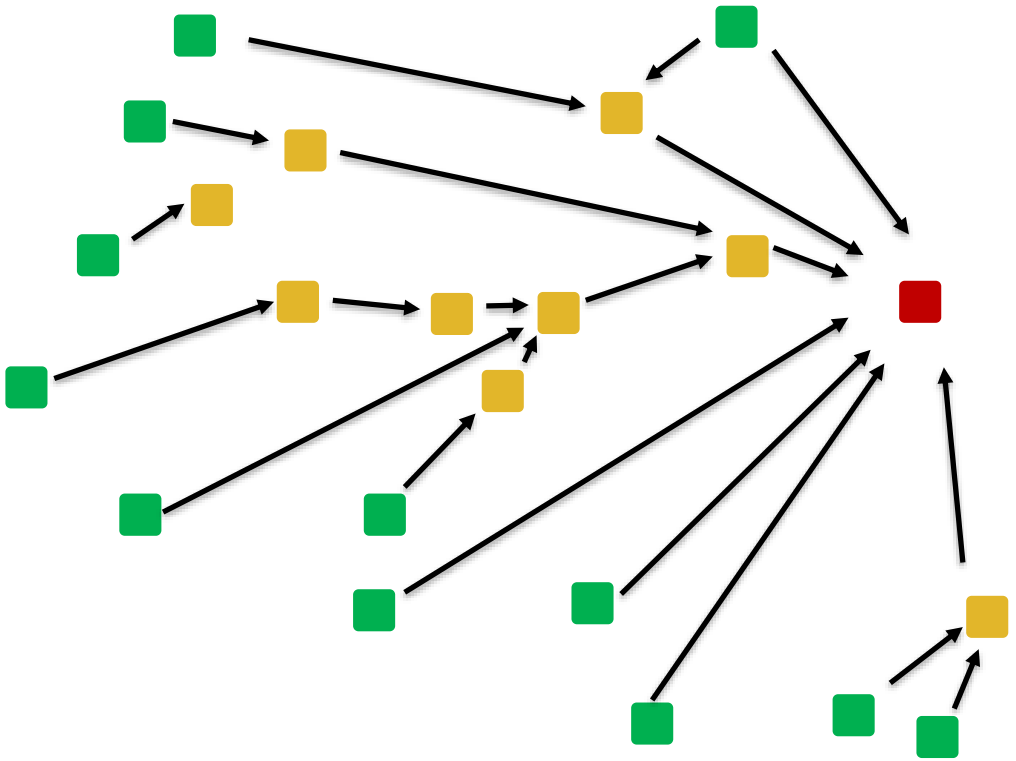
Derek Nexus Endpoint	Number Of Alerts
Carcinogenicity	118
Photocarcinogenicity	5
Mutagenicity	144
Photomutagenicity	6
Chromosome Damage	140
Photo-induced chromosome damage	3
Non-specific genotoxicity	1
Photo-induced non-specific genotoxicity	2
Oestrogenicity	6
Oestrogen receptor modulation	9
Teratogenicity	7
Peroxisome Proliferation	13



Lhasa Limited

Rearranging Derek Nexus Knowledge

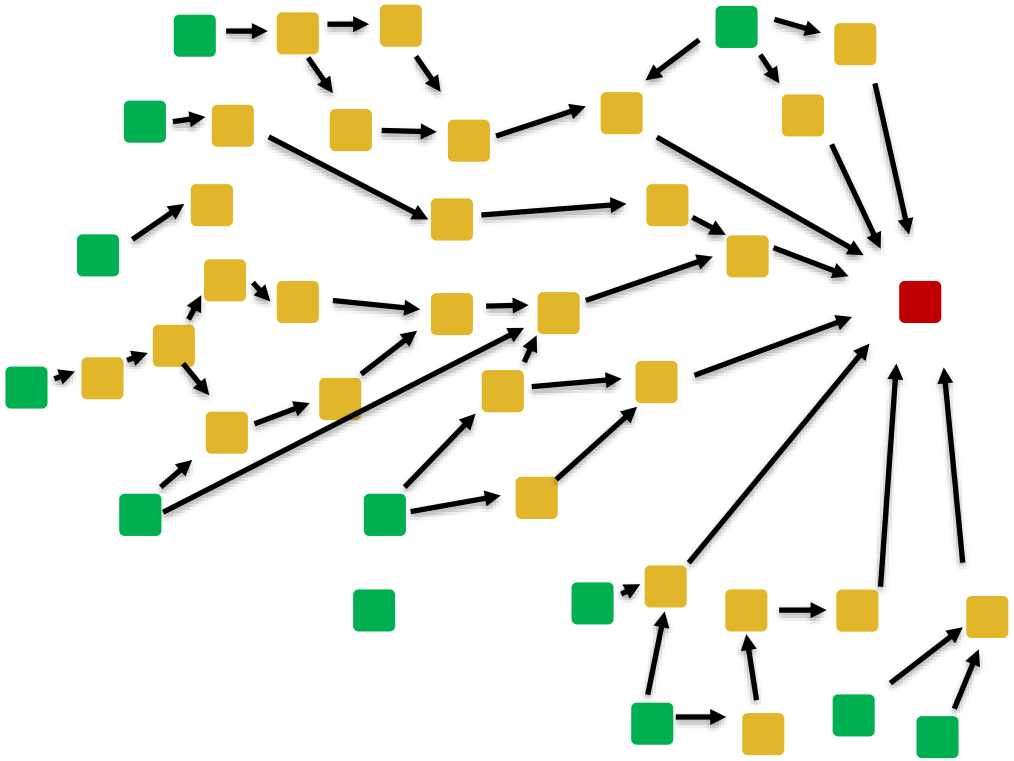
- The pathways were further investigated to add in KEs missing from the Derek Nexus alerts
- Controlled terminology for each event and KER was used



Lhasa Limited

Expanding the Network

- The pathways were further investigated to add in KEs missing from the Derek Nexus alerts
- Controlled terminology for each event and KER was used

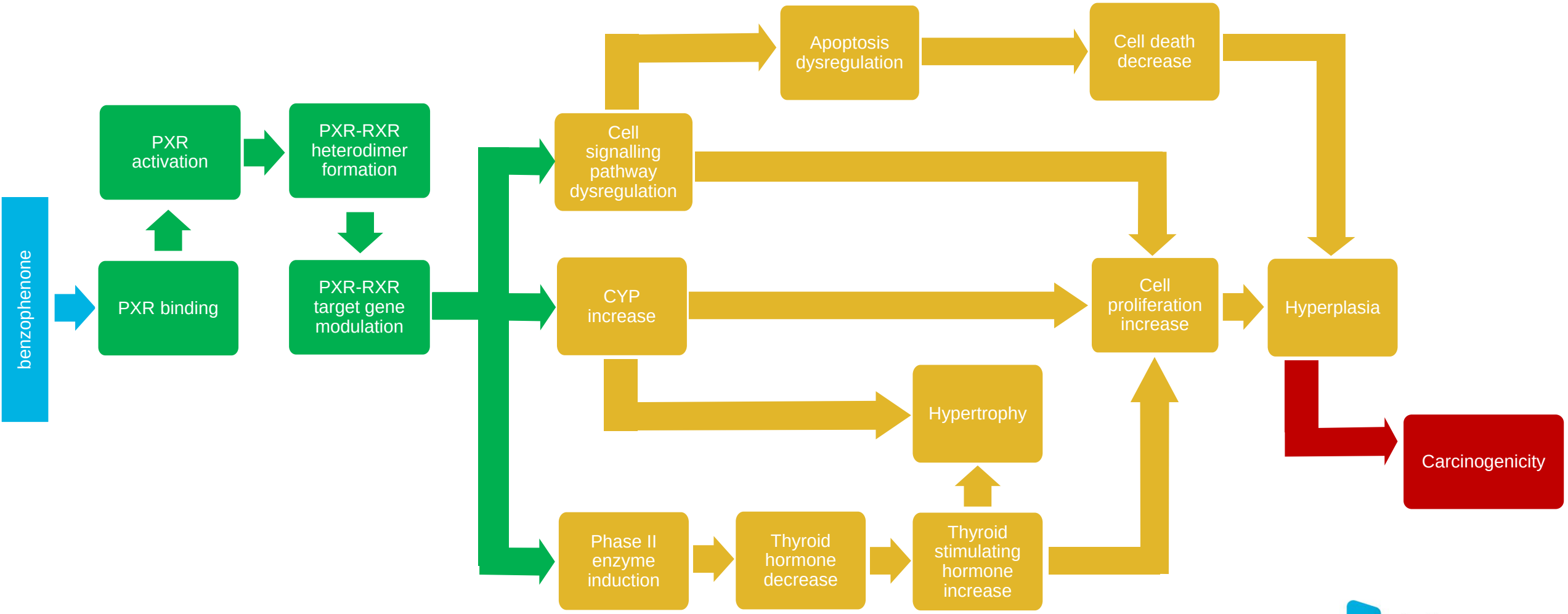


No. MIEs	37
No. AOPs	37
No. Non-Genotoxic AOPs	18
No. Genotoxic AOPs	19

- This network has the potential to be expanded further using additional sources

Lhasa Limited

Expanding the Network

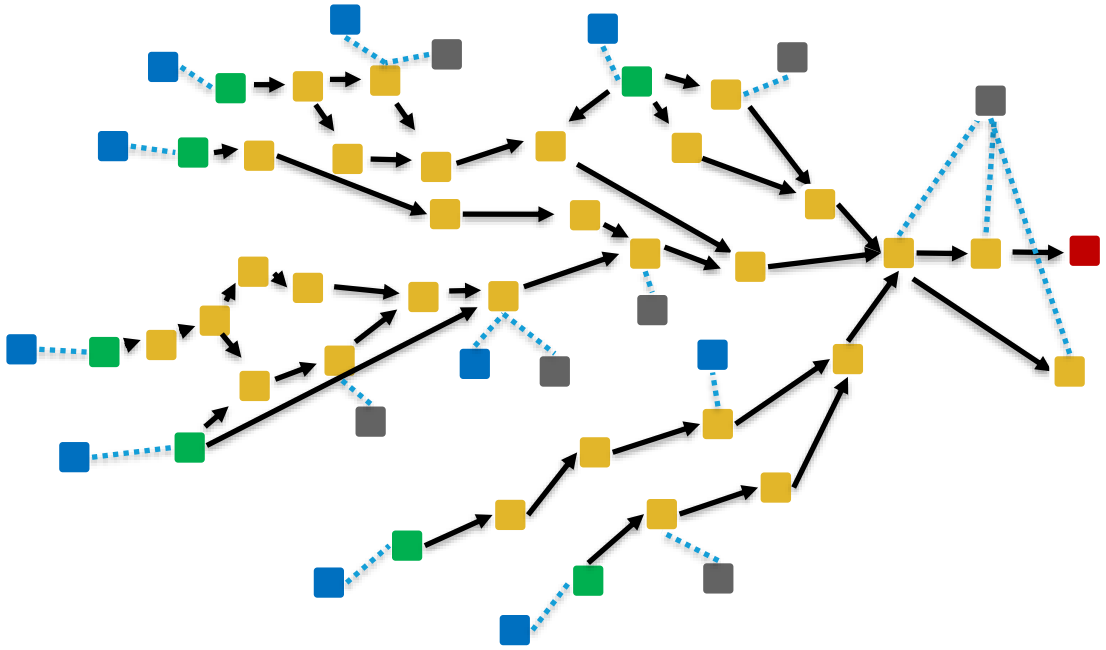


Lhasa Limited

Adding Evidence To The Network

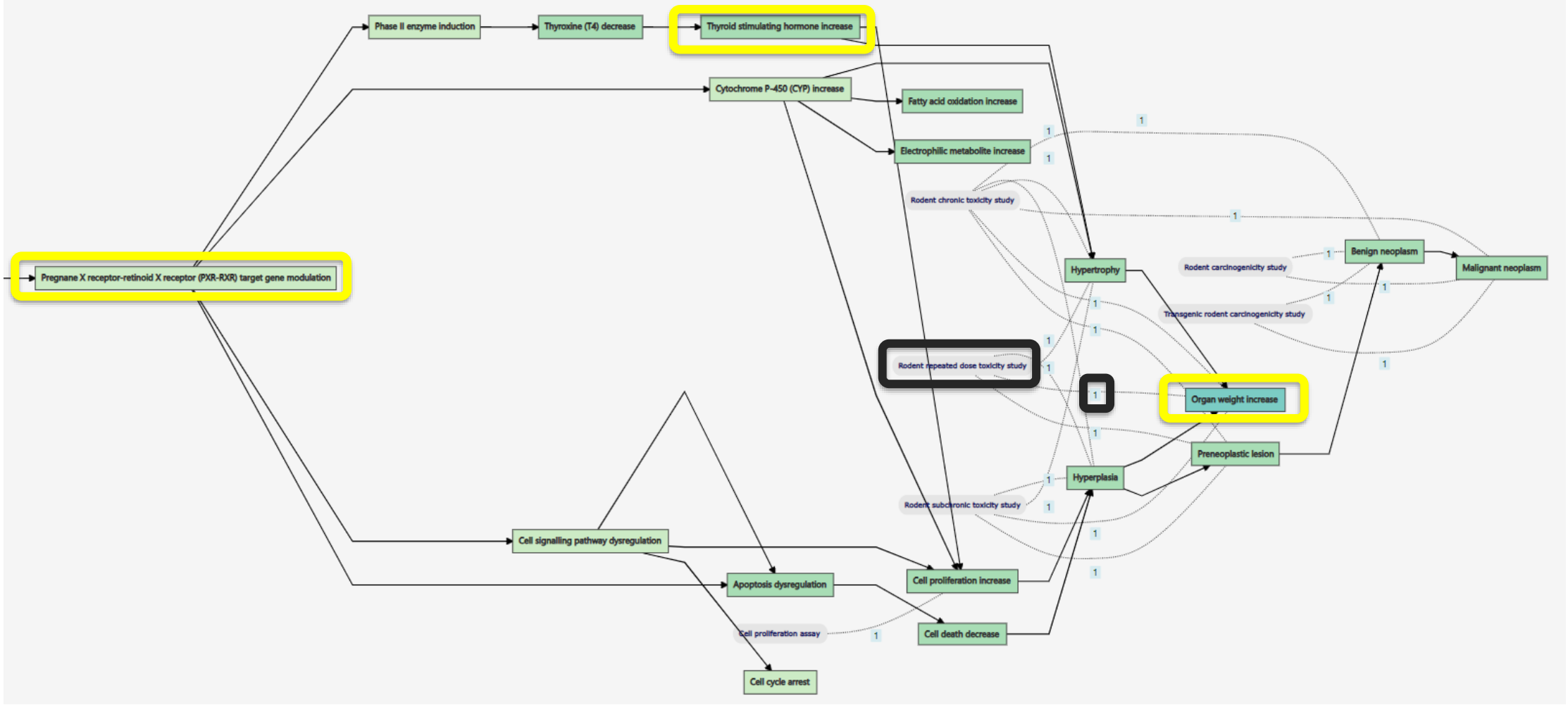
- Associate models with key events
- Associate assays and their measurements with key events
- Add data to the assays

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



Ames test
In vitro chromosome aberration
In vivo micronucleus
ER binding assay
Rodent repeat dose

Kaptis



Molecular

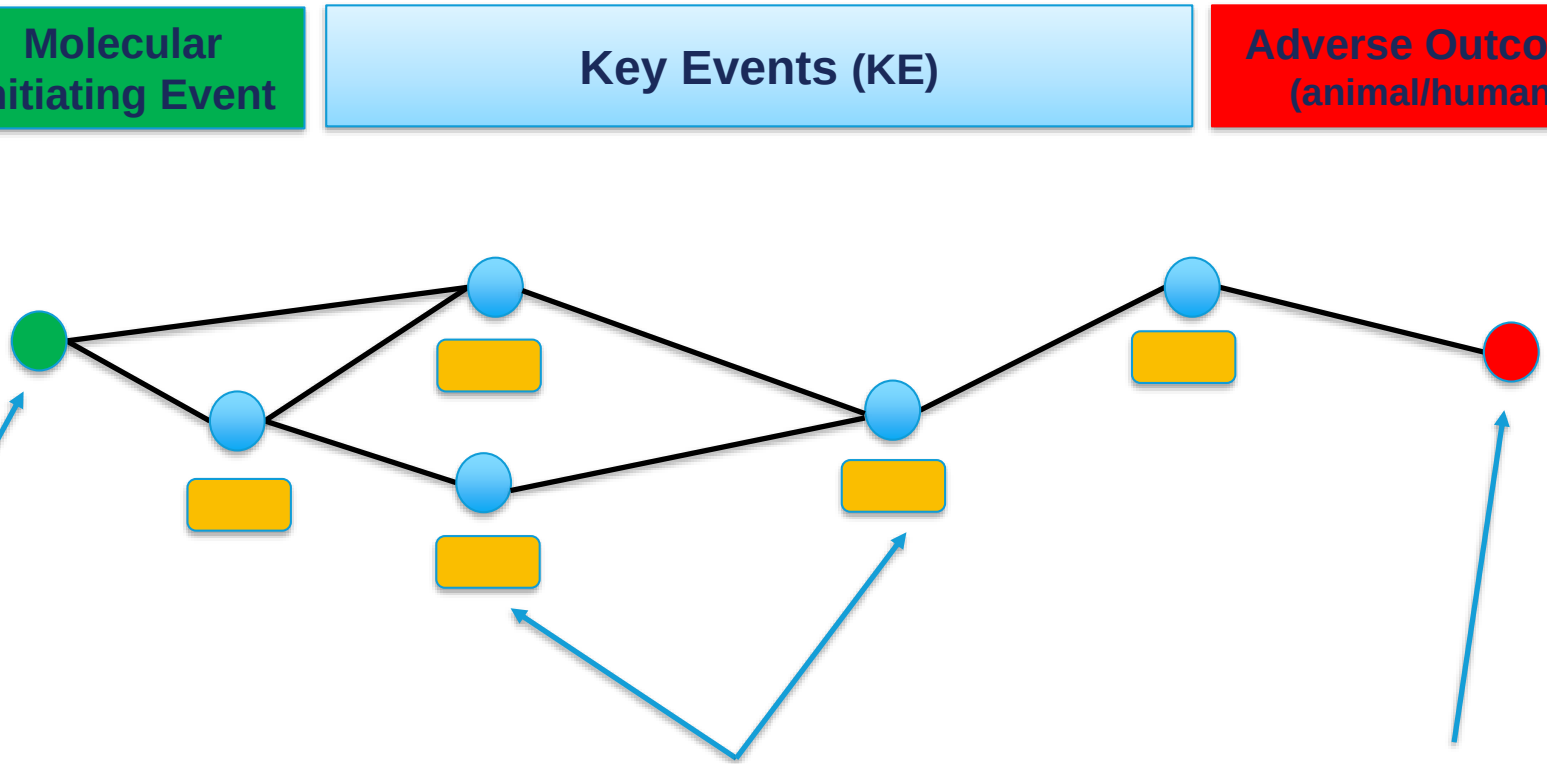
Cellular

Organ

Assay and Assay measurement

Lhasa Limited

Application



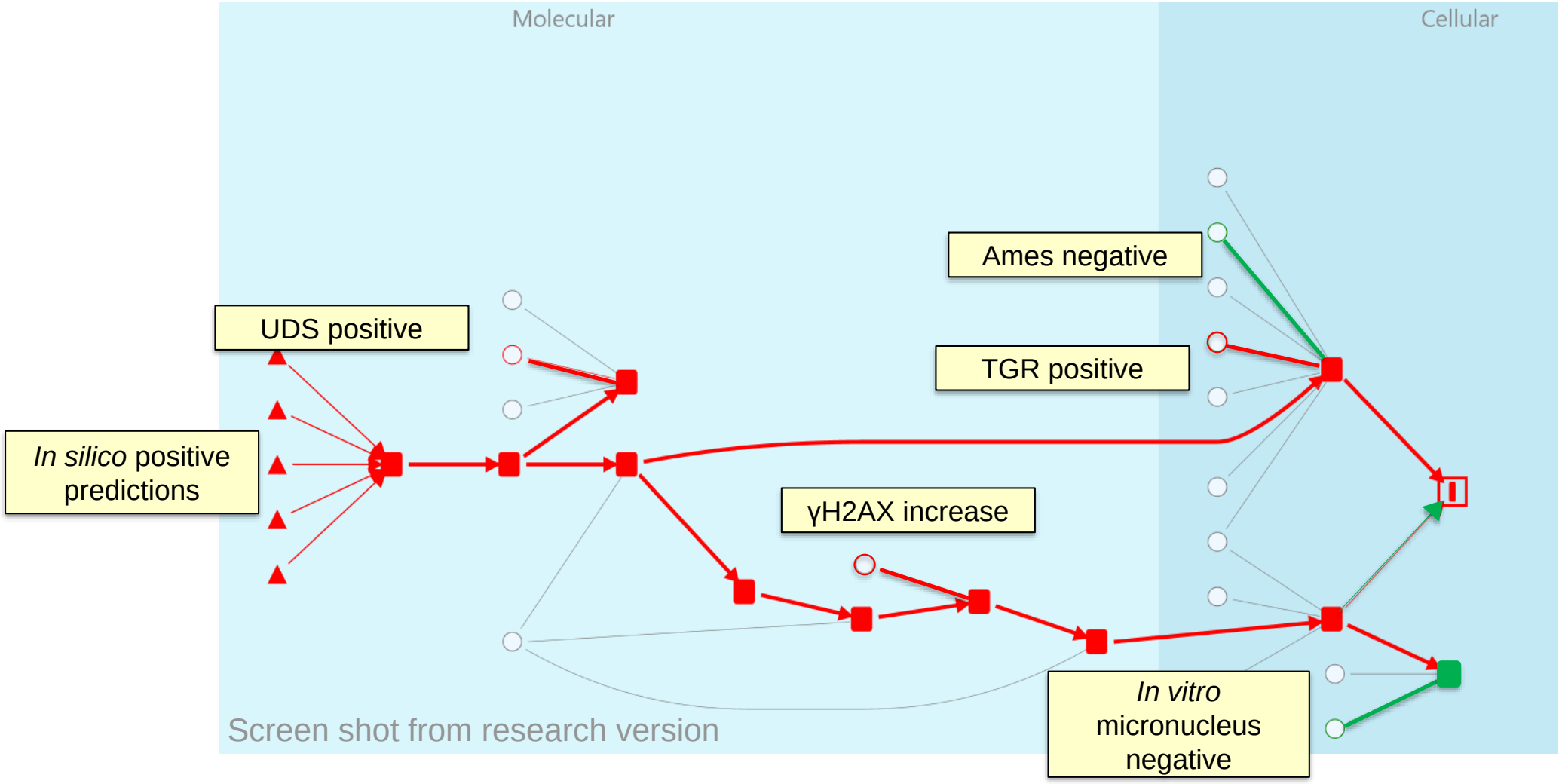
Discovery
"what is the consequence of hitting this target?"

Early Development
"which assay best predicts the (human) outcome?"

Late Development
"what adverse outcome might I see?"

Lhasa Limited

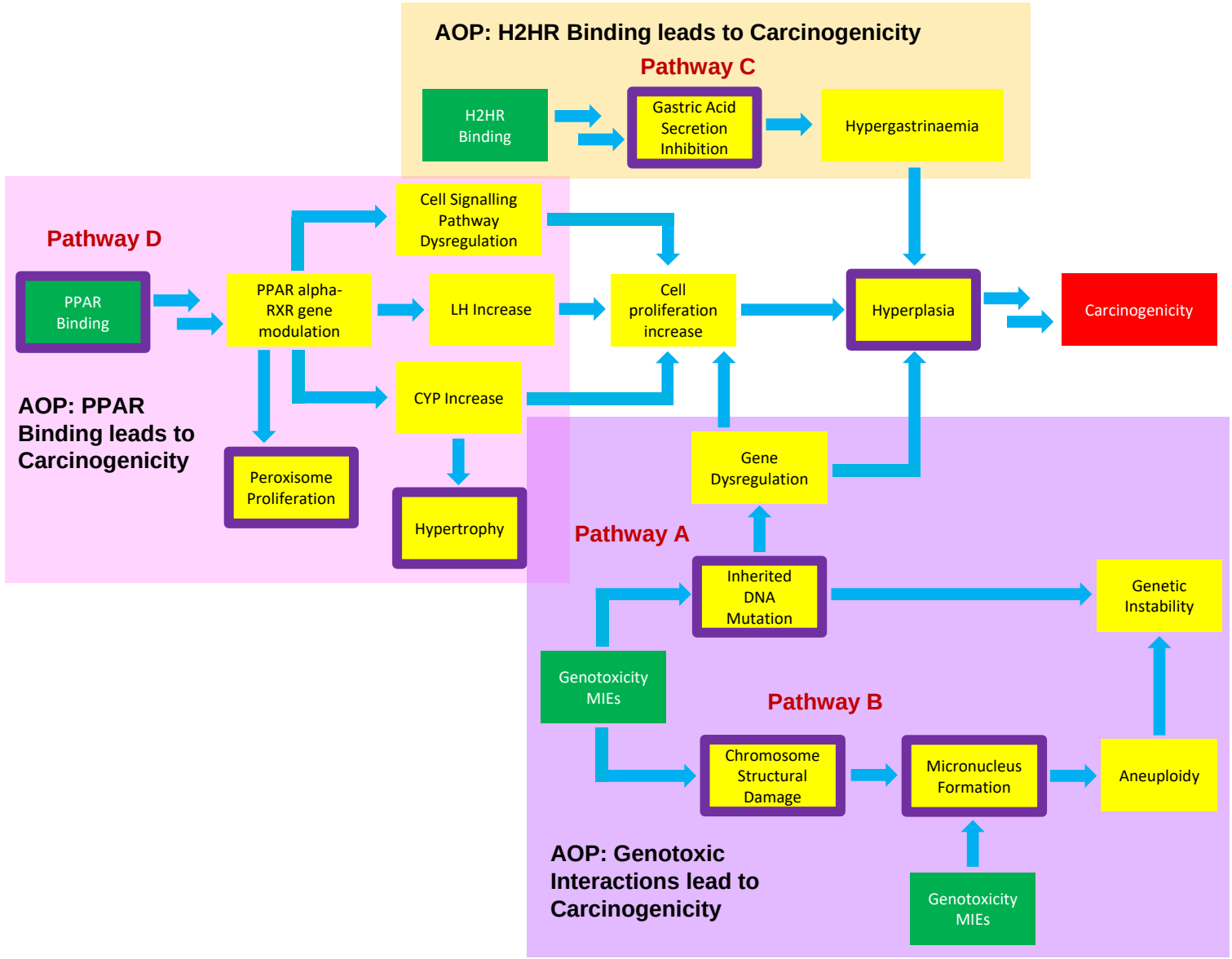
Reasoning Between Evidence

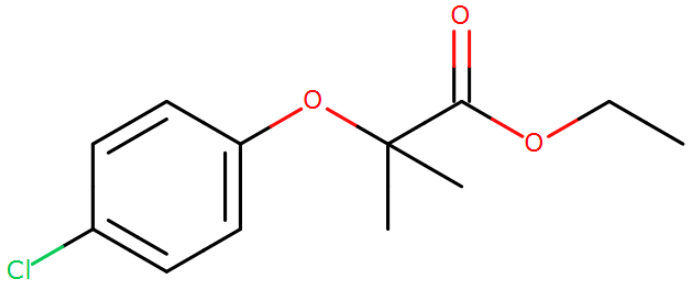


Reasoning is key in using AOPs to make decisions based on the evidence available

Lhasa Limited

Example: Clofibrate

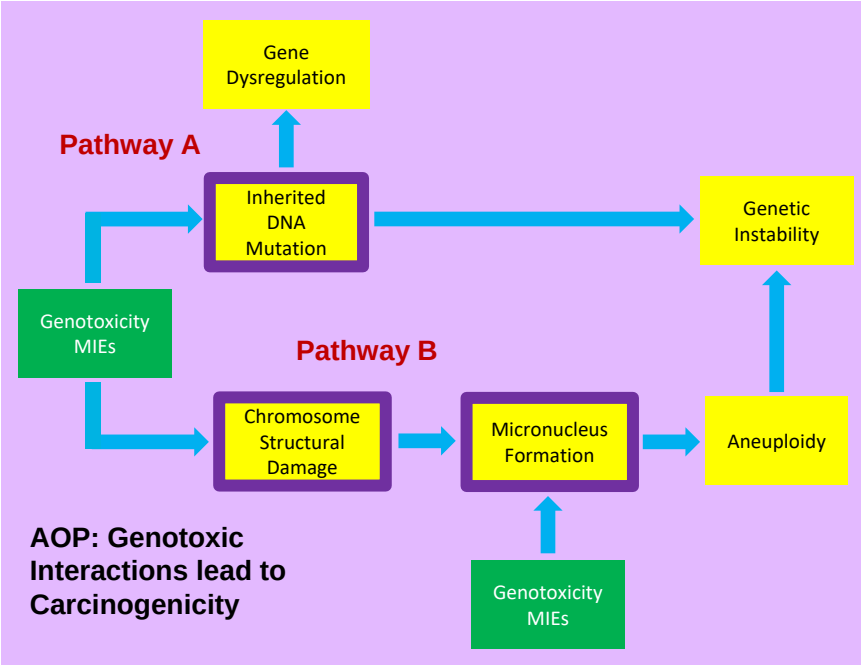




Clofibrate

Lhasa Limited

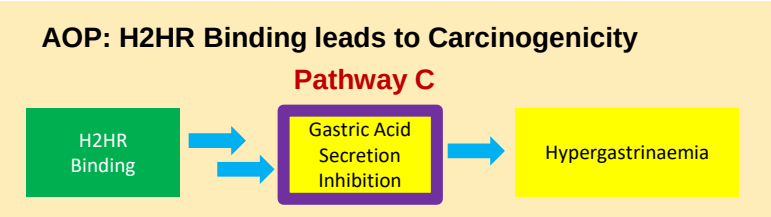
Example: Clofibrate



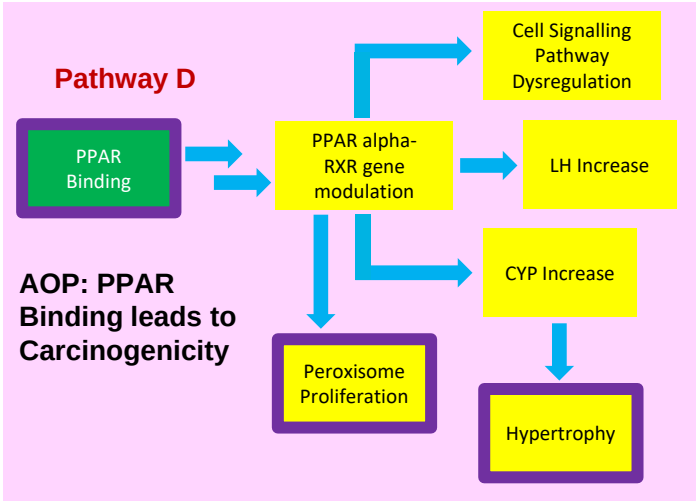
AOP	Human Relevant	Pathway	KE	Evidence	Result	Complexity of System	Acceptability
Genotoxicity*	Likely	A	Inherited DNA Mutation	Ames Test	Negative	*In vitro*	Accepted
				TGR Mutation Assay	Negative	***In vivo***	Accepted
		B	Chromosome Structural Damage	*In vitro* CA Test	Conflicted	*In vitro*	Accepted
				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

Lhasa Limited

Example: Clofibrate



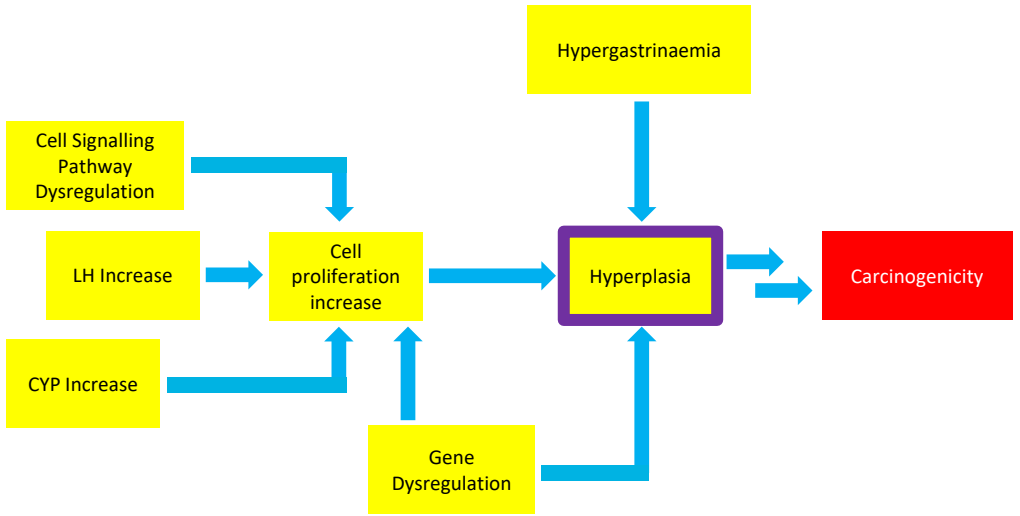
AOP	Human Relevant	Pathway	KE	Evidence	Result	Complexity of System	Acceptability
H2HR Binding	Unlikely	C	Gastric Acid Secretion Inhibition	Derek Nexus Carc Alert	PLAUSIBLE	*In silico*	Accepted



AOP	Human Relevant	Pathway	KE	Evidence	Result	Complexity of System	Acceptability
PPAR Binding	Unlikely	D	PPAR Binding, Peroxisome Proliferation	Derek Nexus Carc Alert	PLAUSIBLE	*In silico*	Accepted

Lhasa Limited

Example: Clofibrate



AOP	Human Relevant	Pathway	KE	Evidence	Result	Complexity of System	Acceptability
All	Unlikely	-	Hypertrophy, Hyperplasia, Peroxisome Proliferation	13-week subchronic study	Increase in organ weight	***In vivo***	Accepted

Lhasa Limited

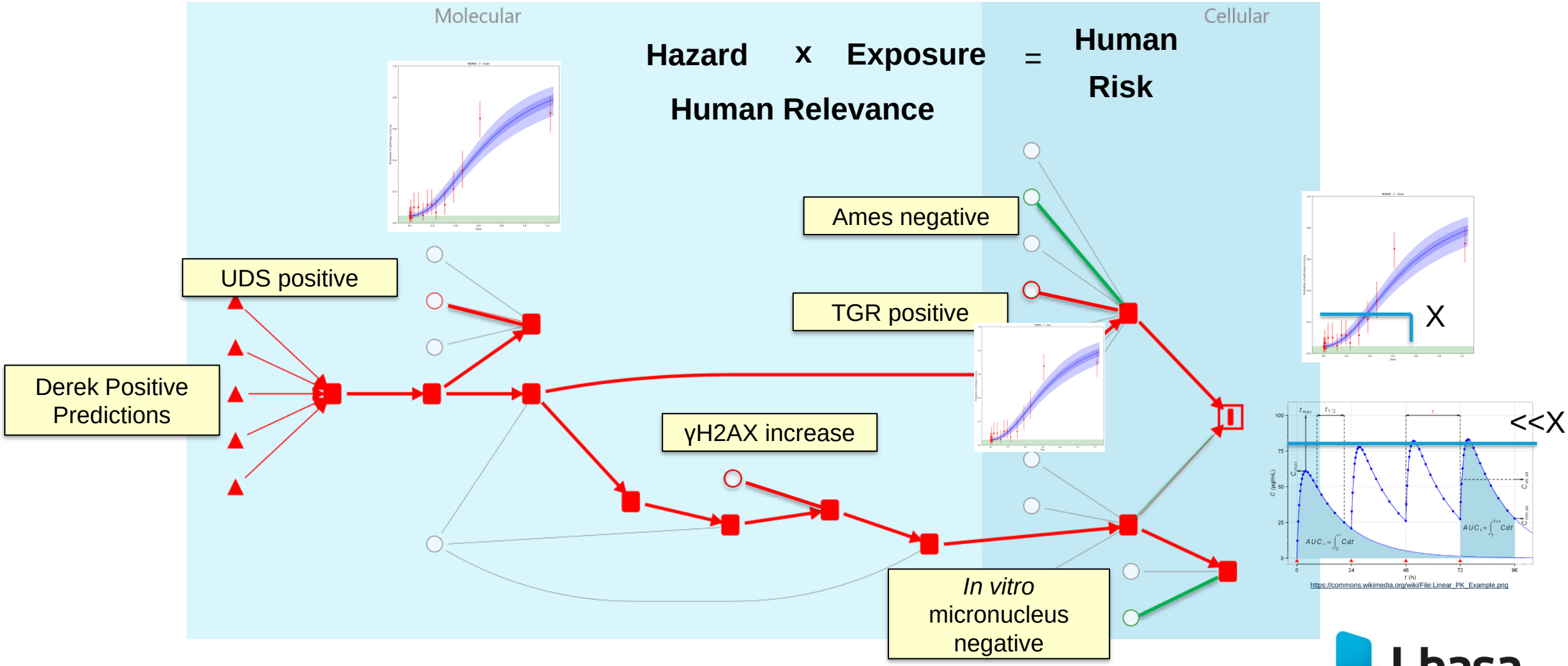
Example: Clofibrate

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

Positive (unlikely human relevant)

Lhasa Limited

Future - Moving From Hazard To Risk



Lhasa Limited

Summary

- An AOP network for carcinogenicity has been built using a literature-based approach of knowledge extracted from Derek Nexus and public literature.
- Lhasa has developed an AOP framework which can be used to:

  -organise and give context to data

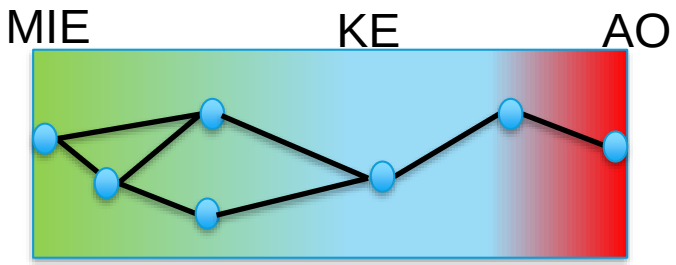
  -visualise the data and knowledge relationships

  …with the aim of aiding decision-making in risk assessment.
- AOPs can be used as a framework for exploring hazard and risk assessment in a broad range of industries

  - from early (discovery) phases in development through to safety assessment

  - evidence organised in such a way help make decisions in a transparent and robust manner

Kaptis



Lhasa Limited

The Lhasa Limited Team

Alex Cayley
Robert Foster
Reza Zarei
Emma Hill
Grace Kocks
Steven Kane
Alun Myden
Dan Newman
Jonathan Vessey

Lhasa Limited

Thank you!

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