Linking Evidence With AOPs to Produce Integrated Approaches to Testing and Assessment (IATA)

The Theory and The Practice

Alex Cayley

Principal Scientist

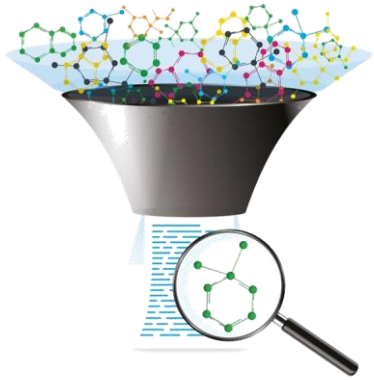
alex.cayley@lhasalimited.org

Outline

- New paradigms in chemical risk assessment
- Where *in silico* approaches fit in with these new methods
- The theory
  - AOPs
  - Evidence organisation
- The practice
- Challenges
  - Associating data

Lhasa Limited

Historical Problems With Safety Assessment



Time

Cost

Ethical

Pesticides
Biocides
Industrial Chemicals
Pharmaceuticals
Veterinary Medicines

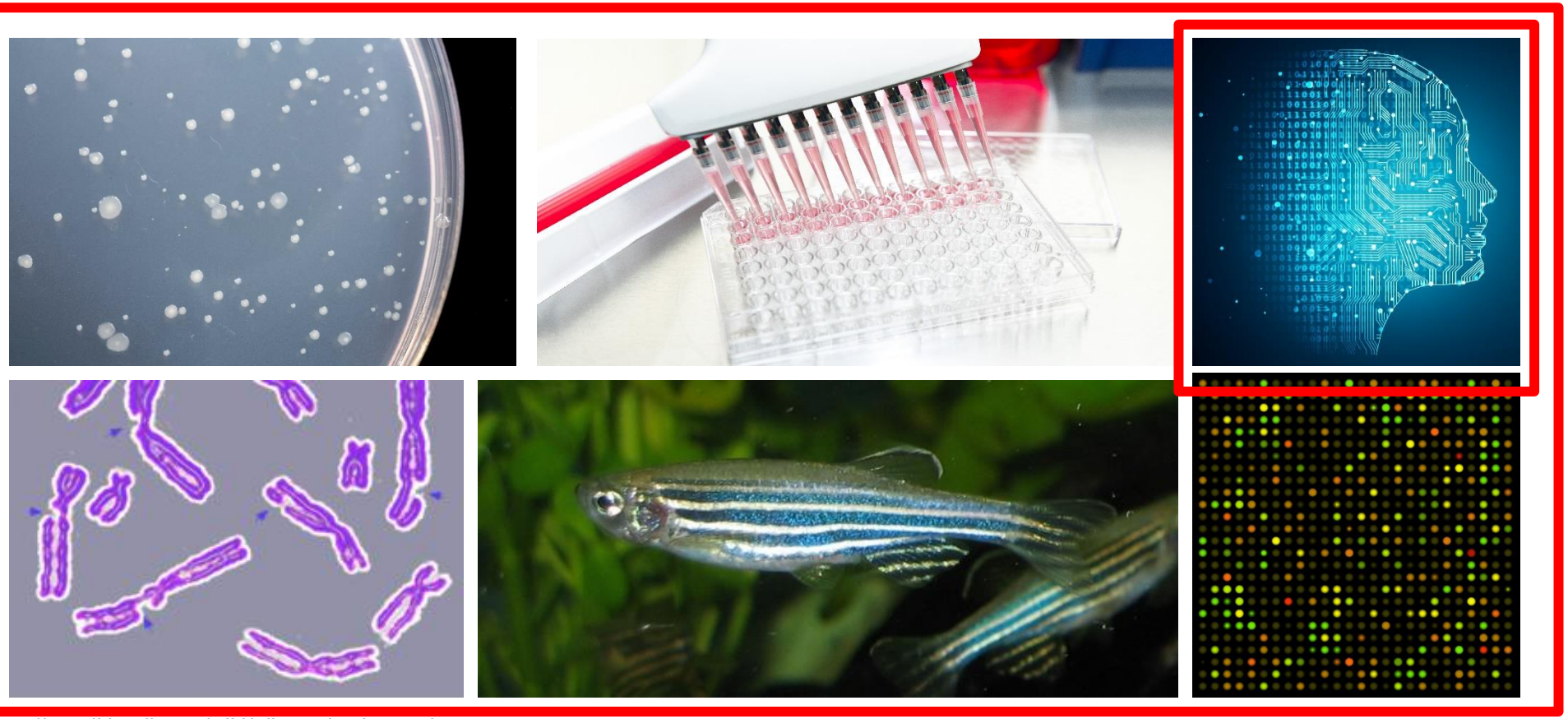
?

Relevance

Lhasa Limited

New Paradigm

***In vitro*, high-throughput and *in silico* models**

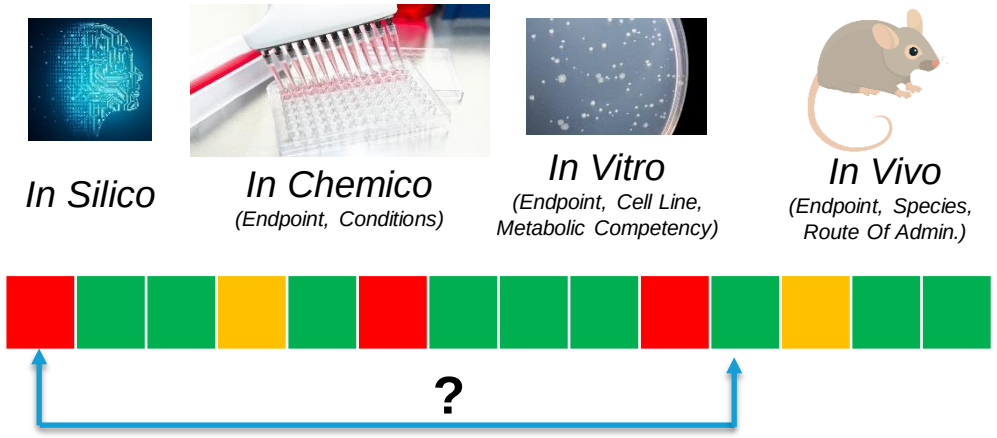


https://en.wikipedia.org/wiki/File:Brokechromo.jpg

Lhasa Limited

Reasoning Between Evidence

- 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?



Lhasa Limited

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

The Theory

Integration Into New Guidance/Regulatory Paradigms

Alternative methods are emerging

and cellular responses to substances. For some endpoints, progress has been made in developing *in vitro* test methods; OECD Test Guidelines using *in vitro* techniques are available for skin/eye corrosion and irritation, skin sensitisation, genotoxicity and endocrine disruption. In recent years, these alternative test methods have influenced regulatory decision-making, especially when coupled with *in silico* approaches and grouping of substances into chemical categories. Thus, a shift is already occurring from a scheme basing toxicity assessment largely on *in vivo* test results to one incorporating results from alternative approaches (e.g. *in silico, in chemico, in vitro,* including HT/HC test methods).

At present, many testing approaches, irrespective of the particular methodology employed, do not result in a mechanistic understanding of the induced toxicity. This is particularly the case with non-animal testing approaches and understanding the relationship between what is tested and the apical toxicity endpoint being predicted. This is one of the reasons why results from novel approaches are not yet widely and consistently used for regulatory decision-making. Therefore, an objective and systematic framework is needed to characterise the individual biological and toxicological relevance of novel methods in predicting an adverse effect. The same framework could also inform their potential use in combination with other tools and methods to benefit from an integrated approach.

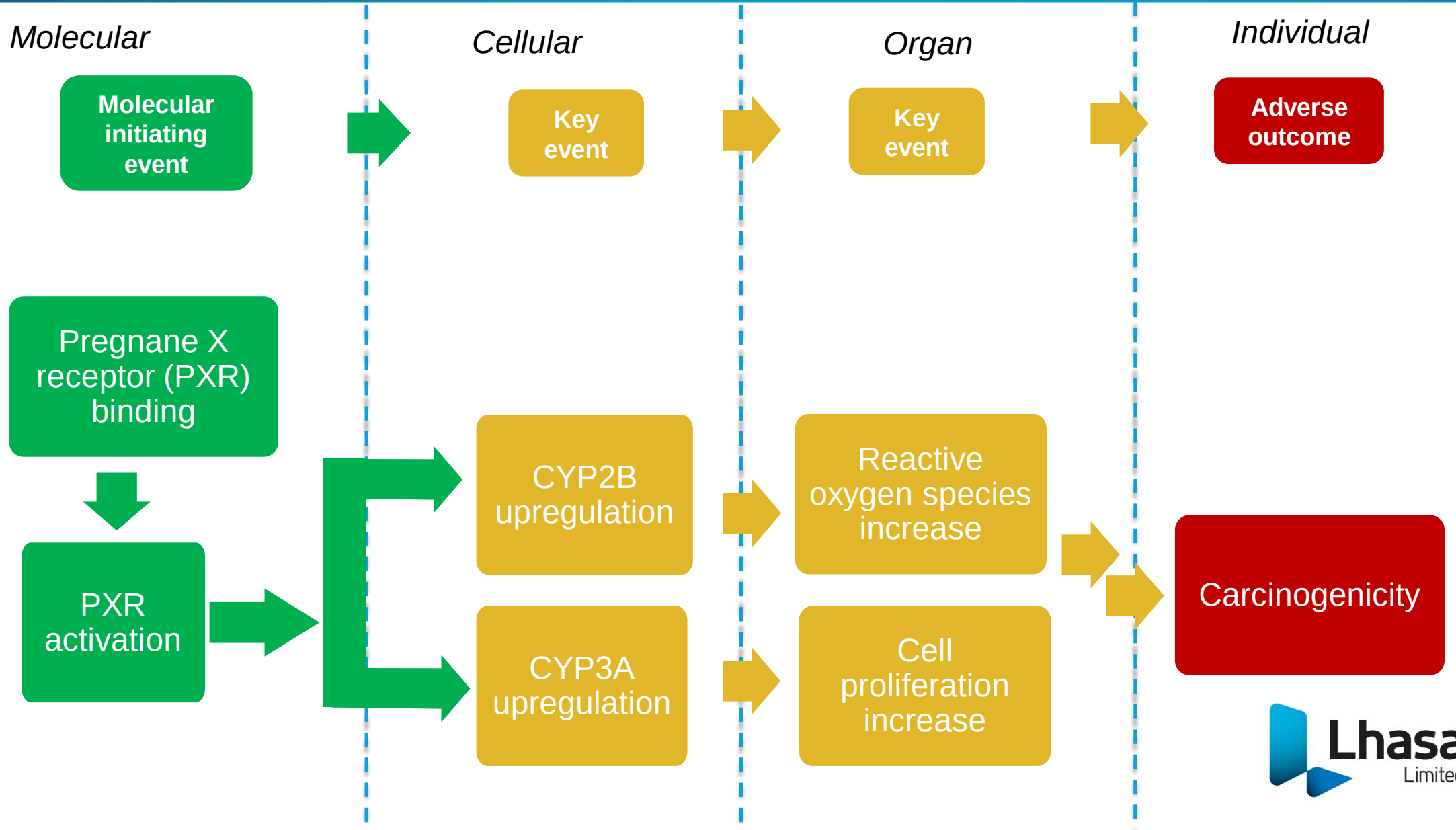
We need to know how to use these

AOPs present a good framework to arrange this knowledge

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

Lhasa Limited

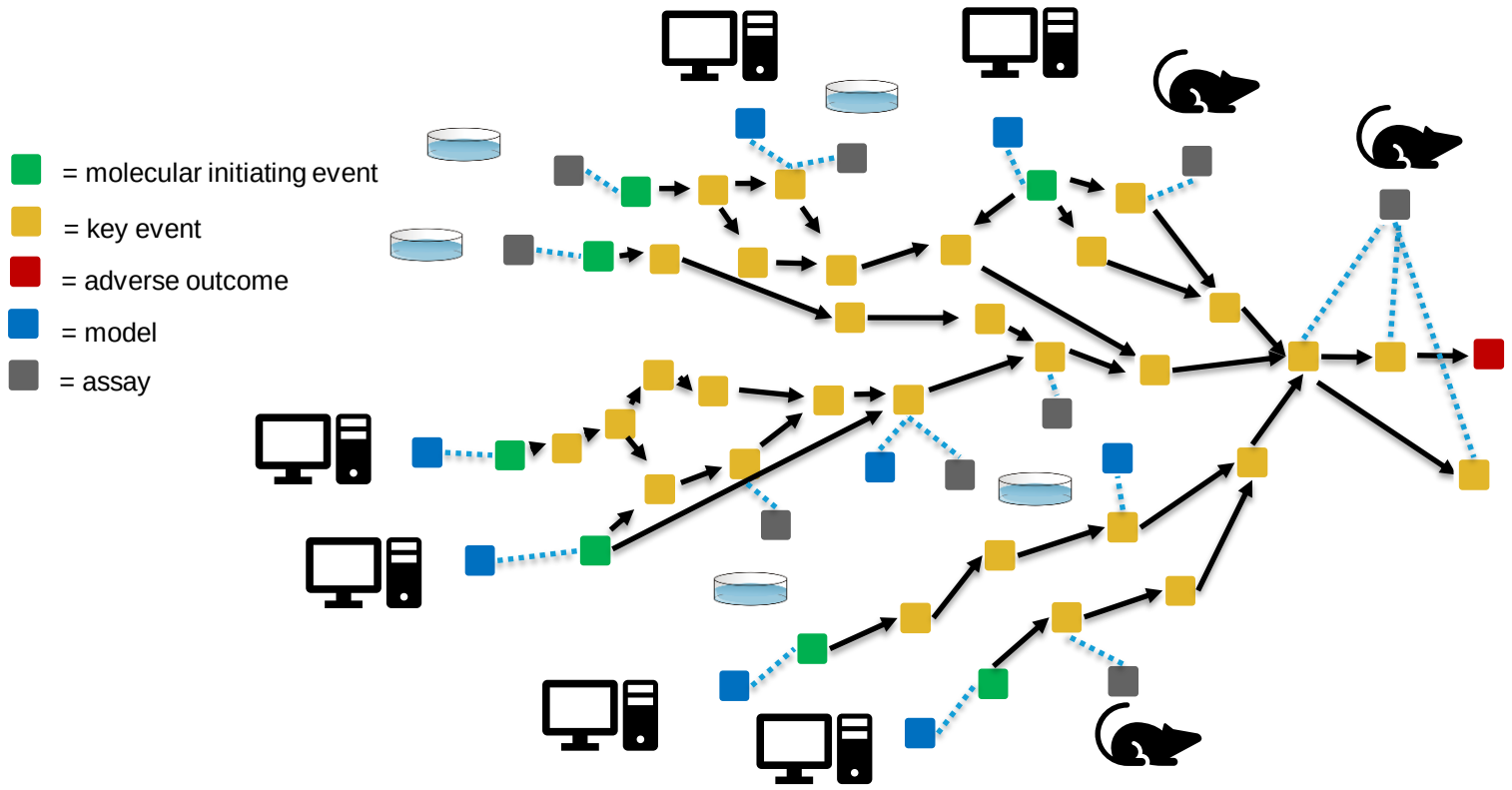
Adverse Outcome Pathways



Lhasa Limited

Adding Evidence To AOP Networks

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



Lhasa Limited

Identifying Knowledge Gaps - New Approaches

Hazard x Exposure = Human Risk

Human Relevance

= molecular initiating event

= key event

= adverse outcome

= *in vitro* assay

= *in vivo* assay

= *in silico* model

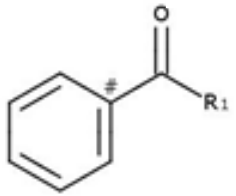
Metabolism
PK

Lhasa Limited

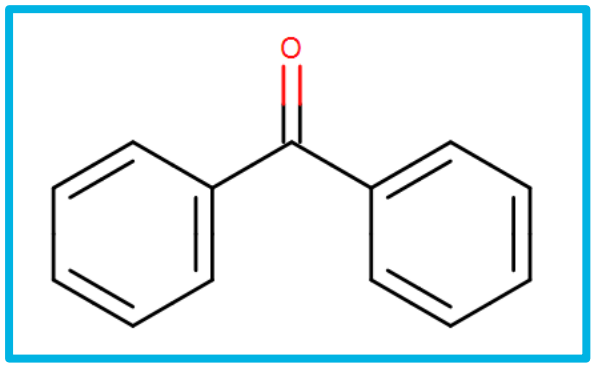
The Practice

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 anywhere



- **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

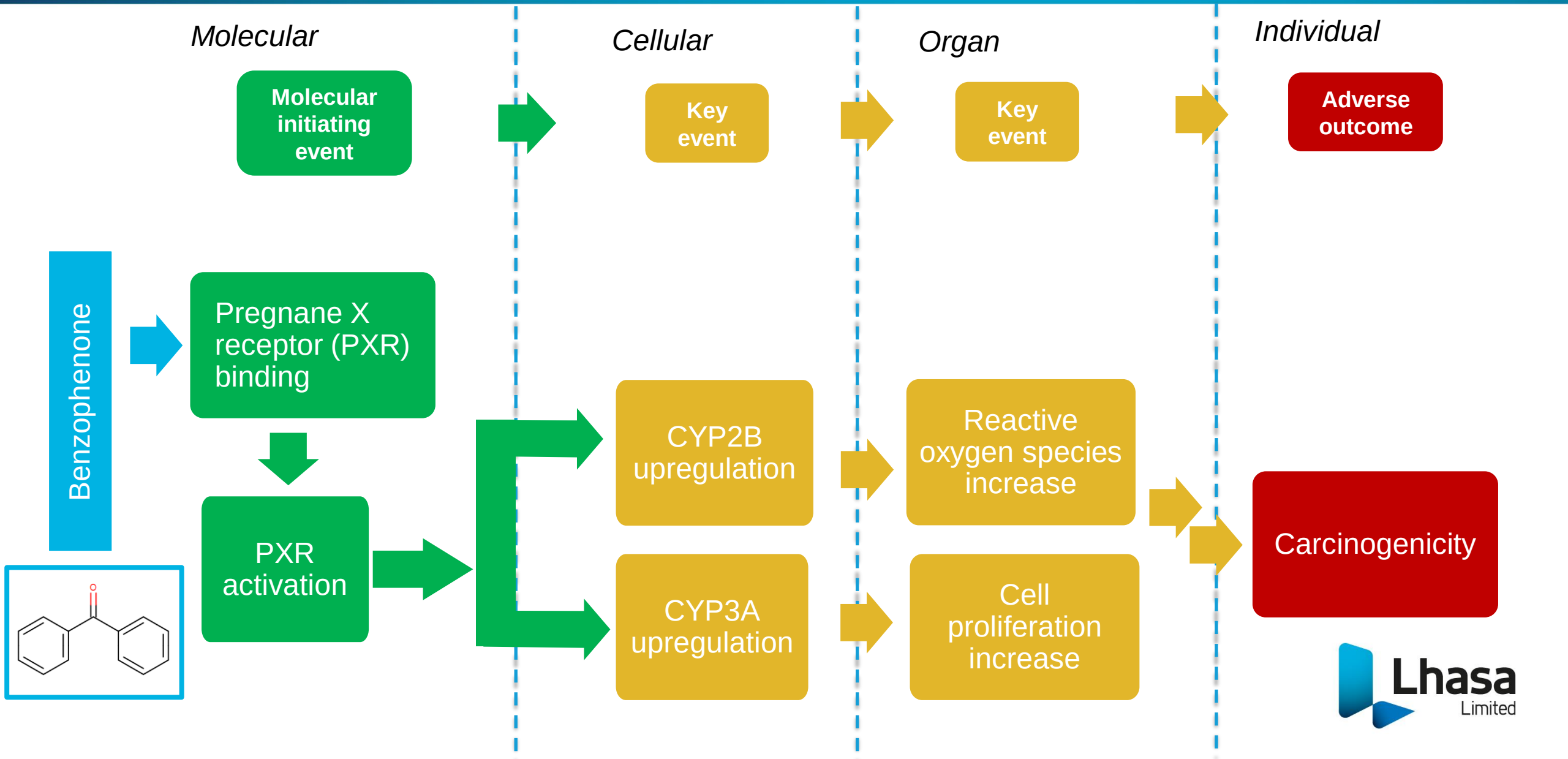
Adverse outcome

Molecular initiating event

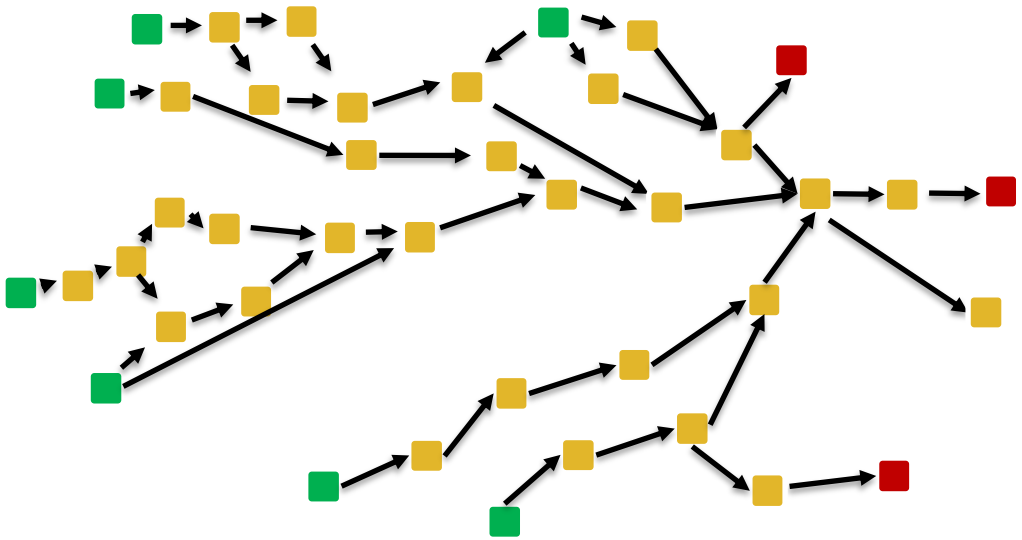
Key event

Lhasa Limited

Adverse Outcome Pathways



Building AOP Networks



Carcinogenicity

No. MIEs	37
No. AOPs	37
No. Pathways	>400
No. Non-genotoxic AOPs	15
No. Genotoxic AOPs	22

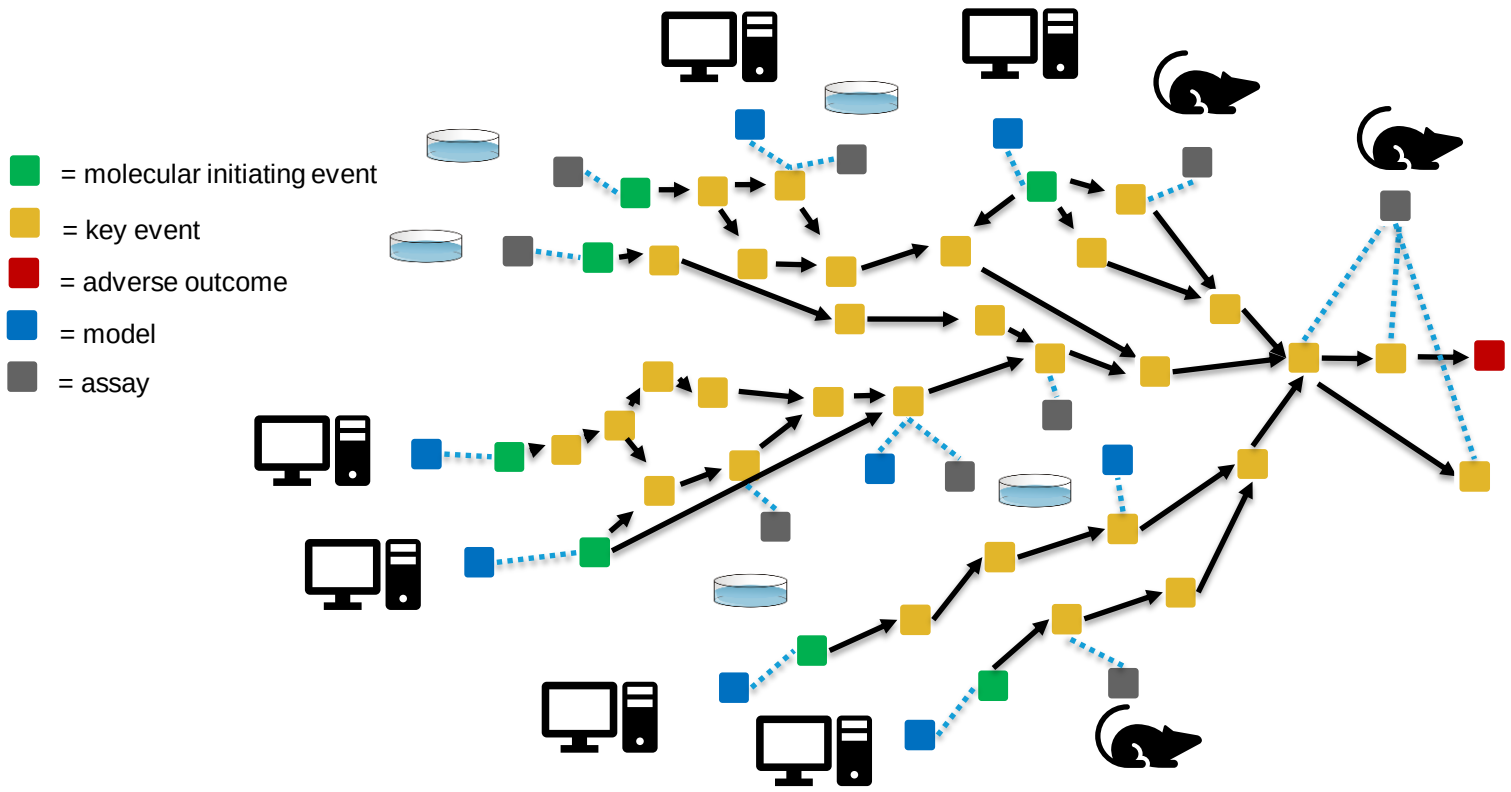
DART

AOP networks	MIEs (excluding isoforms)	Endpoints
RAR	5	Teratogenicity
Thyroid receptor	8	Embryo-foetal lethality, Neurodevelopmental toxicity
24 more developed	40	Teratogenicity, Fertility, Embryo-foetal lethality, Neurodevelopmental toxicity

Lhasa Limited

Adding Evidence To AOP Networks

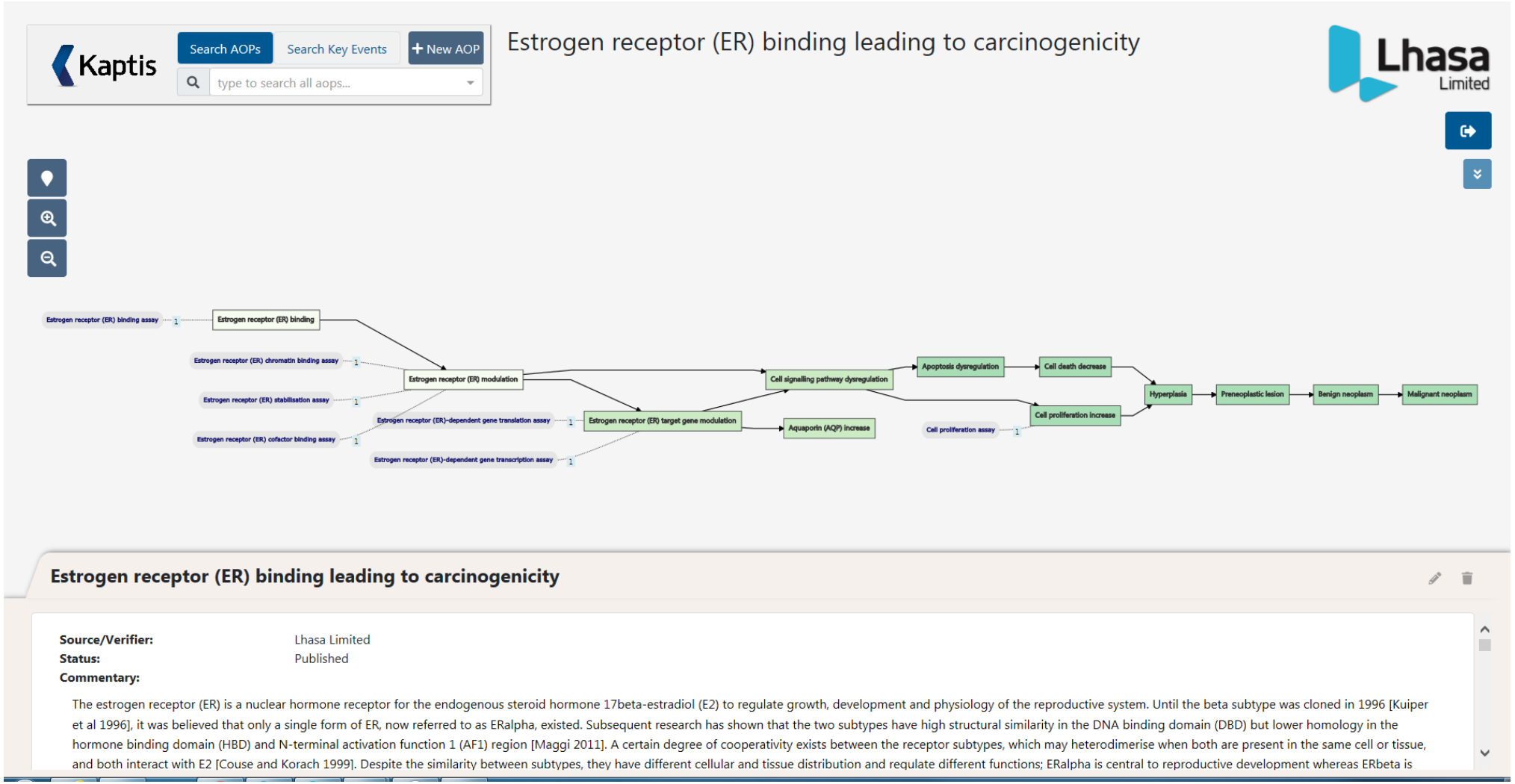
- Associate models with key events
- Associate assays and their measurements with key events
- Add data to the assays



No. Assays	68
No. Measurements	65
In vitro assay	51
In vivo assay	17

Lhasa Limited

Capturing This Knowledge In Kaptis

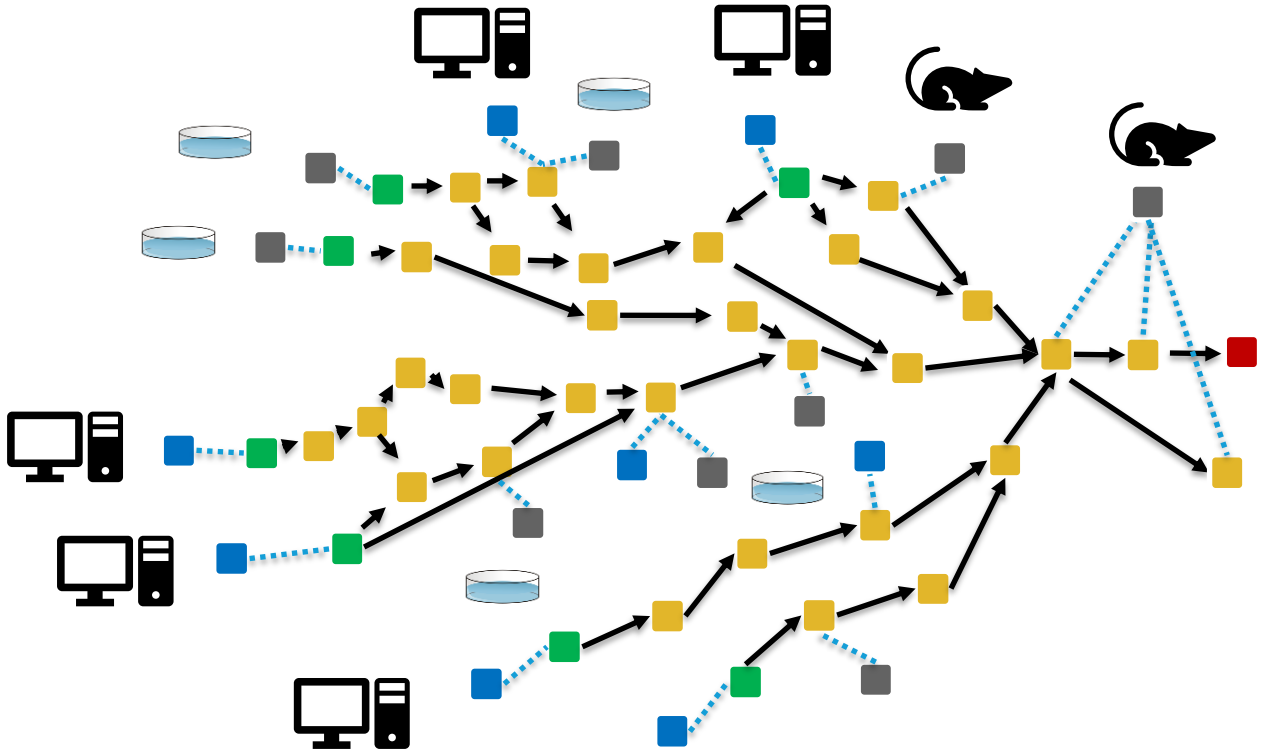


Lhasa Limited

Challenges

Adding Data To AOPs

- Linking data to KEs
  - Standardisation of terms
  - Relationships between terms

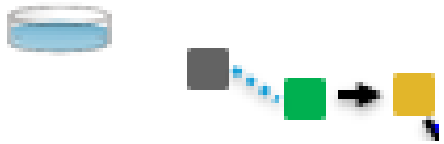


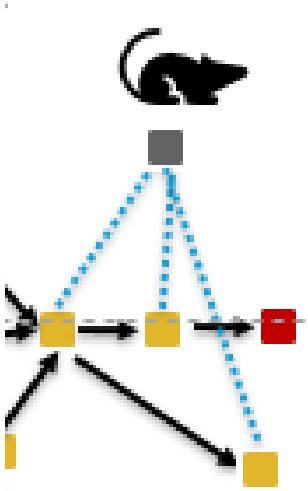
= molecular initiating event
= key event
= adverse outcome
= model
= assay

Lhasa Limited

Standardisation Of Terms

- *In vitro* assays often measure one variable
- *In vivo* assays measure multiple variables which can link to different KEs
- There is limited standardisation of these terms in historical data which sometimes makes linking difficult





cdisc **SEND**

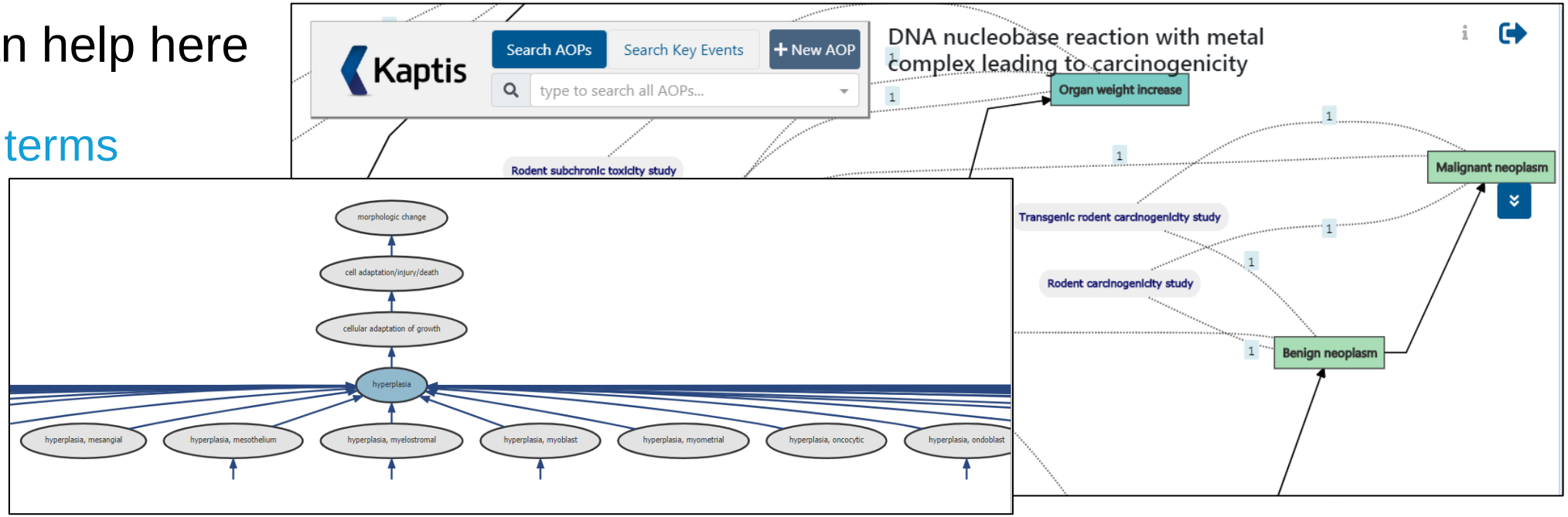
Code	Codelist Code	Codelist Extensible (Yes/No)	Codelist Name	CDISC Submission Value	CDISC Synonym(s)	CDISC Definition
C26791	C120531		Non-Neoplastic Finding Type	HEMORRHAGE		The presence of extravascular erythrocytes.
C161539	C120531		Non-Neoplastic Finding Type	HEPATOCYTES, SUBINTIMAL		Presence of normal hepatocytes in hepatic veins and within t vessel. (INHAND)
C120889	C120531		Non-Neoplastic Finding Type	HEPATODIAPHRAGMATIC NODULE		A congenital abnormality of the liver, characterized by grossl nodule(s) usually located on the median lobe. (INHAND)
C3111	C120531		Non-Neoplastic Finding Type	HYDROCEPHALUS		An enlargement of the ventricles relative to brain tissue.
C123638	C120531		Non-Neoplastic Finding Type	HYDROMYELIA		Dilation of the central canal of the spinal cord.
C35541	C120531		Non-Neoplastic Finding Type	HYPERKERATOSIS	Increased Keratinization	Thickening of the outermost layer of stratified squamous epit
C3113	C120531		Non-Neoplastic Finding Type	HYPERPLASIA		Increase in the number of resident cells, generally with an in figures present, per unit area in an organ or tissue.
C120890	C120531		Non-Neoplastic Finding Type	HYPERPLASIA/METAPLASIA	Metaplasia/Hyperplasia	A finding that generally has features of hyperplasia and meta
C3124	C120531		Non-Neoplastic Finding Type	HYPERTROPHY		Cell size enlargement due to the increase in the amount of c constituent organelles. The cells are larger but otherwise the unchanged.
C120891	C120531		Non-Neoplastic Finding Type	HYPERTROPHY/HYPERPLASIA	Hyperplasia/Hypertrophy	A finding that generally has features of hypertrophy and hype
C120892	C120531		Non-Neoplastic Finding Type	HYPERTROPHY/KARYOMEGALY	Karyomegaly/Hypertrophy	A finding that generally has features of hypertrophy and kary
C120893	C120531		Non-Neoplastic Finding Type	HYPOPLASIA		Incomplete or underdevelopment of a tissue or organ. (NCI)

Lhasa Limited

https://www.cancer.gov/research/resources/terminology/cdisc#standard-for-the-exchange-of-nonclinical-data-send

Relationships Between Terms

- Findings are captured in toxicity studies at different levels of detail depending on the source
- The level of detail in the term captured in the study may differ from that used to link to the key event
- There may be no direct match but a link via a related term may be possible
- Ontologies can help here
  - Synonyms
  - Parent-child terms



Conclusions

- New and emerging methods (NAMs) present a bright future as alternatives to animal testing
- Methods of combining evidence from these NAMs to reach a meaningful conclusion must be developed
- AOPs present an attractive framework for organising this knowledge
- *In silico* methods can take advantage of evidence organised in this way
- There are practical challenges that need to be overcome to make this vision a reality but these can be tackled
- New solutions are emerging all the time!

Lhasa Limited

Acknowledgements

Science

Susanne Stalford
Steve Kane
Rob Foster
Adrian Fowkes
Emma Hill
Alun Myden
Reza Zarei
Grace Kocks
Jonathan Vessey
Dan Newman

Global Alliances

Crina Heghes

External Collaboration

Industry Members
Regulators

Software Development

Helen Brown
David Blaney
Alan Haywood
Tom Roberts
Leah Shields
William Bradwell
Charlie Dyson
Christos Wild
Ben George
Ian Addison
Andrew Markwell
Mark Nolan
James Cook
Pip Everard

Lhasa Limited

Questions?

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.