In Silico Prediction Of Genotoxicity: Current Applications And Future Perspectives

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Overview

- Who Lhasa are and what we do
- General approaches to *in silico* genotoxicity prediction
- How *in silico* predictions can be used in the pharmaceutical development process
- How *in silico* predictions for toxicity (in Derek Nexus) currently account for metabolism
- How *in silico* predictions might develop and be used in the future

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Introduction to Lhasa Limited

- Established in 1983
- HQ located in Leeds, United Kingdom
- Not-for-profit & Educational Charity
- Facilitate collaborative data sharing projects in the chemistry-related industries
- Controlled by our members
- Creators of knowledge base, statistical and database systems

Derek nexus

Zeneth

Meteor nexus

Sarah nexus

Mirabilis

Vitic

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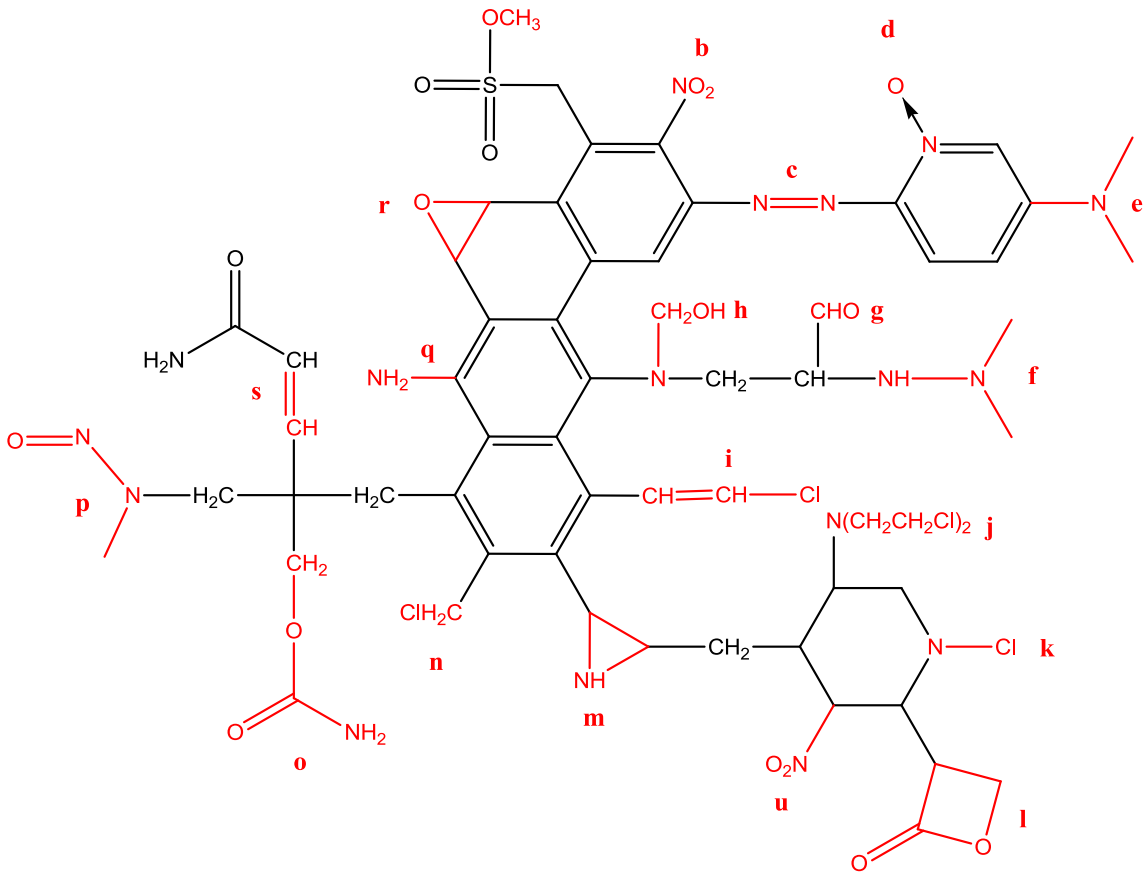
Origins of *in silico* genotoxicity prediction

Mutat Res. 1988 Jan;204(1):17-115.

Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP.

Ashby J[1], Tennant RW.



$C(X)_4$ t
X= H, F, Cl, Br, I (*in any combination*)

DEREK
Oncologic
Toxtree (Benigni-Bossa rulebase)

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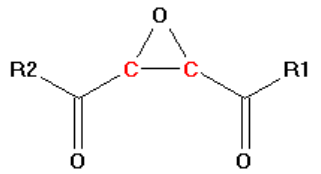
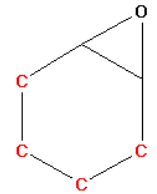
Development of *in silico* genotoxicity prediction

2004	2005	2018
4	7	8





132 alerts for Mutagenicity in Derek Nexus 2018.1

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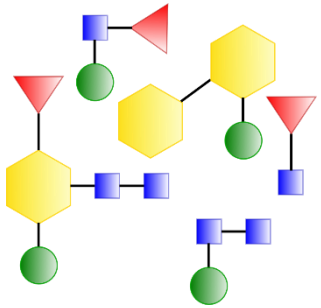
Ridings et al., Toxicology; 1996; 106; 267-279
https://www.ncbi.nlm.nih.gov/pubmed/8571398

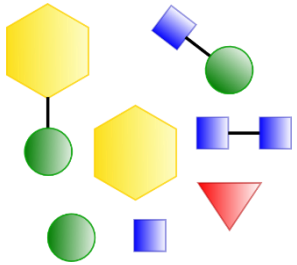
Origins of *in silico* genotoxicity prediction



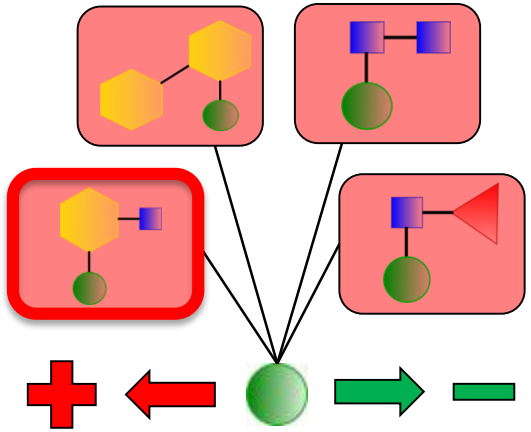
Experimental Data

Fragmentation

Fragment Dictionary



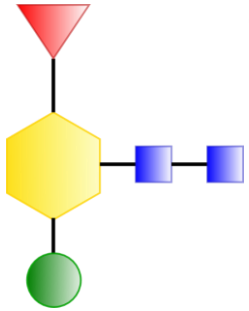
Fragment Association



Predict

Fragment

Match Fragments



Query Compound

MCase
TOPKAT

Sarah nexus

Leadscope
Lazar

Hanser et al.; J. Cheminform.; 2014; 6; 21

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4048587/

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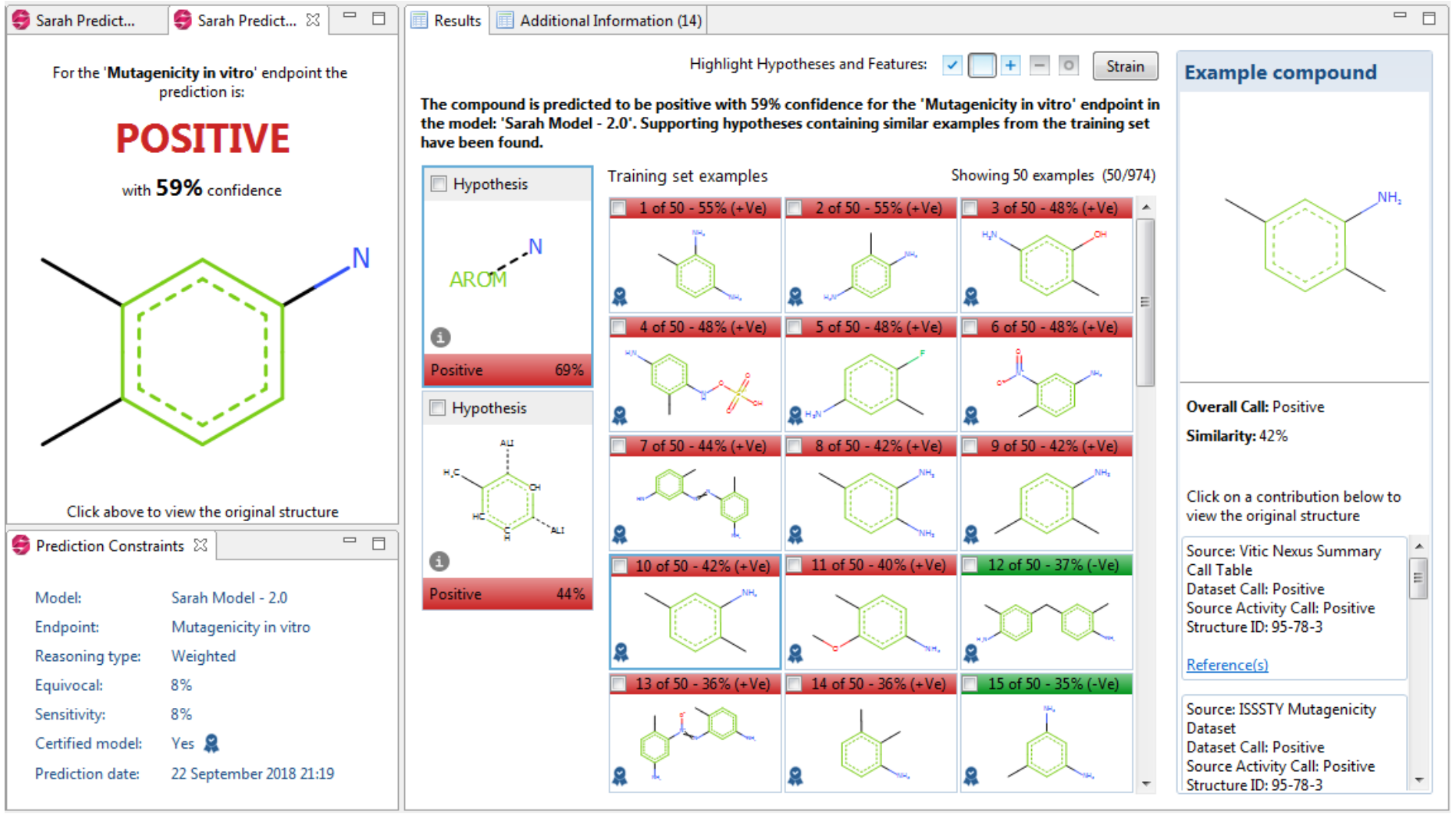
Development of *in silico* genotoxicity prediction



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Features Of Different Approaches

- Physicochemical properties can also be used as descriptors by both types of model (CLogP, HOMO, LUMO)

Expert Rule	Statistical Approach
Correlation usually causative	Correlation may not be causative
Slower To Implement	Quick To implement
Rules can be based on theory alone	Large data set required
Highly interpretable	May not be as interpretable
Able to deal with ‘noise’ in the data	More prone to errors in data
Risk of overfitting	Risk of overfitting

Barber *et al*.; Regul. Toxicol. Pharmacol.; 2017; 84; 124-130
https://www.ncbi.nlm.nih.gov/pubmed/28057482

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How Well Do They Perform?

- Against Ames mutagenicity data sets, pretty well but will depend on chemical space (better against published data than private)
- Expert review improves results
- For other genotoxicity endpoints more work is required

Performance Metric	Average Performance (Public)	Average Performance (Proprietary)
Balanced Accuracy	77%	66%
Sensitivity	74%	58%
Specificity	81%	73%
Coverage	95%	92%

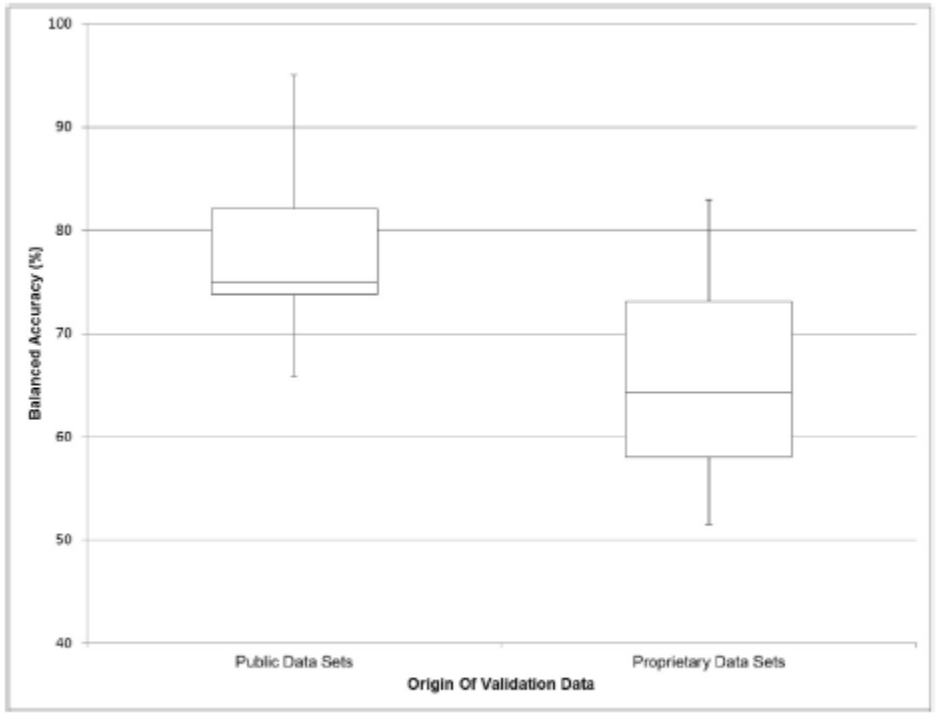


Fig. 1. A box and whisker plot comparing the balanced accuracy (sensitivity + specificity/2) of Ames mutagenicity predictions for publicly available versus proprietary data. The top and bottom edges of each box represent the third and first quartile balanced accuracy values for each set and the line in the middle of the box represents the median value. The whiskers illustrate the maximum and minimum values for each set.

Barber *et al.*; Reg. Tox. Pharm.; 2016; 76; 7-20
https://www.ncbi.nlm.nih.gov/pubmed/26708083

Dobo *et al.*; Reg. Tox. Pharm.; 2012; 62; 449-455
https://www.ncbi.nlm.nih.gov/pubmed/22321701

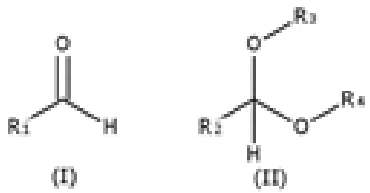
Considerations Of Metabolism

Why is metabolism important?

- Non-genotoxic species may have genotoxic metabolites
- Potentially genotoxic species may be deactivated by metabolism

How Do We deal With Metabolism Currently?

- Let the assay take care of it and model the assay results
- Encode the metabolism in the model
- Predict metabolism and then predict toxicity



R1, R2 = H, C (not multiply bound or attached to any heteroatoms)
R3, R4 = H, C

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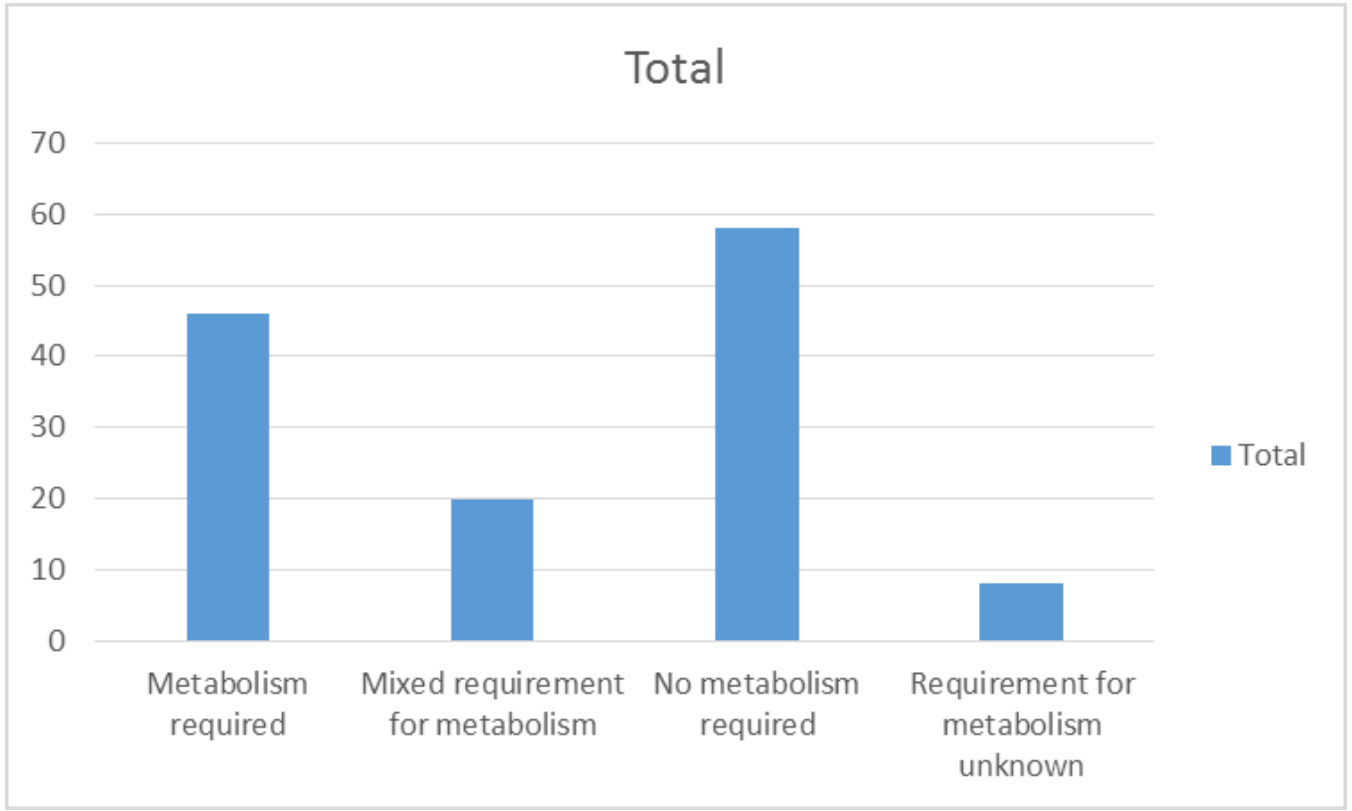
Considerations Of Metabolism

Type Of Alert	Number Of Alerts
All mutagenicity endpoint	132
Mutagenicity requiring metabolism	46
Direct mutagenicity	58
Mixed requirement	20
Unknown	8



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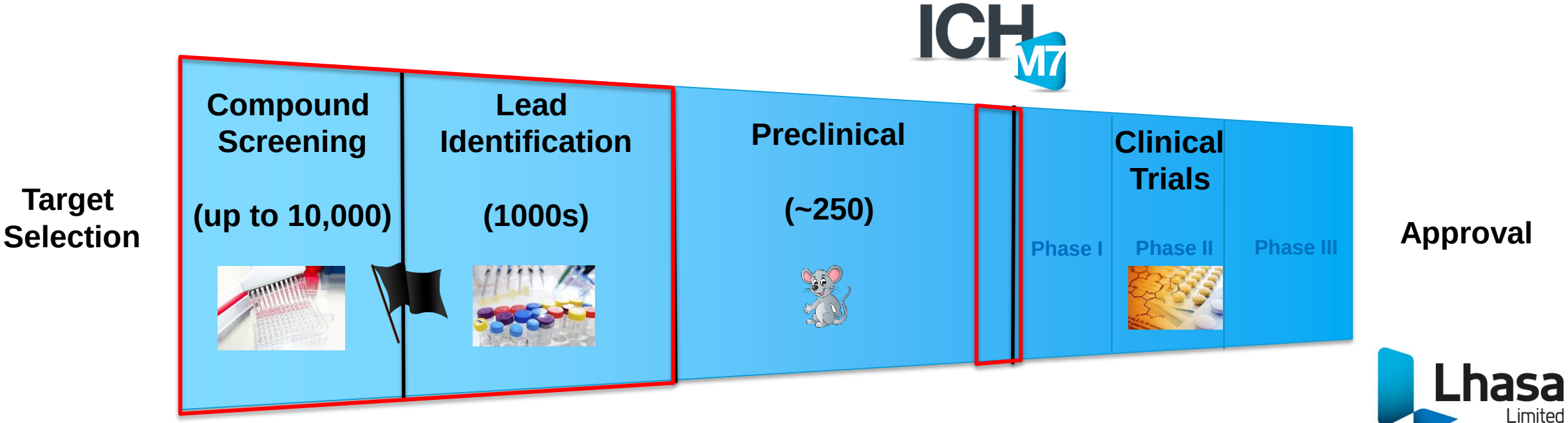
When Are *In Silico* Predictions Used?

- Where a chemical is not available for testing
- Where testing would be prohibitively expensive (large number of chemicals)
- Where time is of the essence (large number of chemicals)
- To prioritise future work
- Where guidelines indicate that *in silico* predictions can be used in place of other tests



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What Could Be Improved?

- Better coverage of mechanisms/endpoints leading to genotoxicity not covered by Ames test (mainly electrophilic reactivity)
- Better advice about what to do next following a prediction
- Better integration with other available evidence (*in vitro*, *in vivo* genotoxicity assays and bioassays)
- More transparent information about mechanism causing toxicity, leading to better models
- More information about metabolism activating or deactivating compound
- Relevance beyond the species/test being modelled
- Moving from hazard to risk prediction (less binary, more quantitative predictions)

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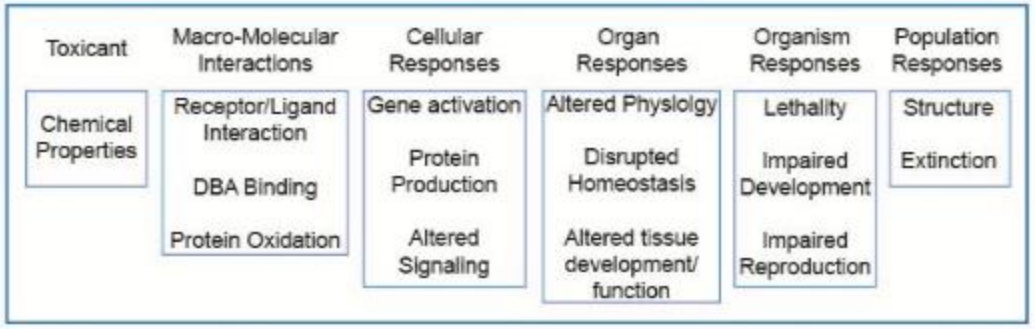
The Adverse Outcome Pathway Framework

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

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What Could Be Improved?

- Better coverage of mechanisms/endpoints leading to genotoxicity not covered by Ames test (mainly electrophilic reactivity)

Easier To Identify Holes And Use More Data To fill Them

- Better advice about what to do next following a prediction
- Better integration with other available evidence (*in vitro*, *in vivo* genotoxicity assays and bioassays)
- More transparent information about mechanism causing toxicity, leading to better models
- More information about metabolism activating or deactivating compound

Easier To Predict Metabolism In Correct Context

- Relevance beyond the species/test being modelled
- Moving from hazard to risk prediction (less binary, more quantitative predictions)

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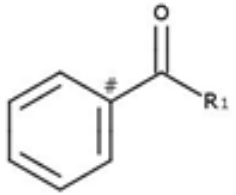
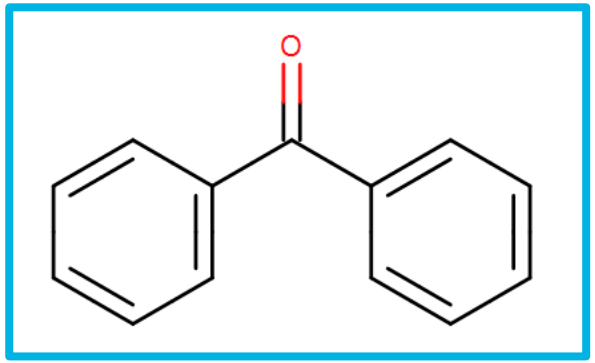
The Future - AOPs

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

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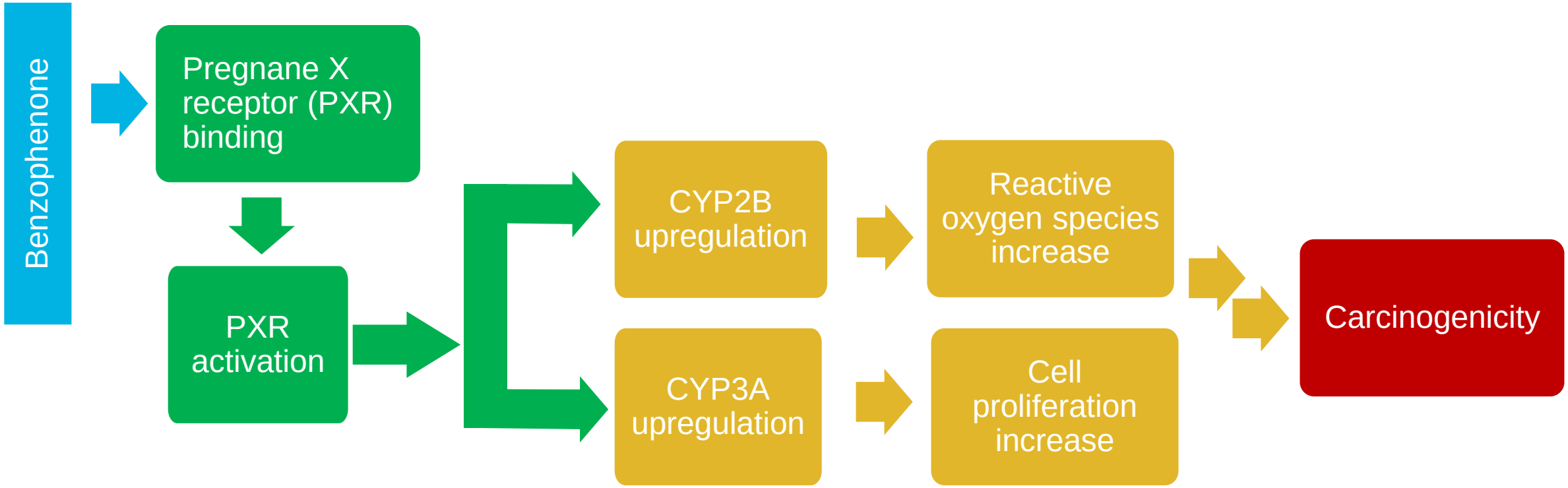
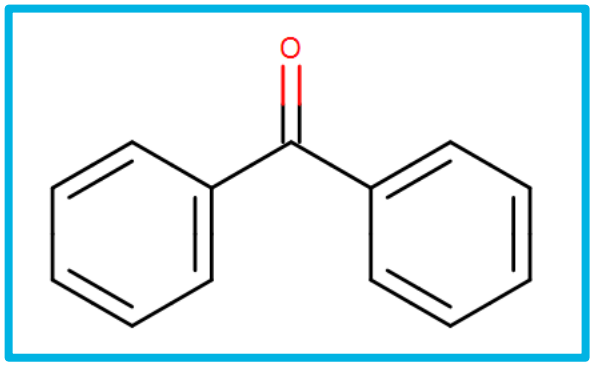
The Future - AOPs





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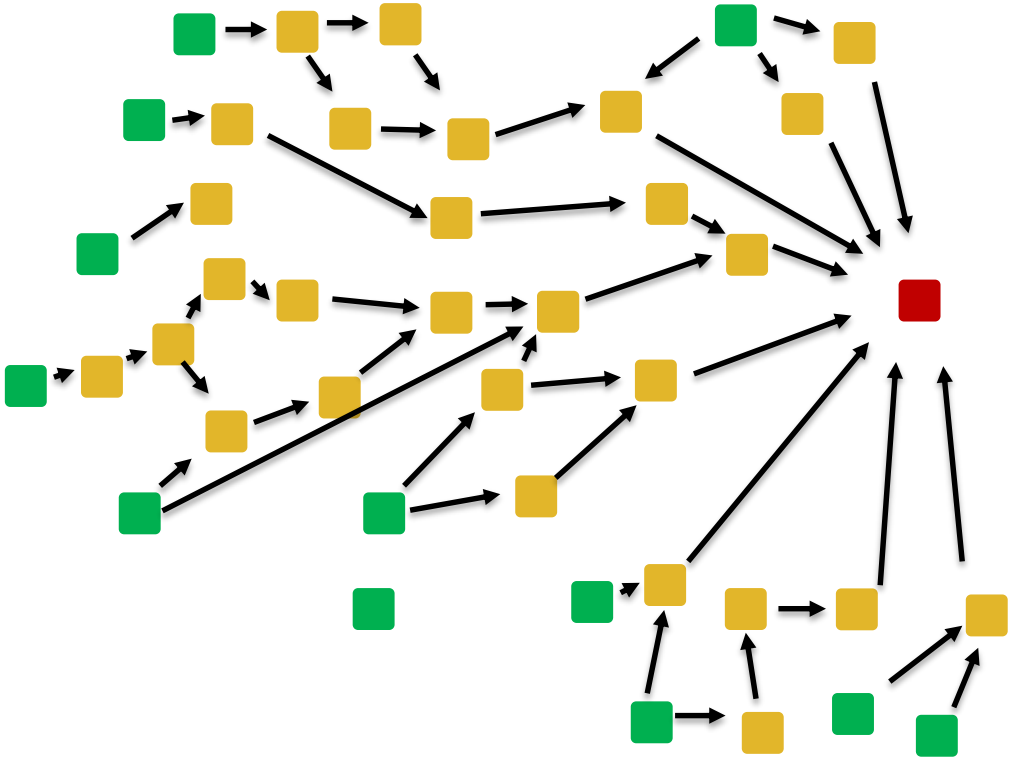
The Future - AOPs

I am only interested in pathways leading to direct damage to DNA



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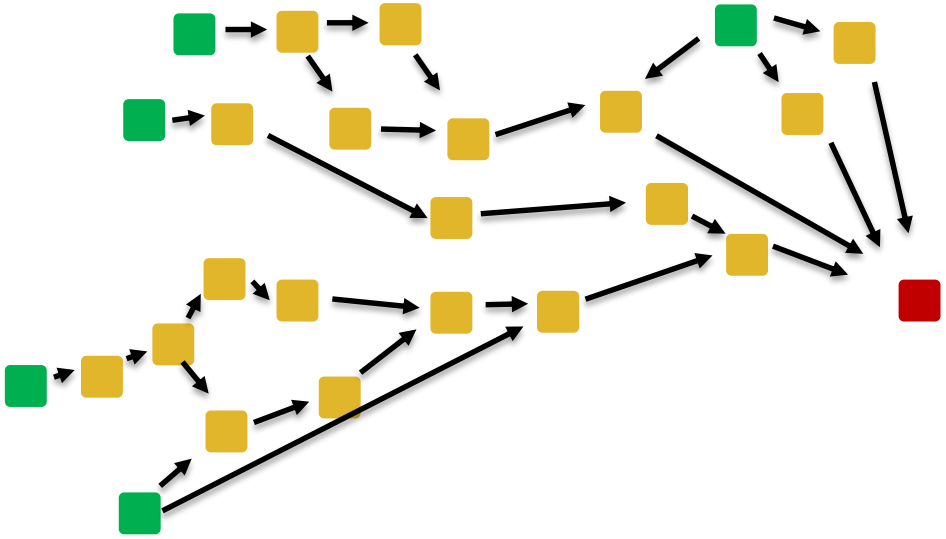

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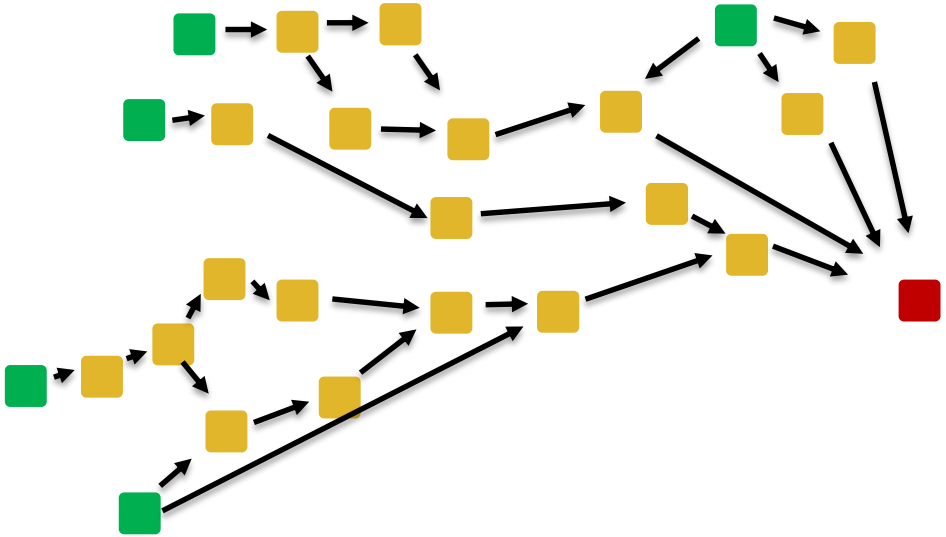

Integrate other information with network

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The Future - AOPs

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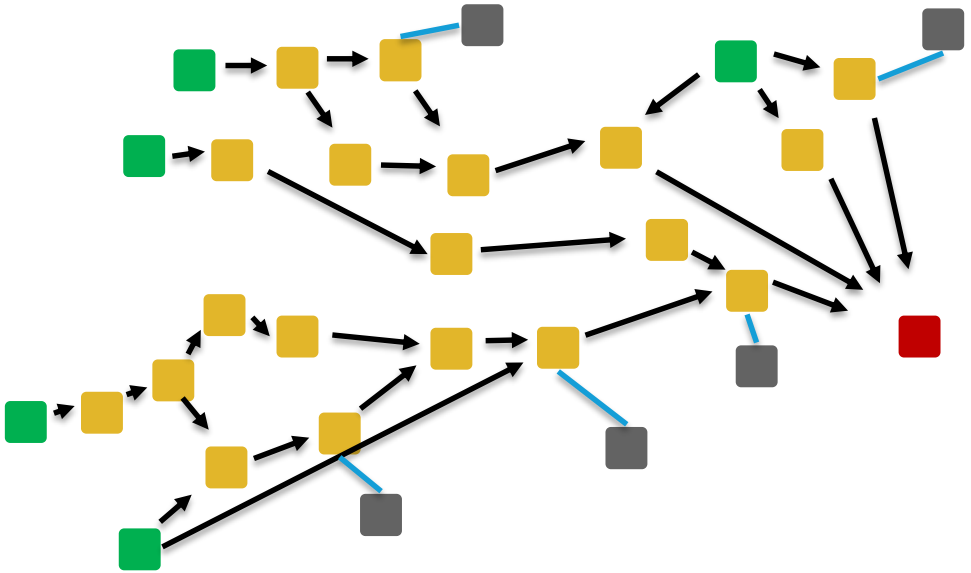

Integrate other information with network

In Vitro Assays

In Vivo Assays

Gene/Protein expression

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The Future - AOPs

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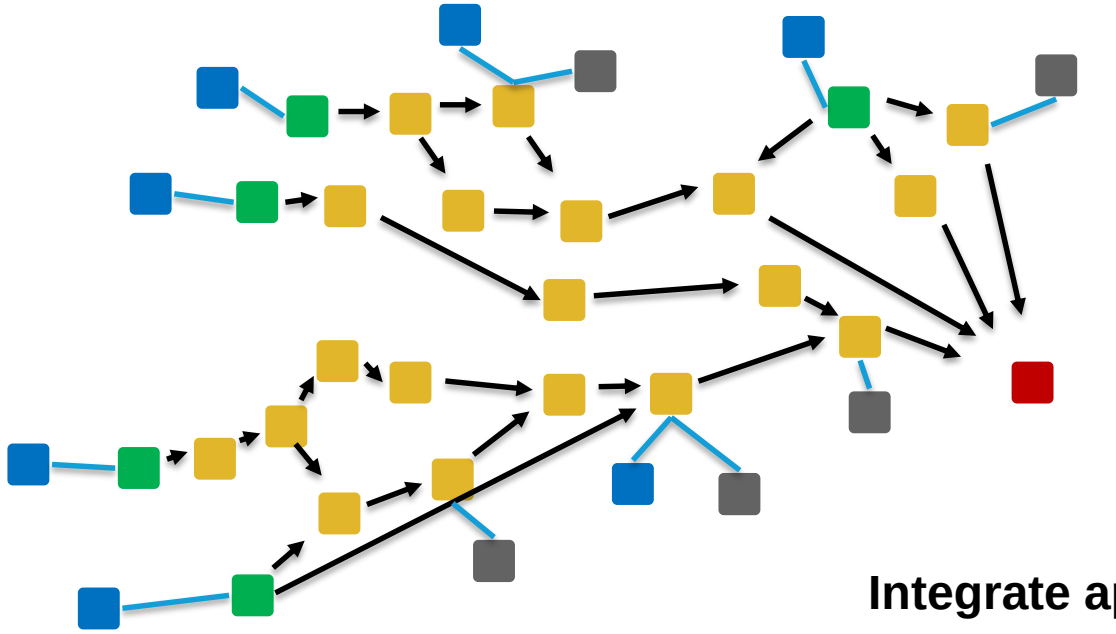

Integrate appropriate models with the network

Better selection of descriptors/
modelling techniques
More data available

Integrate other information with network

In Vitro Assays
In Vivo Assays
Gene/Protein expression

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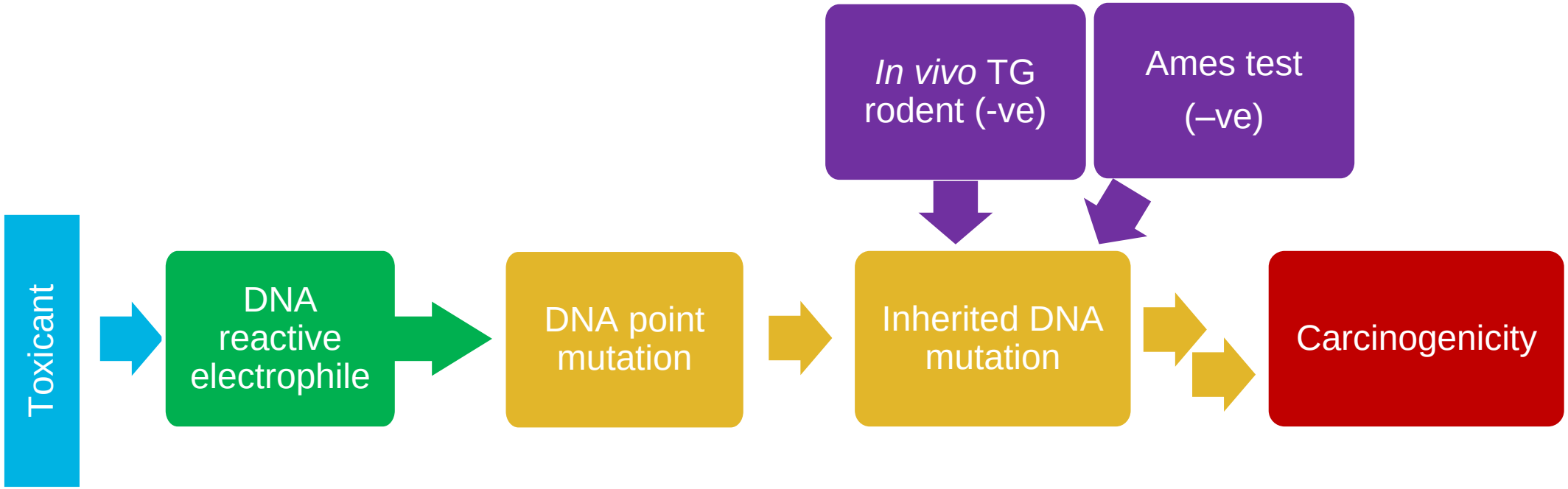
Integrating Pathways With Assay Data



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Integrating Pathways With Assay Data

Environ Mol Mutagen. 2016 Apr;57(3):171-89. doi: 10.1002/em.21996. Epub 2016 Jan 13.

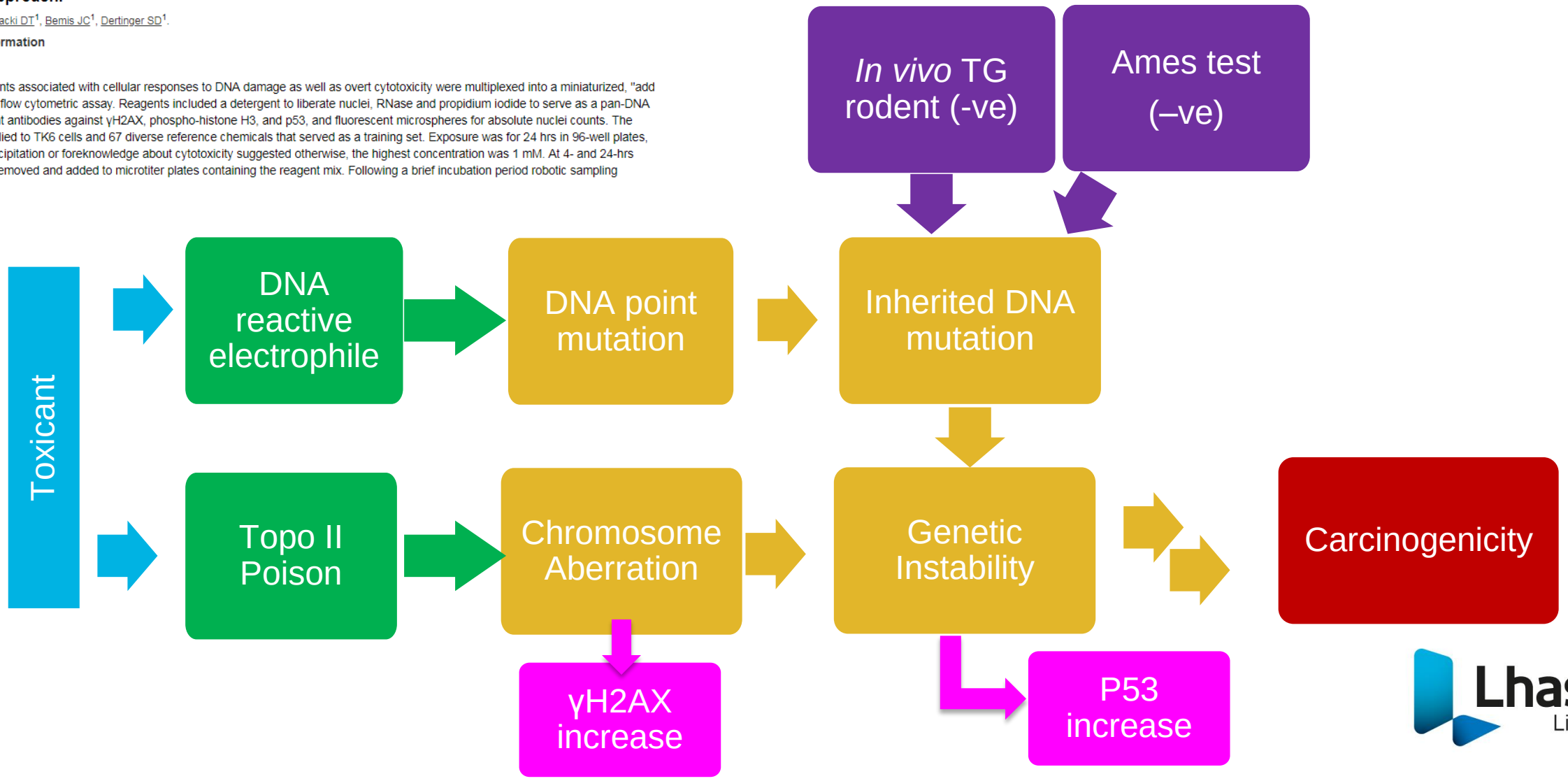
Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach.

Bryce SM[1], Bernacki DT[1], Bemis JC[1], Dertinger SD[1].

Author information

Abstract

Several endpoints associated with cellular responses to DNA damage as well as overt cytotoxicity were multiplexed into a miniaturized, "add and read" type flow cytometric assay. Reagents included a detergent to liberate nuclei, RNase and propidium iodide to serve as a pan-DNA dye, fluorescent antibodies against γH2AX, phospho-histone H3, and p53, and fluorescent microspheres for absolute nuclei counts. The assay was applied to TK6 cells and 67 diverse reference chemicals that served as a training set. Exposure was for 24 hrs in 96-well plates, and unless precipitation or foreknowledge about cytotoxicity suggested otherwise, the highest concentration was 1 mM. At 4- and 24-hrs aliquots were removed and added to microtiter plates containing the reagent mix. Following a brief incubation period robotic sampling



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The Benefits

1) Allows for cutting of the knowledge in different ways depending on use case
2) Allows for custom model building using descriptors appropriate to that MIE/KE
3) Allows for more precise hypothesis testing following a prediction
4) Allows for closer integration with emerging *in vitro* and *in vivo* assays
5) Allows for integration with other toxicity endpoints
6) Many more…

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Additional Considerations

1) Assess exposure qualitatively and quantitatively (ADME)

2) Make predictions quantitative

3) Make *in vitro* to *in vivo* extrapolations (IVIVE)

4) Make risk assessments

5) Make an assessment of human relevance

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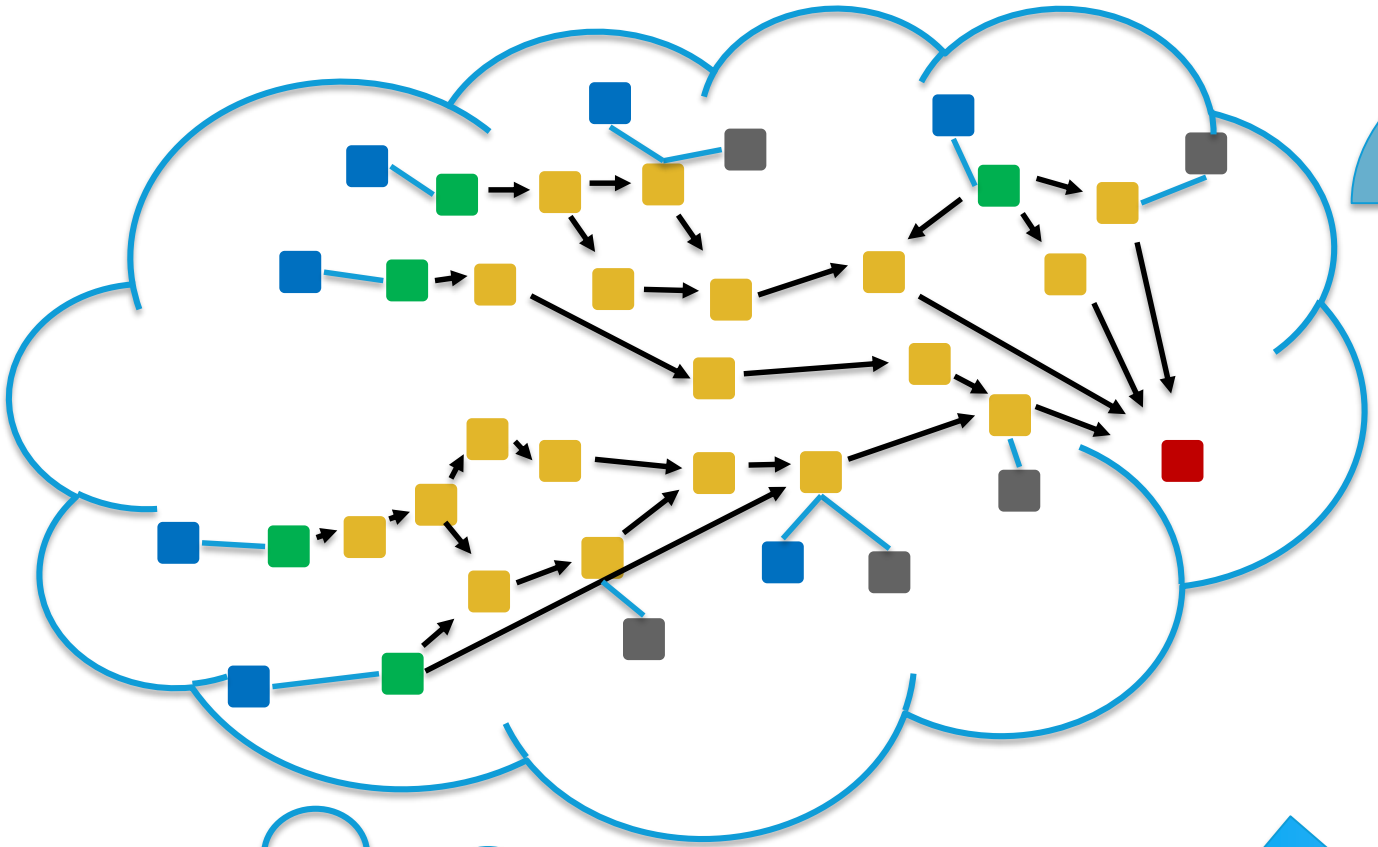
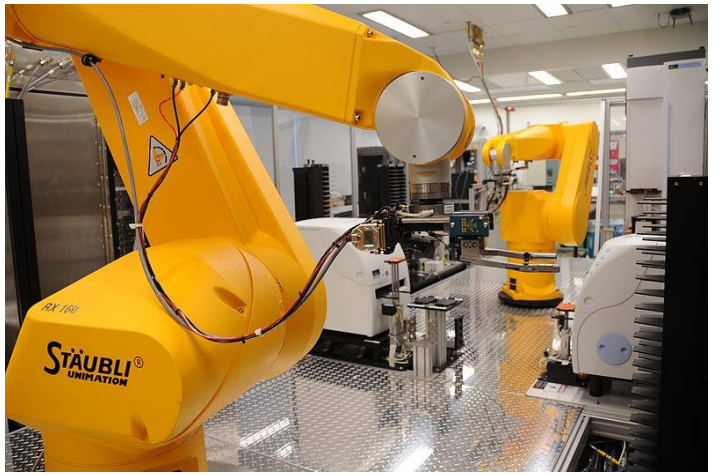
The Future





https://www.nih.gov/news-events/nih-research-matters/finding-compounds-inhibit-zika

Report
+
Acceptable
Intake

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Conclusions

- *In silico* prediction of genotoxicity has come a long way since its inception
- The predictions for these endpoints are embedded in the development process in a number of different places and are already proving their worth
- There is still room for improvement, particularly for predictions which do not relate to direct reaction with DNA
- These predictions may be improved by,
  - More work
  - Better models utilising all data available
  - Better integration of all available evidence
- AOPs are a promising way of achieving these things as well as providing scope for extending beyond hazard prediction to prediction of human risk

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Questions?

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