Mutagenicity Prediction Using *In Silico* Methods:

Gigabyte-Sized Petri Dishes.

Antonio Sánchez Ruiz
Knowledge Base Scientist
antonio.sanchez-ruiz@lhasalimited.org

Predictive Toxicology

- **What is it?**
  - Collection of strategies employed to forecast the interaction between chemical compounds that lead to adverse effects in biological systems.

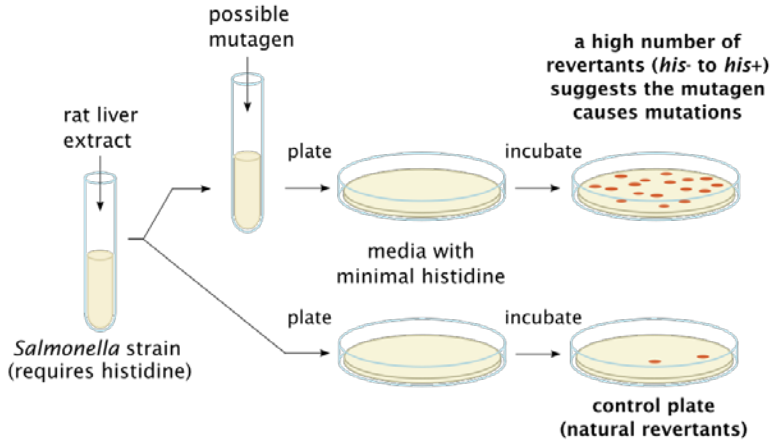
- **Why is it important?**
  - ICH M7 guidelines for the assessment of mutagenic impurities in drugs explicitly include the use of *in silico* methods to fulfil regulatory requirements.
  - Replaces animal testing (follows the 3R initiative: Replacement, Reduction and Refinement).
  - Fast, cost effective, reliable and reproducible approach.

Predictive Toxicology

- **Genotoxicity is the capacity of a chemical compound to cause damage to DNA.**
  - It covers polyploidy, aneuploidy, mutagenicity, chromosome damage and non-inheritable DNA damage.
  - Predicting genotoxicity is essential to ensure the safety of drugs, foods, etc...

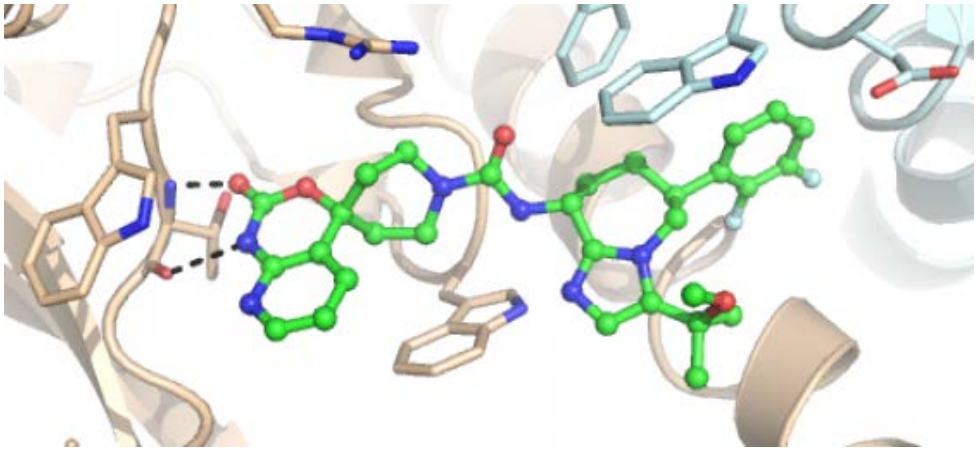
- **How is genotoxicity predicted?**
  - *In vitro* assays: **Replacement** of animal testing for cell/bacterial experiments.
  - *In silico* prediction methods: **Replacement** of *in vitro* assays for algorithms in a computer.

Predictive Genotoxicity



- ***In vitro* tests**
  - ⊙ Study the genotoxic effect of a specific chemical in bacterial, yeast or mammalian cells.
  - ⊙ They are fast, easy to set up, cheaper than animal tests, simple to run, amenable for automation and provide quick results.
  - ⊙ Main limitations come from the extrapolation of the *in vitro* data to *in vivo* systems and from the need to have the test substance isolated and purified in sufficient quantities to conduct the tests.

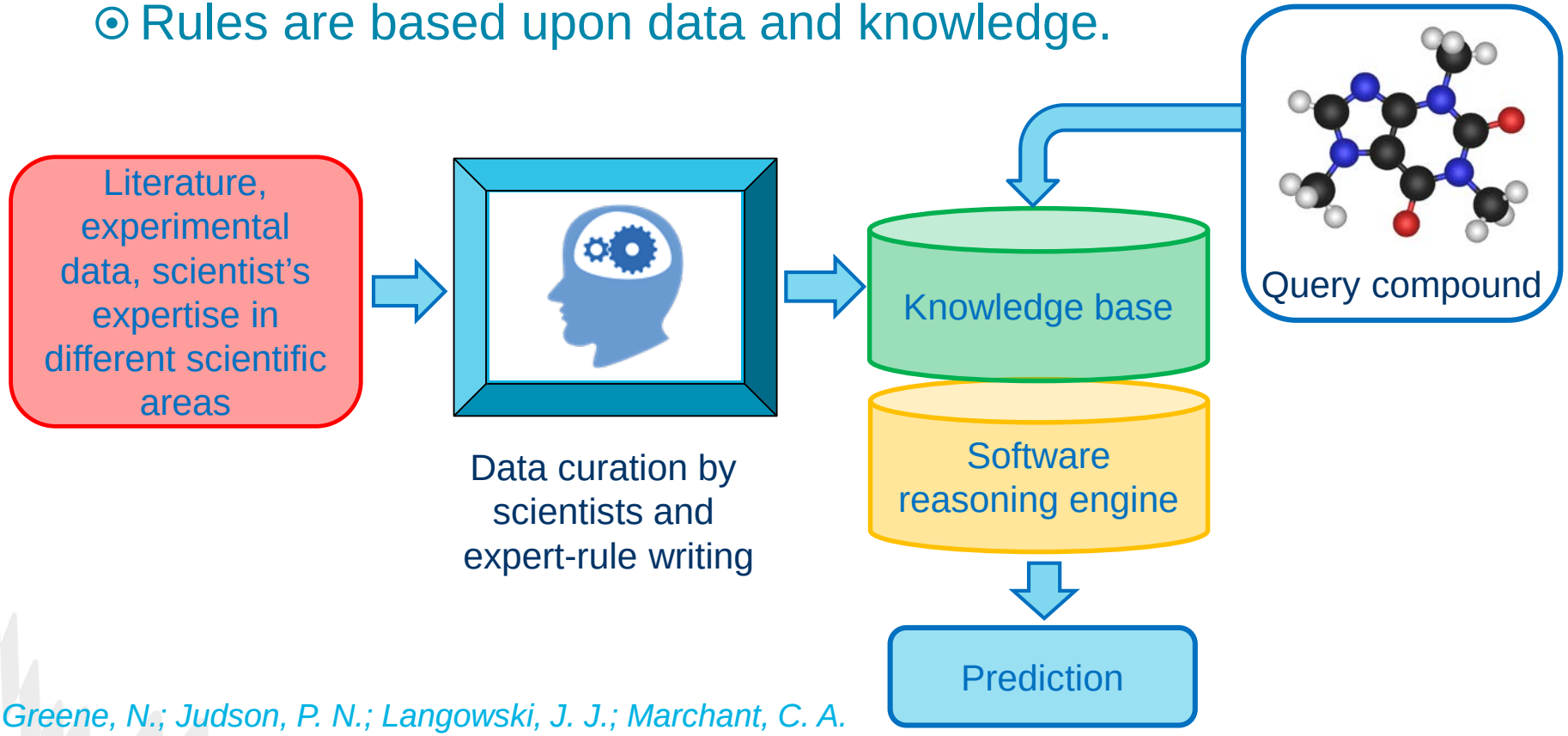
Predictive Genotoxicity



- ***In silico* tests**
  - Toxicity assessment that uses computational methods to model, simulate or predict toxicity of chemicals.
  - Higher throughput, faster, cheaper than *in vitro* methods and present a high reproducibility if the same model is used. Information about the mechanism of action can also be obtained.
  - Limitations root in the quality of the dataset, not accounting for ADME features and the complexity of certain endpoints (e.g. neurotoxicity).

In silico Predictive Genotoxicity

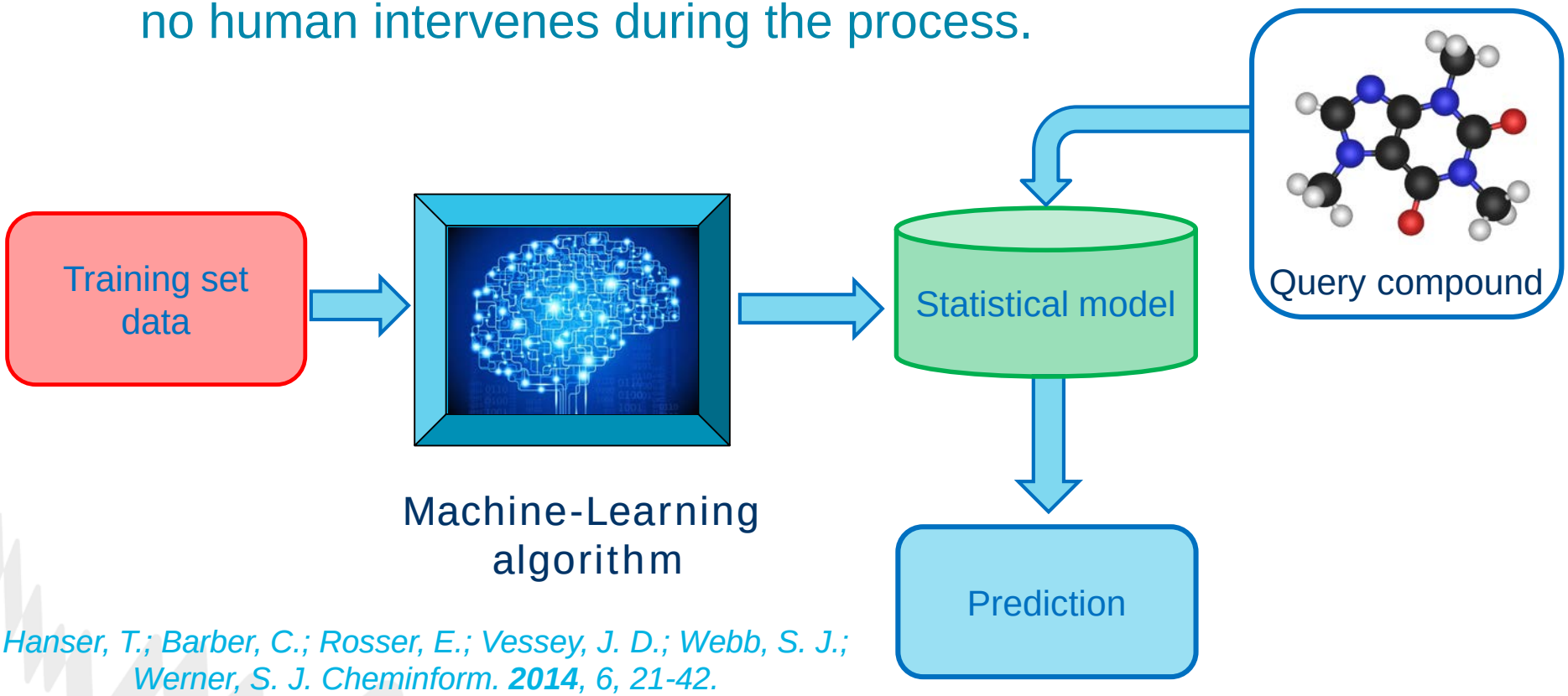
- **Expert Knowledge Software.**
  - Scientists write expert rules that relate chemical structure to toxicity.
  - Rules are based upon data and knowledge.



Greene, N.; Judson, P. N.; Langowski, J. J.; Marchant, C. A. SAR QSAR Environ. Res. ***1999****, 10, 299–314*

In silico Predictive Genotoxicity

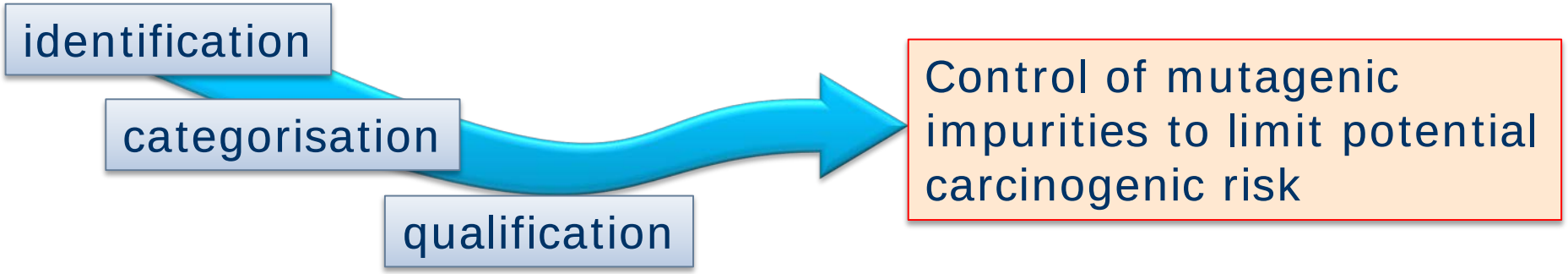
- **Statistical Modelling Software.**
  - A training dataset is used to construct a statistical structure-activity model through machine learning algorithms.
  - This model is then used to predict the query compound toxicity; no human intervenes during the process.



Hanser, T.; Barber, C.; Rosser, E.; Vessey, J. D.; Webb, S. J.; Werner, S. J. Cheminform. ***2014****, 6, 21-42.*

Importance of *in silico* methods: ICH M7

- "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk"
  - ⊙ 'Global' guidelines – America, Europe, Japan
  - ⊙ Finalised - June 2014
  - ⊙ In force since Jan 2016



http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html

Importance of *in silico* methods: ICH M7

Class	Definition	Proposed action for control
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown mutagenic potential (bacterial mutagenicity positive, no rodent carcinogenicity data)	Control at of below acceptable limits (TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (acceptable TTC) or conduct bacterial mutagenicity assay; If non-mutagenic=Class 5 If mutagenic=Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity.
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity.

http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html

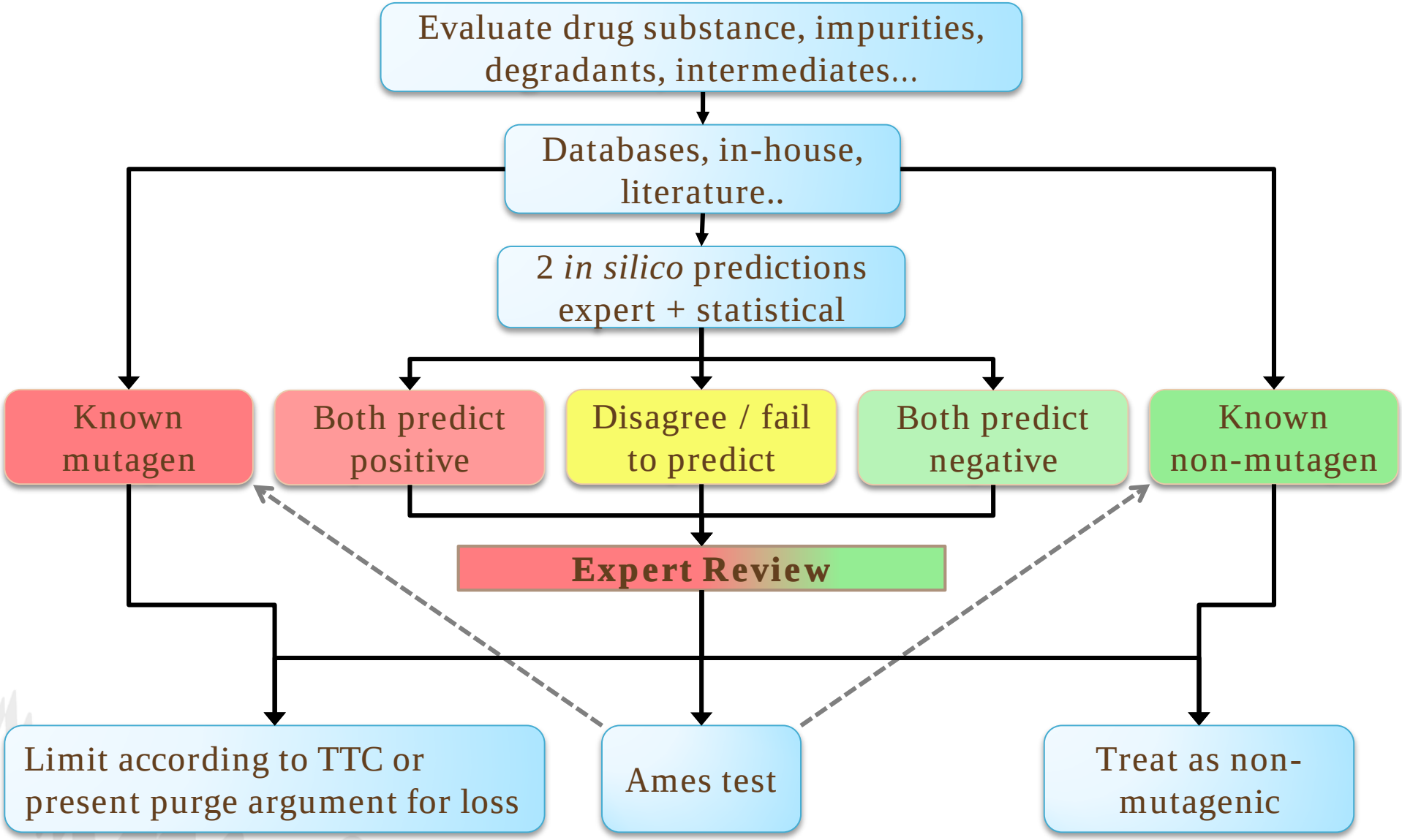
Focussing on the *in silico* predictions…

- Q(SAR) Requirements:
  - must predict the outcome of a bacterial mutagenicity assay
  - one expert rule-based; the second statistical-based
  - both should follow the OECD principles

> 1. *a defined endpoint;*
> 2. *an unambiguous algorithm;*
> 3. *a defined domain of applicability;*
> 4. *appropriate measures of goodness-of-fit, robustness and predictivity;*
> 5. *a mechanistic interpretation, if possible.*

- The absence of structural alerts from both is sufficient to conclude that the impurity is of no mutagenic concern
  - Expert review can provide
    - additional supportive evidence
    - reason to dismiss an *in silico* prediction
    - rationale to support the final conclusion

In silico workflow under ICH M7



Lhasa Limited Solutions

Derek Nexus – Expert knowledge-based toxicity prediction software

Meteor Nexus – Expert decision support software for predicting the metabolic fate of chemicals in mammals

Vitic Nexus - Chemical database and information management system, offering researchers and scientists rapid access to searchable toxicological information

Zeneth - Expert decision support software for predicting the forced degradation pathways of organic compounds

Sarah Nexus - Statistical-based software for the prediction of mutagenicity

Lhasa Limited Solutions

Derek Nexus – Expert knowledge-based toxicity prediction software

Meteor Nexus – Expert decision support software for predicting the metabolic fate of chemicals in mammals

Vitic Nexus - Chemical database and information management system, offering researchers and scientists rapid access to searchable toxicological information

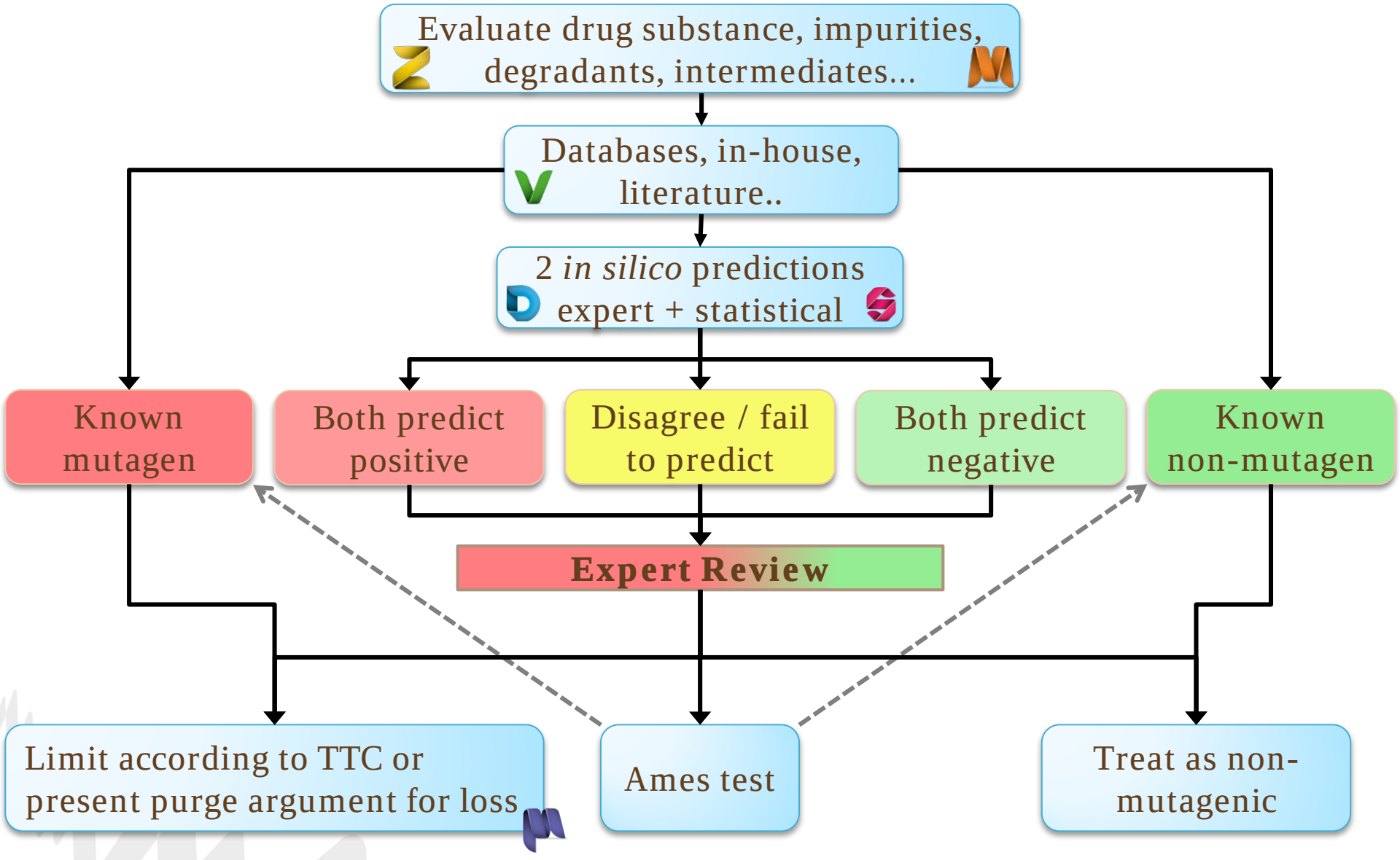
Zeneth - Expert decision support software for predicting the forced degradation pathways of organic compounds

Sarah Nexus - Statistical-based software for the prediction of mutagenicity

Of expert and statistical systems...

	Expert system	Statistical system
Data	• Public *and confidential data* • Wider knowledge - mechanism, reactivity, related assays…	• Transparent systems can only show non-confidential data
Methodology	• Human-written rules based upon data & knowledge	• *Machine-learnt fragments* • Machine-learnt significance of each fragment
Scope of alert	• Human defined (Markush)	• Fragments learnt by model
Interpretability	• Expert commentary • Mechanistic explanation • Supporting examples • References • Confidence • Highlights areas of uncertainty	• Transparent methodology • No uninterpretable descriptors or definitions of applicability • Learning summarised • Direct link to training set • Confidence in prediction

How Lhasa fits into ICH M7 workflow



Conclusions

- **Predictive Toxicology** tries to forecast toxicity by studying the perturbation of biological pathways on a molecular basis.
- It moves away from the direct observation of adverse effects in animals to *in vitro* and *in silico* tests.
- *In vitro* assays provide experimental data in a fast way.
- *In silico* methods are faster, more cost effective and have a higher throughput than *in vitro* assays.
- Reliability of *in silico* methods results has motivated the possibility of being employed as a substitute for *in vitro* assays, as stated by ICH M7 guidelines for mutagenicity.
- Improvements in computational power and new algorithms will cause a sheer increase in the predictive capabilities of *in silico* methodologies.

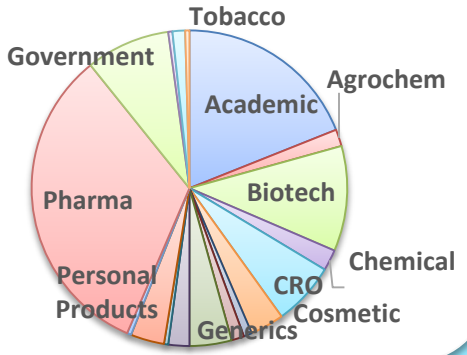
Thank you for your attention!

Not-for-profit Educational charity

- Teaching lectures
- Interns
- Sponsor PhDs
- Undergrad projects

Located in Leeds, UK

A membership organisation



Lhasa Limited

Predictive software (expert & machine learnt)

Purge

Degradation

Toxicity

Metabolism

Regulators (members & collaborators)

FDA

NIHS

OECD
BETTER POLICIES FOR BETTER LIVES

Data & knowledge sharing Honest broker

Vitic intermediates

Vitic excipients

eTOX

MIP-DILI

iPiE

Proprietary data mining