Modelling Local and Systemic Toxicity: Incorporation of *In Silico* Predictions in the Development of Adverse Outcome Pathways

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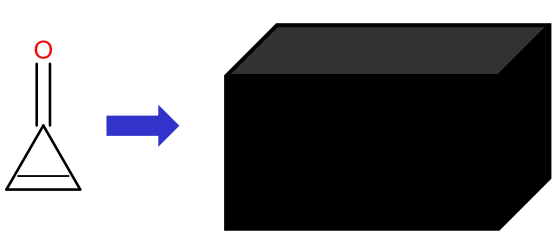
In Silico Prediction

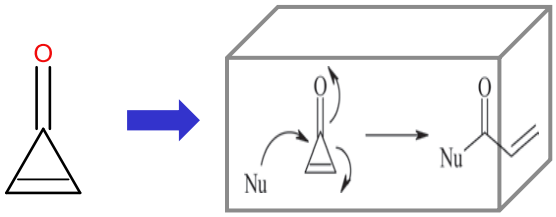
- Activity (e.g. toxicity) of a chemical ∝ Molecular (structural) properties

- Certain endpoints easier to predict than others

- Methods need to be transparent

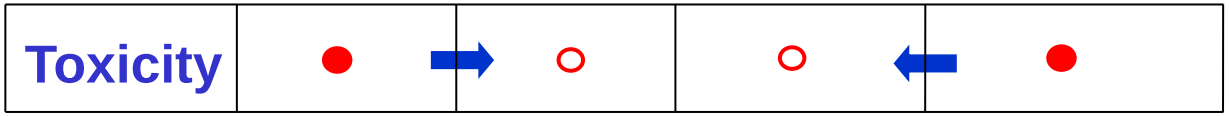


= toxic

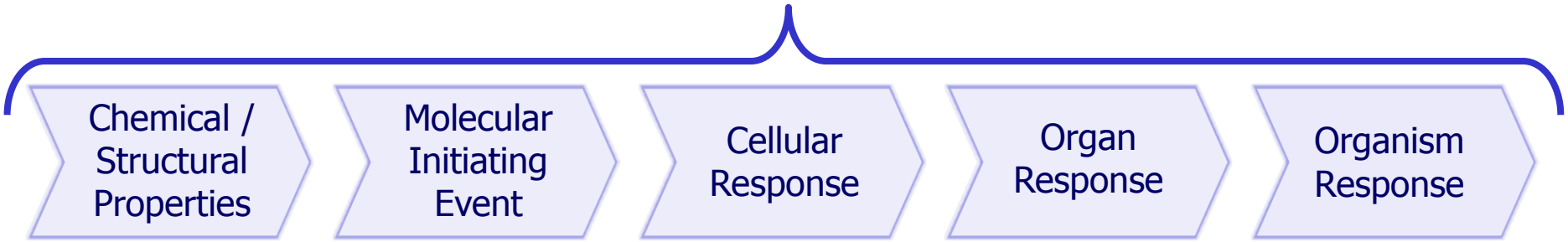


= covalent protein binding

- Newer methods such as category formation (**grouping**) & read-across can be applied to complex endpoints & are transparent



Adverse **O**utcome **P**athway for Skin Sensitisation



- The AOP provides a method to represent the **Key Events** involved
- **Key Events** are measurable and toxicologically relevant

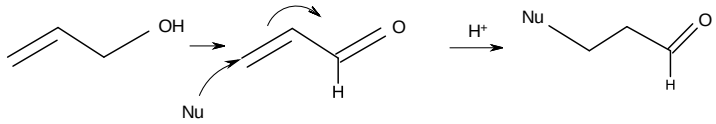
- NB pathway may be non-linear; adaptive & regulatory responses may be involved
- Realistic exposure scenario need to be considered

Key Events in Skin Sensitisation AOP

Key Event 1 — Molecular Initiating Event

Covalent binding interaction

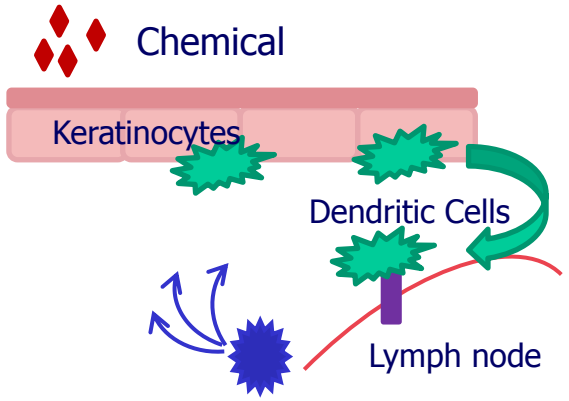
Electrophile



Nucleophile on skin protein (cysteine/lysine)

Key Event 2 — Cellular response

Keratinocyte inflammatory response



Key Event 3 — Cellular response

Dendritic cell activation

Key Event 4 — Organ Response

Lymph node - activation of T-cells
proliferation of activated T-cells

Adverse Outcome

Skin:
Inflammation on challenge with antigen

Key Events in Skin Sensitisation

Key Event 1 — Molecular Initiating Event

Covalent binding interaction

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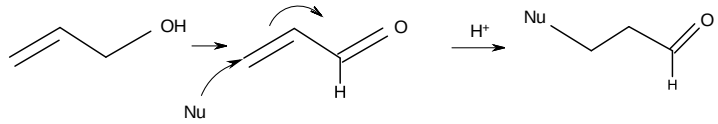

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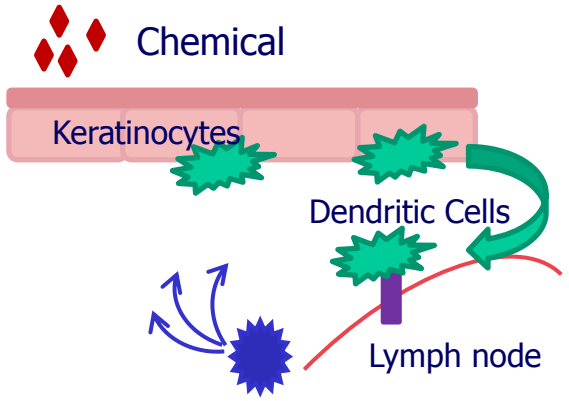

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Adverse Outcome

Skin:
Inflammation on challenge with antigen

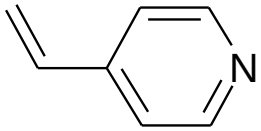
Accumulating Information to Develop AOPs

- The Molecular Initiating Event (MIE) (initial interaction between chemical and biological system) is a **Key Event**
  - Hypothesis or evidence required for Key Events
  - Evidence can be accumulated from a range of sources

In Silico	*In Chemico*	*In Vitro*	*In Vivo*
Creating Structural Alerts / Profilers Identifying MIEs	Direct Peptide Reactivity Assay Glutathione depletion	MUSST/h-CLAT Ames Test Mitochondrial Damage Oxidative Stress	GPMT LLNA Human Patch Test Toxicity Assays

- Capturing the chemistry underlying an MIE can be used to develop Structural Alerts / Profilers for ***grouping*** chemicals into categories

Identifying Groups in Chemical Space



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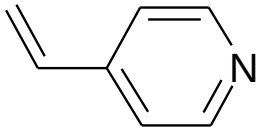

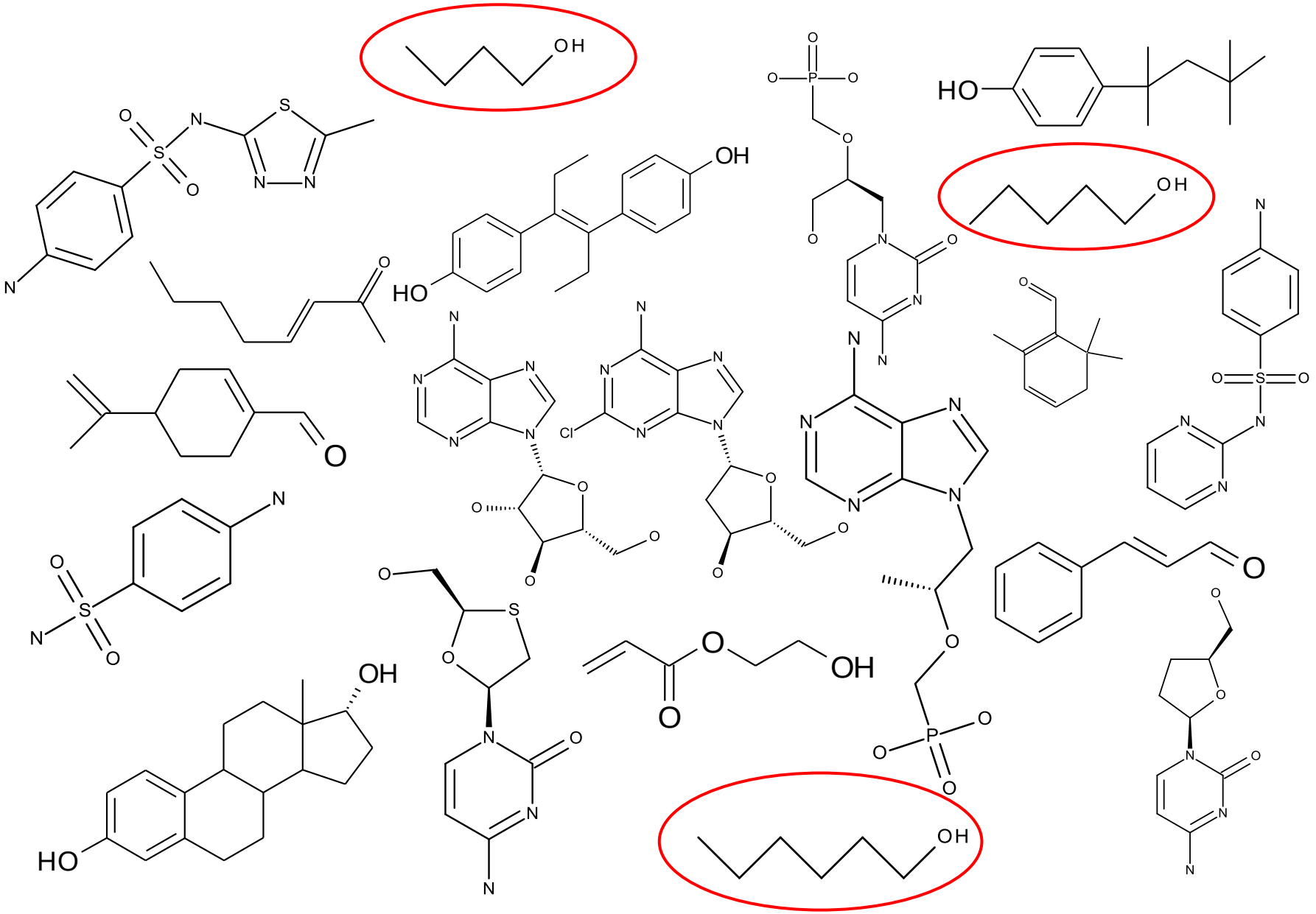

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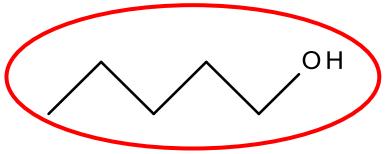
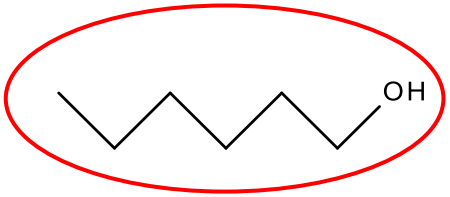


Structural Analogues



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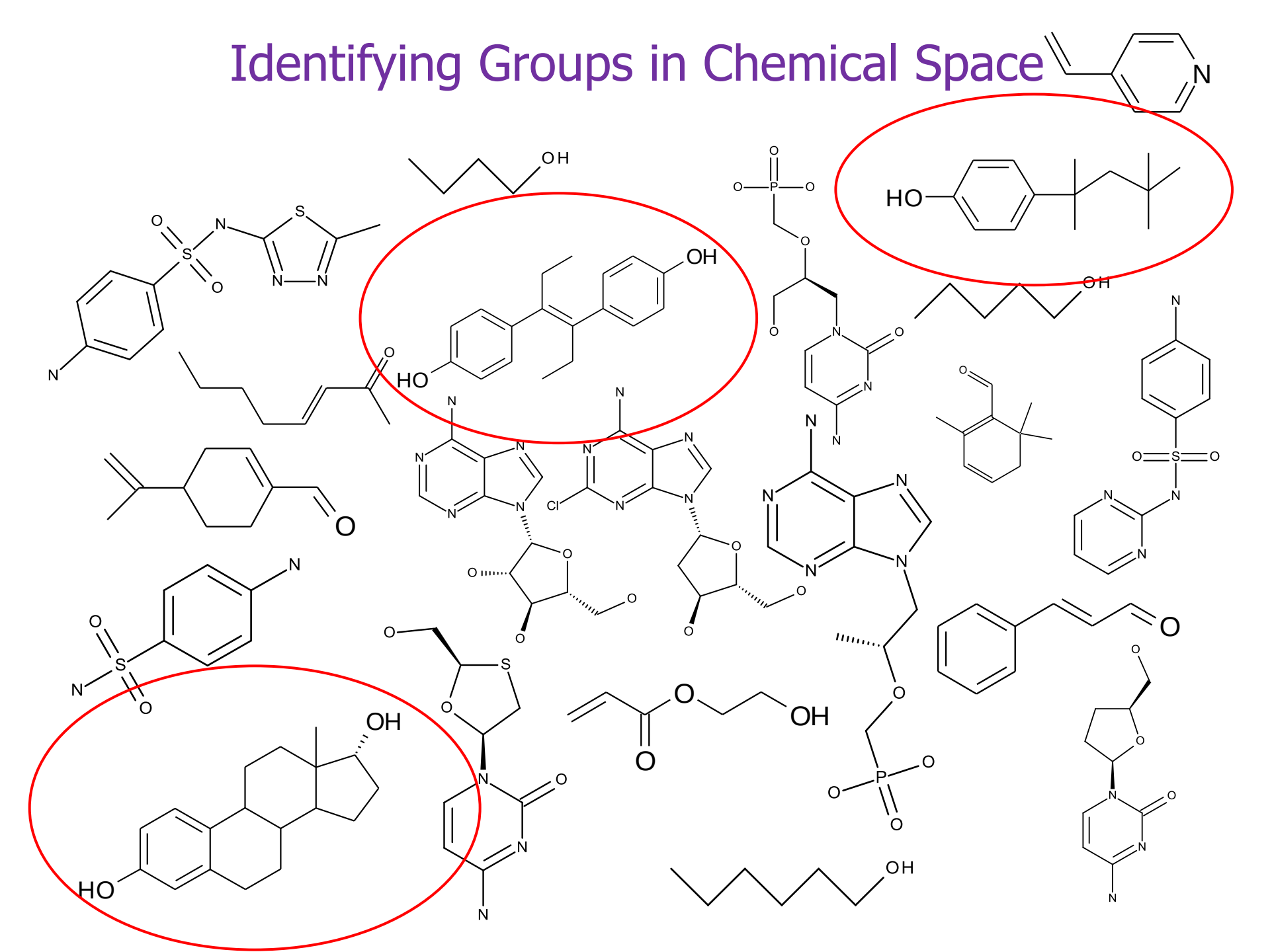

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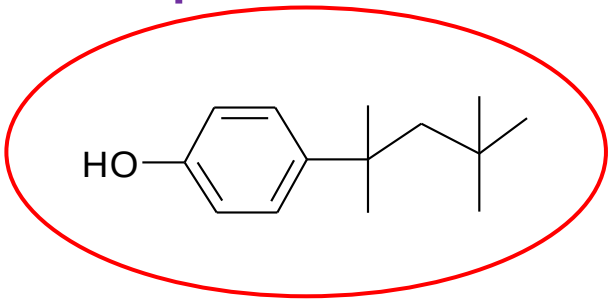
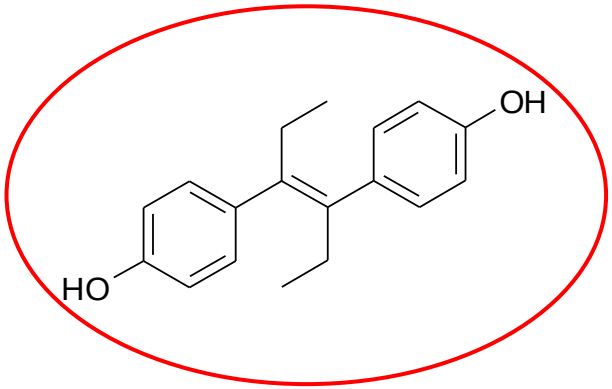
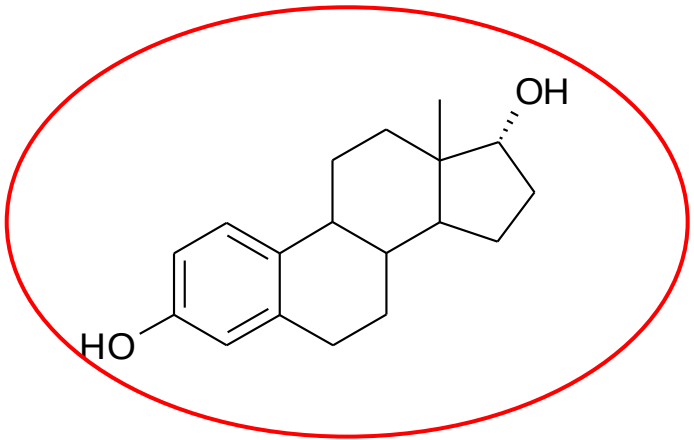




Mode of Action Analogues
(Binding to oestrogen receptor)



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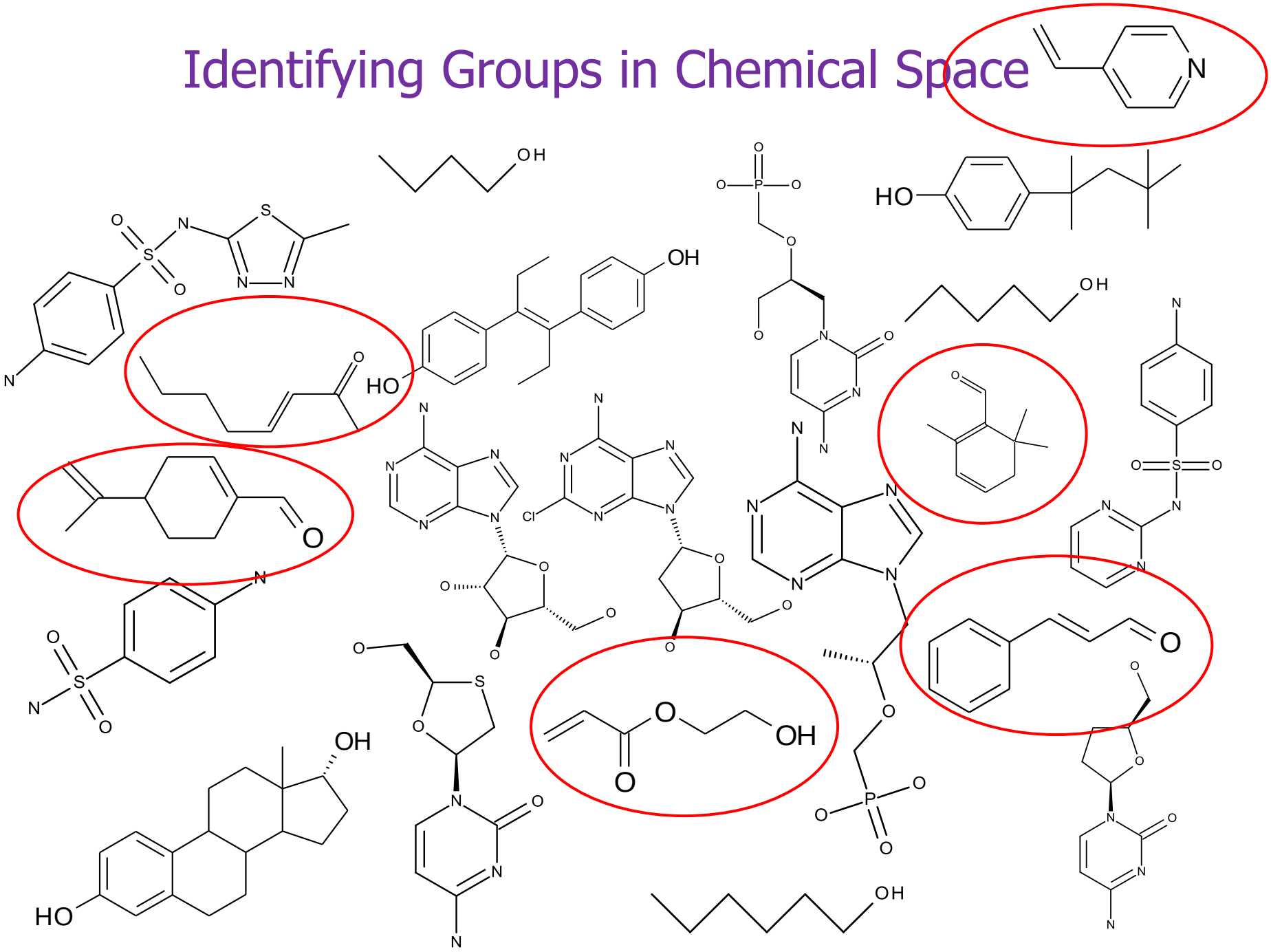

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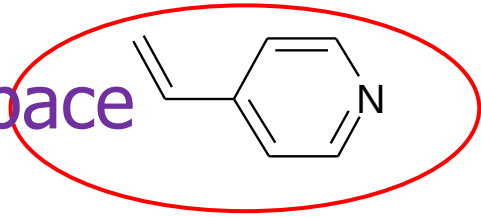
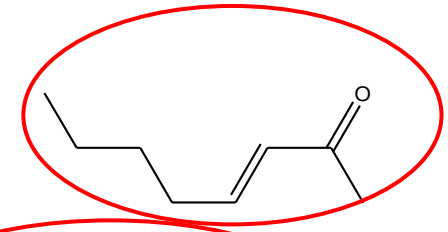
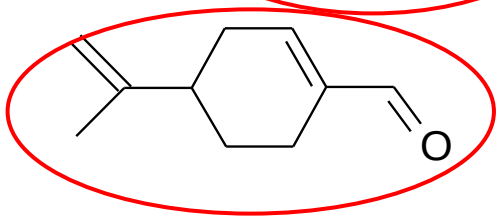
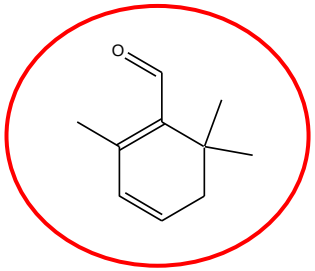
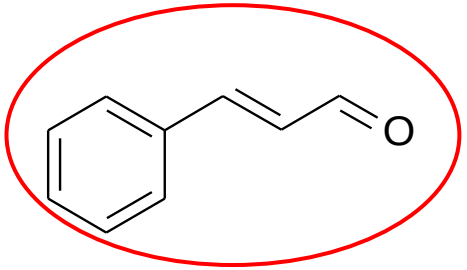


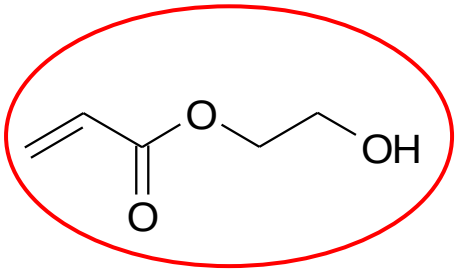
Mechanistic analogues
(Michael addition reaction)







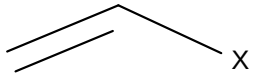




Mechanistic Structural Alerts and Profilers Developed:

Protein binding relating to skin & respiratory sensitisation

Use knowledge of mechanistic chemistry domain e.g.
Acylation
Schiff Base Formation
Michael Addition$^{s>r}$ → Mechanistic Alert: (e.g. polarised alkenes) → Structural Alerts: e.g. α,β unsaturated aldehydes, ketones, amides



104 structural alerts developed for protein binding (46 associated with skin sensitisation)[2];
52 structural alerts for respiratory sensitisation

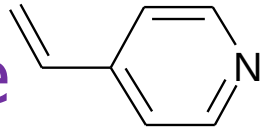
Also 57 structural alerts developed for DNA binding[2]

Derive associated SMARTS[1] patterns (e.g.CC=OC; C=NCCR)

Encode into profilers

[1] freely available – also encoded in KNIME workflows
[2] *available in OECD QSAR Toolbox;*

Identifying Groups in Chemical Space



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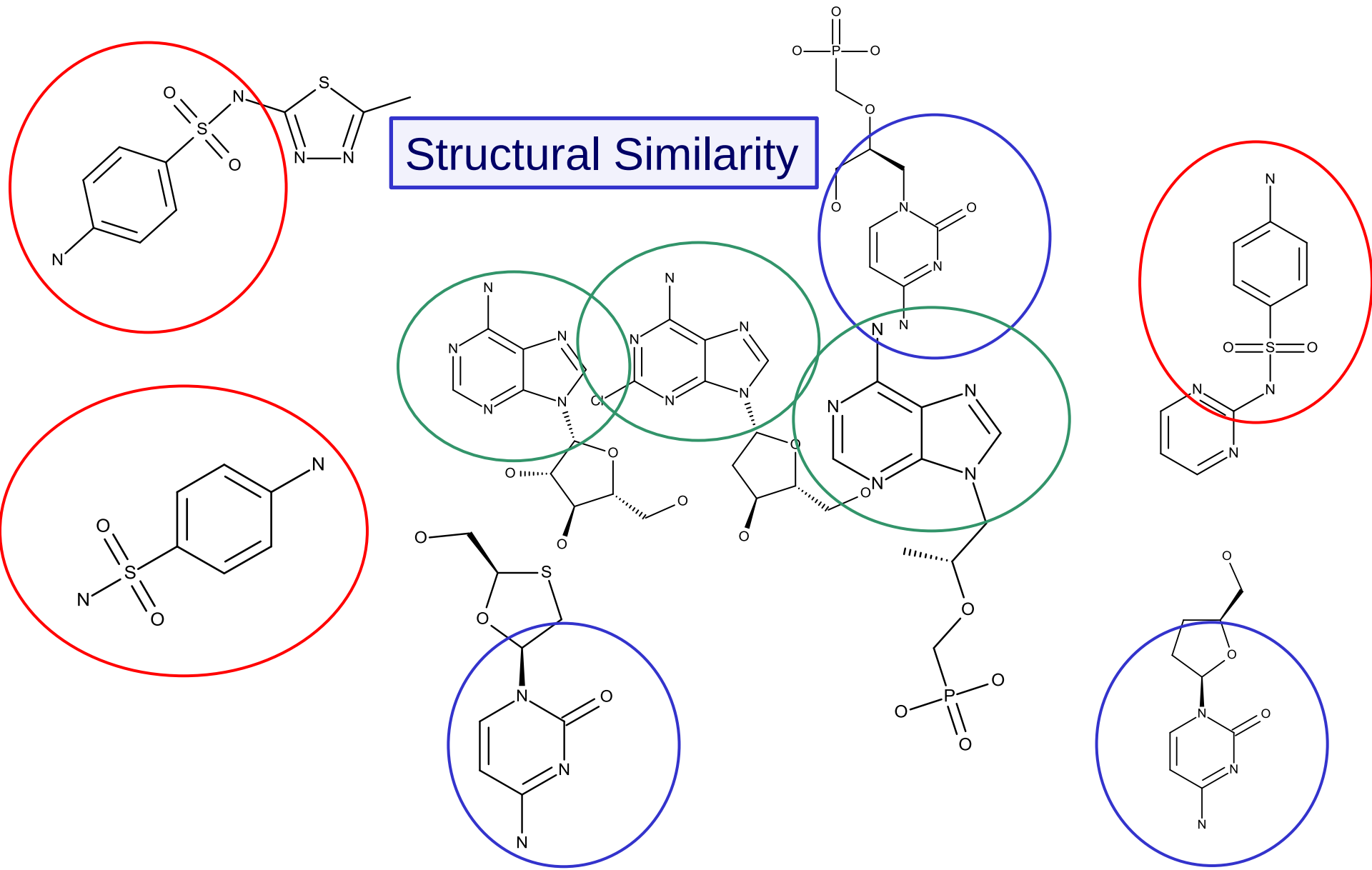
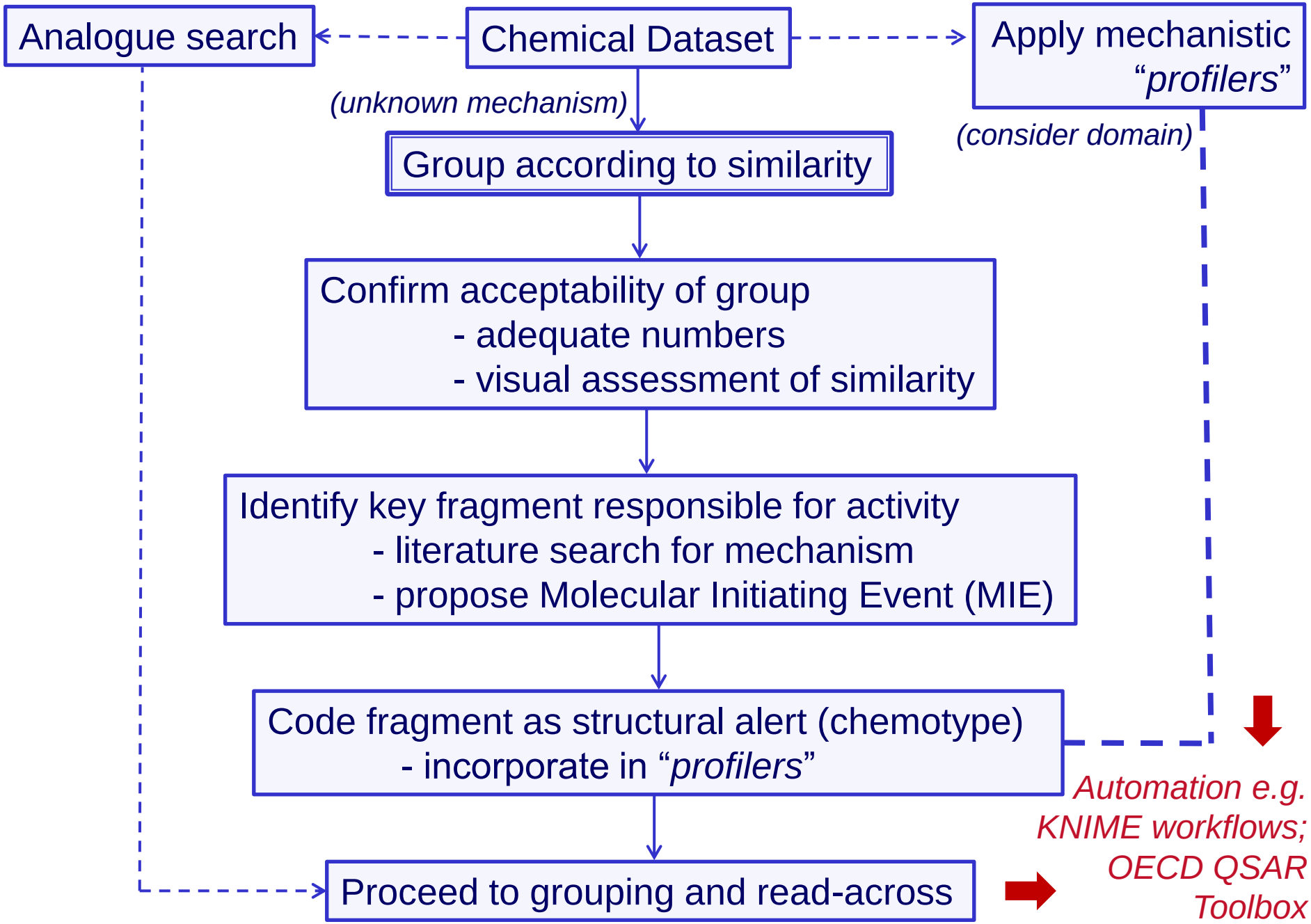


Grouping Chemicals for Read-Across



Structural Alerts for Hepatotoxicity

951 compound dataset* (650 +ve)

↓

Grouped by structural similarity
- using Toxmatch software

↓

Group (category) acceptable if:
contained ≥ 6 compounds
and similarity index ≥ 0.6
and visually appeared similar

↓

Alerts used to re-screen dataset
↑no. category members

↓

Literature search for putative mechanisms of toxicity

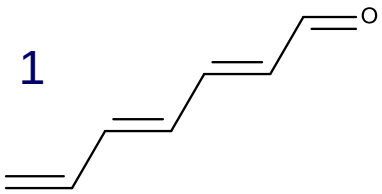
↓

Mechanisms proposed for ~25% of hepatotoxicants in dataset

16 structural alerts[#] (key fragments) identified

Example SMARTS Patterns
1. C=CC=CC=CC=O
2. ClCCNCCCl
3. O=C1CCC2C3CCC4CCCC4C3CCC2=C1

1



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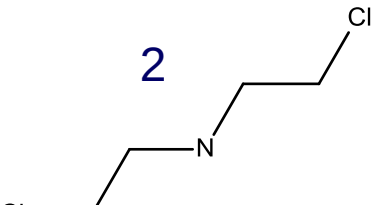
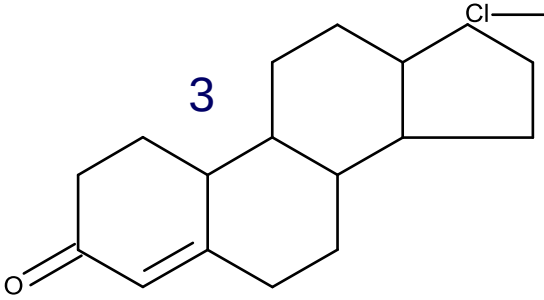


3



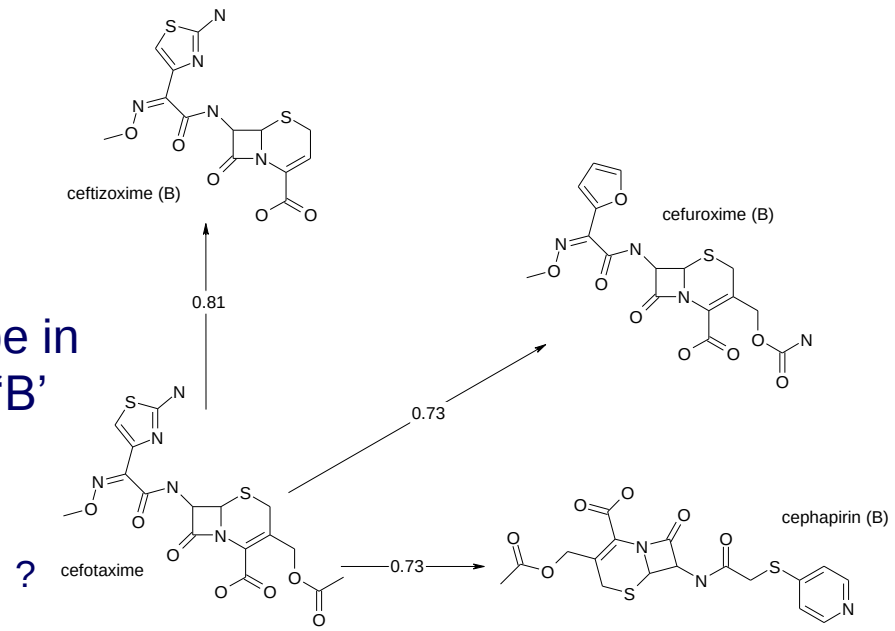
*Fourches et al (2010) Chem Res Toxicol **23***

[#]*Hewitt et al (2013) Crit RevToxicol **43***

Using the Information to Predict Toxicity

- Profilers can be used to form groups of compounds (categories)
- Structural or mechanistic knowledge of category members can be used to infer information concerning an (unknown) compound of interest i.e. a read-across prediction

Cefotaxime predicted to be in toxicity class 'B'



- Enables transparent, justifiable predictions to be made

Enoch SJ *et al* (2009) *QSAR Comb. Sci.* 28: 696-708.
Formation of Structural Categories to Allow for Read-Across for Teratogenicity

Tools for Category Formation & Read-Across:
OECD QSAR Toolbox

Input query chemical

↓

Profile (identify key features) ← Profilers:
Mechanistic
Empiric
Toxicological
Endpoint specific
or by affiliation

↓

Gather endpoint information

↓

Develop and refine category

↓

Use compounds of known activity to fill data gap

↓

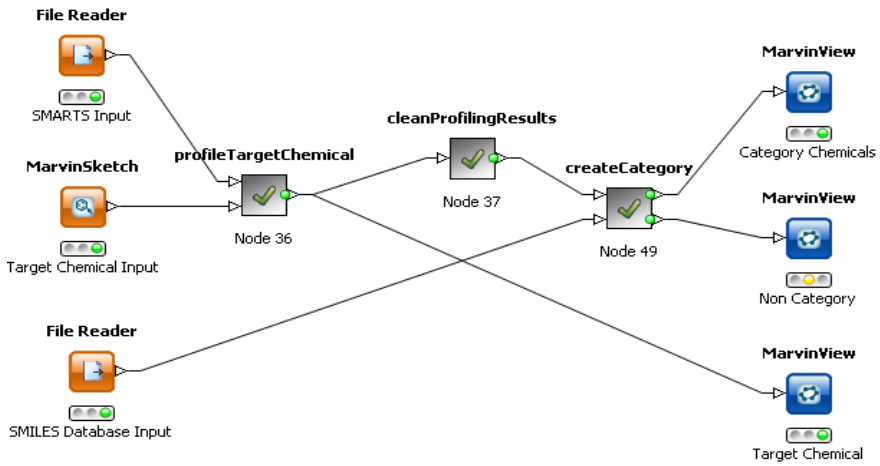
Produce report

Under further development...

The COSMOS Project

Integrated *in silico* models for the prediction of repeated dose toxicity of **COSM**etics to **O**ptimise **S**afety

- Developing tools for predicting repeat dose toxicity
  - database of relevant toxicity & ADME data
  - building (Q)SARs
  - identifying structural alerts – creating profilers for category formation
- Freely available as KNIME workflows



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Conclusions and Outlook

- AOPs provide a framework for organising information
  - The MIE is a key event () in an AOP
- Understanding mechanistic chemistry enables structural alerts / profilers to be built associated with an MIE
- Categories (groups) can be based on structural similarity & potential mechanisms investigated
- Literature or experimental evidence provides support for AOPs
  - Link *in silico* investigation to directed *in vitro* analysis
    - Confirmation of effects; defining chemical space of alert
- Improvements needed in tools to capture and use information
  - Toolbox; Effectopedia
  - Mitigating factors need to be considered
  - More quantitative predictions in future

Category formation (grouping) & read-across provide more transparent, acceptable methods of predicting toxicity

Acknowledgements

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COSMOS

QSAR TOOLBOX

eTOX

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Thank you for your attention!