Computational Methods in Next-Generation Risk Assessment of Consumer Products

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The author declares no conflict of interest.
Abbreviations

- TTC – Threshold of Toxicological Concern
- DST – Dermal Sensitisation Threshold
- EBW – Exposure-Based Waiving
- EcoTTC – Ecotoxicological Threshold of Concern
- TOSCA – Toxic Substances Control Act
- REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals
- HPV – High Production Volume
- CPDB – Carcinogenic Potency Database
- 3Rs – Replacement, Refinement and Reduction (in animal use)
- ICCR – International Cooperation on Cosmetic Regulation
- QSAR – Quantitative Structure-Activity Relationship
- AOP – Adverse Outcome Pathway
- MIE – Molecular Initiating Event
- MCSS – Maximum Common Substructure
- AA2aR – Adenosine A2a Receptor
- SE – Sensitivity
- SP – Specificity
- Q – Quality
- MCC – Matthews Correlation Coefficient
- SMILES – Simplified Molecular Input Line Entry System
- MCSS – Maximum Common Sub-Structure
- CoMFA – Comparative Molecular Field Analysis
Agenda

• Risk Assessment in Consumer Product Industry
• Leading with Exposure
• Example 1 – Threshold of Toxicological Concern
• Mechanistic Understanding
• Example 2 – Molecular Initiating Event Predictions
• Conclusions
What is covered by “Consumer Products”
Risk Assessment for Consumer Products

- **Context...**
  - Classification and labelling
    - Favours a cautious approach
    - Hazard based rules
    - Occupational focus

- **Screening/product development**
  - Many potential lead chemicals
  - Often only hazard prediction methods are used
  - Performance of models is less critical
  - Exposure may be considered by the use of threshold-based approaches e.g. TTC, DST, EBW, EcoTTC

- **Risk Assessment including actual exposure**
  - Requires a high degree of accuracy
  - Route and amount of exposure dictate the need for toxicology data
  - High level of scrutiny (internal and external)
Why Does (Eco)toxicity Testing Need to Change?

**Practical Considerations**
- Large numbers of chemicals with limited toxicity information
  - HPVs EPA (TOSCA 90,000)
  - REACH (140,000 preregistered under REACH ~70,000 will require tox data)
- Only ~1600 chemicals have ever been tested in a rodent carc bioassay (CPDB 2010)
- Need to consider metabolites, degradation products, process intermediates, mixtures
- Novel materials are entering the market all the time e.g. nano

**Scientific Considerations**
- Accuracy of risk assessment based on lab species
  - Do they cover all endpoints and sub-populations?
- Species: species extrapolation
- Use of “arbitrary” uncertainty factors
- Use of animals in toxicity testing – 3Rs
  - Legislation
ICCR principles of risk assessment without animal testing

- The overall goal is human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm (i.e. distinguish between adaptation and adversity)
- Using a tiered and iterative approach
- Following an appropriate appraisal of existing information
- Using robust and relevant methods and strategies
- The logic of the approach should be transparently and explicitly documented
- Sources of uncertainty should be characterised and documented

Dent M. et al., Computational Toxicology, 2018
Leading with exposure
Next Generation Risk Assessment

- Start with exposure rather than toxicity

‘....... Human safety depends on exposure and toxicity. Indeed, the 2012 European Commission report on addressing the New Challenges for Risk Assessment states, “A paradigm shift is likely from a hazard-driven process to one that is exposure-driven” ....’

Pastoor et al, Critical Reviews in Toxicology, 2014
Exposure estimate

Hazard estimate

Margin of Safety

Exposure / Dose

Hazard

Exposure

[Diagram showing the relationship between exposure, dose, hazard, and margin of safety]
Exposure estimate

Hazard estimate

Exposure estimate

Hazard

Exposure
Example 1 - TTC
Threshold of Toxicological Concern (TTC)

- Describes a level of exposure to an untested or unidentified chemical that is likely to be without harm, such that chronic exposures below the threshold can be assumed to be without appreciable risk over a lifetime

- Extended to
  - Dermal Sensitisation Threshold – skin allergy
  - EcoTTC – aquatic environmental risk

Munro et. al., Food and Chemical Toxicology, 1996
Importance of SARs to define genotox chemicals

A SAR is a (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological effect

Ashby and Tennant, Mutation Research, 1988
Importance of SARs to define genotox chemicals

Important to understand and encode exclusion rules as well as structural alerts e.g. tri- and tetra-substituted epoxides
Cramer Classification

Cramer classification based on structural rules → in silico tool now in e.g. toxtree

<table>
<thead>
<tr>
<th>Cramer Class</th>
<th>Description</th>
<th>TTC (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Substances of simple chemical structure with known metabolic pathways and innocuous end products which suggest a low order of oral toxicity.</td>
<td>1,800 (30 µg/kg bw/d)</td>
</tr>
<tr>
<td>II</td>
<td>Substances that are intermediate. Possess structures that are less innocuous than those in Class 1 but do not contain structural features that are suggestive of toxicity like those in Class 3.</td>
<td>540 (9 µg/kg bw/d)</td>
</tr>
<tr>
<td>III</td>
<td>Substances with chemical structures that permit no strong initial impression of safety and may even suggest a significant toxicity.</td>
<td>90 (1.5 µg/kg bw/d)</td>
</tr>
</tbody>
</table>

Mechanistic understanding
An AOP is the sequence of events from understanding the chemical structure of a target chemical, through the molecular initiating event (MIE) to an in vivo toxicity outcome of interest.

It is the ‘capture’ of the mechanistic processes that initiate and progress through the levels of biology to give rise to an adverse outcome in living organisms for given chemical toxins.
Example 2 – MIE Predictions
The Molecular Initiating Event (MIE)

- An MIE is the initial interaction between a molecule and a biomolecule or biosystem that can be linked to an outcome via a pathway.
- Different MIEs can lead to the same Adverse Outcome Pathway (AOP).
- Most chemicals can interact with more than one target with different affinities and effects.

The MIE is just a chemical-chemical interaction....

Matrix composition

\[ \text{Cl}^- \quad \text{Na}^+ \quad \text{Mg}^{2+} \quad \text{K}^+ \quad \text{O}^- \quad \text{Ca}^{2+} \]

Lipid

Enzyme

Protein

Vesicle

Partitioning & interactions with endogenous material

Dynamic system

3-Dimensional Structure

Competition

pH effects

\[ \text{H}^+ \quad \text{H}^+ \quad \text{H}^+ \]

Carlsson et. al, Nature Chemical Biology 2011
**SIMPLE CONCEPT**

1. **Identify MIEs**
2. **Develop in chemico assay**
3. **Generate training set**
4. **Build in silico model from in chemico data**
5. **Test in silico model performance on blind test set**
6. **SARs to group chemicals by MIE**
7. **QSARs to predict MIE dose response**
8. **Correlate MIE with next plausible level of organisation**

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**References**

Gutsell and Russell, Toxicology Research, 2013
Towards an MIE Atlas

• MIE Research at the University of Cambridge
Towards an MIE Atlas

Input Data
- MCSS finds largest fragment common to 2% of training set
- Hits removed from training set

Alert coded

Performance stats calculated (on test set)

Alerts and Performance Data Output

Expert Interpretation
- >5 molecules in training set AND more hits than FPs in test set
- >2 molecules in training set

Alerts selected for Risk Assessment
Alerts selected for Screening

Allen, T.E., Goodman, J.M., Gutsell, S., Russell, P.J., Toxicological Sciences, 2018
Towards an MIE Atlas

- STRUCTURAL ALERT
  - AA2aR 80
  - All other structural alerts for AA2aR

- MODEL
  - for AA2aR activity

- TOOL
  - for MIE prediction
  - All other models for targets
Model performance

Average across all biological targets

<table>
<thead>
<tr>
<th>Model</th>
<th>Alerts</th>
<th>Binders</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen Model</td>
<td>100</td>
<td>1517</td>
<td>81.92</td>
<td>93.45</td>
<td>93.49</td>
<td>0.569</td>
</tr>
<tr>
<td>RA Model</td>
<td>59</td>
<td>1517</td>
<td>66.30</td>
<td>98.58</td>
<td>97.80</td>
<td>0.666</td>
</tr>
</tbody>
</table>

*SE* = sensitivity (percentage of active chemicals the models correctly assigns)

*SP* = specificity (percentage of negative chemicals the model correctly assigns)

*Q* = overall quality (percentage of chemicals the model correctly assigns)

*MCC* = Matthews correlation coefficient (Score from -1 to 1 with a higher score indicating a better model. Scores account for imbalance in dataset).
P(Ki) values for chemical categories of adenosine A2A receptor (AA2AR) binders. The categories are defined by specific structural alerts. The box is the mean value +/- the standard deviation and the whisker shows the absolute range. The categories are ordered from lowest to highest mean P(Ki). Some of the category alerts are shown for clarity.
Quantitative Activity Predictions

2D SARs provide an incomplete picture of receptor binding interactions, as molecules and targets are 3D environments.

Can a Three Dimensional approach provide improved molecular activity estimates and more insight into these MIEs?

Comparative Molecular Field Analysis (CoMFA) provides a potential tool for the construction of 3D QSAR models based on the data and fragments already identified.

2D structural alerts provide chemical categories for the use of these higher level calculations.

This requires the alignment of molecules in the training set and calculation of steric and electronic fields around them. These fields are then analysed using PLS regression analysis to give a quantitative predictive model.

Tosco, P., Balle, T., J Mol. Model., 2011
Mu Opioid Receptor 3DQSAR Results

MOR 3D QSAR Predictions

RMSE (Test) = 0.59
RMSE (Train) = 0.20

Glucocorticoid Receptor Interactions
MIE Characterisation

Only make predictions where a plausible connection can be made from the MIE to an endpoint at an appropriate level of biological organisation

» Provides mechanistically transparent categories for pathway-specific Read Across
» Allows screening/prioritisation of chemicals during innovation
» Indicates pathways of possible concern for further experimental investigation
» Insights can be used across an number of species
Conclusions

- Risk assessments are exposure led and tiered
- Threshold-based approaches can be used where exposures are extremely low
- AOPs summarise existing knowledge in a logical mechanistic framework
- MIE characterisation can be the entry point to predicting pathways of concern and may be relevant across a number of species
- AOPs are just as relevant for Ecotox as for Human tox
- Multidisciplinary, collaborative research is essential
  - Chemists, biologists, informaticians, (eco)toxicologists, mathematical modellers etc
- **Aim to turn toxicology from a science based on observation to one based on informed prediction**
References

• M. Dent M. et al., Principles underpinning the use of new methodologies in the risk assessment of cosmetics ingredients, Computational Toxicology, 2018, 7, 20-26
• I.C. Munro, R.A. Ford, E. Kennesohl, J.G. Sprenger, Correlation of Structural Class with No-Observed-Effect Levels: A Proposal for Establishing a Threshold of Concern, Food and Chemical Toxicology, 1996, 5, 829-867
• SCCS, SCHER, SCENIHR, Joint Opinion on the Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on Cosmetics and Consumer Products, 8 June 2012
• R.W. Tennant and J. Ashby, Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 39 chemicals tested for carcinogenicity by the U.S. National Toxicology Program, Mutation Research, 1988, 257(3), 209-27
• J. Carlsson, R.G. Coleman, V. Setola, J.J. Irwan, H. Fan, A. Schlessinger, A. Sali, B.L. Roth, B.K. Schoichet, Ligand discovery from a dopamine D3 receptor homology model and crystal structure, Nature Chemistry and Biology, 2011, 7(11), 769-78
• S. Gutsell and P. Russell, The Role of Chemistry in Developing Understanding of Adverse Outcome Pathways and their Application in Risk Assessment, Toxicology Research, 2013, 2 (5), 299-307
• P. Tosco, T. Balle, Open3DQSAR: a new open-source software aimed at high-throughput chemometric analysis of molecular interaction fields, Journal of Molecular Modelling, 2011, 17(1), 201-208