TRANSLATIONAL CHEMISTRY IN MECHANISTIC TOXICOLOGY

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Analytical

Predictive

Mechanistic Understanding

Chemistry
• Matrix
• Biological geography
• Tertiary structure
• Exposure
• Dynamic system
ANALYTICAL CHEMISTRY – DRIVING CHANGE

1920’s

Today

Sensitivity

Accessibility

1920s

Today
CHEMICAL CHARACTERISATION

Structure

Purity

Physical chemical properties

- HPLC
- GC
- UV
- MS
- FTIR
- NMR
- CE
- ELISA
CHARACTERISING CHEMICAL-BIOLOGICAL INTERACTIONS

Rate of interaction is critical to understanding effect

Monitoring of the chemical reaction between 4-Nitrobenzyl chloride (100mM) and N-Butylamine (910mM) in Methanol-d₄ @ 310K using ¹H NMR spectroscopy

Mechanistic understanding of molecular initiating events (MIEs) using NMR spectroscopy; Sanderson, P.N et al; Toxicology Research; 5 (2016); 34-44
PEPTIDE REACTIVITY

Adduct Formation Assay
Products of reactions of test chemical and synthetic peptides studied by LC-MS
• Confirm mechanism
• Select read across candidates
• Provide input into mathematical models (e.g. skin allergy)

Kinetics Assay
Rate of reaction between test chemical and synthetic peptides studied by fluorescence spectrometry
• Compare relative reactivity
• Provide a surrogate value for in vivo reactivity in mathematical models (e.g. skin allergy)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Kinetic Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>3.0 (\times) 10^-6 mM(^{-1}) s(^{-1})</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>2.3 (\times) 10^-6 mM(^{-1}) s(^{-1})</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>1.8 (\times) 10^-5 mM(^{-1}) s(^{-1})</td>
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</tbody>
</table>

Example – reactivity of MIT
CONSIDERING CONCENTRATION AT TARGET \textit{IN VITRO}

*Additional interactions introduced by \textit{in-vitro} assays compared to an \textit{in-vivo} system.

Emphasis should be placed on understanding the free concentration available to reach the target and have an effect.

Gutsell, S., Russell, P.J.; Toxicology Research; 2 (2013); 229-307
FREE CONCENTRATION MEASUREMENTS

Ultracentrifugation

Rapid Equilibrium Dialysis

Solid Phase Microextraction (SPME)

Ultrafiltration
Predictions of:

- bond angles/lengths (geometry)
- charges
- orbital energies
- heat of formation
- activation energies
- volume/surface area
- pKa
- logP
- etc etc
Binding of 4-Nonylphenol to the estrogen receptor β
Binding of β-Sitosterol to the glucocorticoid receptor
MECHANISTIC CHEMISTRY AND STRUCTURAL ACTIVITY RELATIONSHIPS

By linking an MIE to effects at any organisational level, we may not need to understand subsequent parts of the pathway

Understanding the molecular interactions at the MIE allows reliable predictions which have less variability brought in by subsequent complex biological networks
METABOLISM

Prediction

![Chemical structures of Caffeine and its metabolites]

Paraxanthine (84%), Theobromine (12%), Theophylline (4%)

Measurement

![Mass spectrometry diagram]

BS

PL
PHYSIOLOGICALLY BASED KINETIC MODELLING

PBK modelling can help identify target organs of concern

Knowledge of physical chemical properties is critical
MOLECULAR DYNAMIC MODELLING

Measurement

Prediction

4-hydroxytamoxifen in 3ERT

AutoDock
CDOKER
Glide
SurflexDock
Libdock
Vina
**Objective:** mathematical model scope should be simplest representation of the chemistry and biology capable of reproducing the induction of contact allergy to enable prediction of a safe level of skin exposure (i.e. to inform risk assessment)
TRANSLATION

‘The conversion of something from one form or medium into another’

Data → Knowledge

Problem Solving
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.

Allen, T.E., Goodman, J.M., Gutsell, S., Russell, P.J.; Chemical Research in Toxicology; 27 (2014); 2100-2112
WHAT DRIVES AN MIE?

- 3D structure & Steric effects
- Physchem properties
- Systemic location
- Individuals (variance, susceptibility)
- Metabolism
- Exposure
SUMMARY

Chemistry is fundamental to understand how molecules interact with biology

- Analytical measurements
- Computational predictions

Translating the chemistry into meaningful information requires a collaborative, cross-discipline approach
THANK YOU

ANY QUESTIONS?
EXPOSURE MEASUREMENTS

• Measurement of free concentrations and binding

• Environmental and biological sampling