CHALLENGES IN DEVELOPING SYSTEMS MODELS FOR SAFETY DECISION MAKING.

ANDREW WHITE
CAN WE USE A NEW INGREDIENT SAFELY?

Will it be safe
- For our consumers?
- For our workers?
- For the environment?

Any information we use must be robust, internationally accepted and underpins the safety of our products.
NEEDS & BENEFITS FOR APPLICATION OF SYSTEMS MODELS

Want to utilise systems models that can **predict** the relationship between **exposure** and **response** using **mechanistic understanding**

- Understand molecular events that lead to adverse effects and select appropriate biomarkers and toxicity pathways based on relevant biology
- Understand the behaviour of an integrated system

Build capability in how to apply this approach

- so that adverse effects measured in *in vitro* systems could be correctly interpreted in the context of risk for human health

Q: Is ingredient X safe to use in product Y at Z%?
Why do we have confidence in this approach?

**CONFIDENCE IN SAFETY ASSESSMENT**

- **Tox Endpoint**
- **NOAEL**
- **Conc. of ingredient due to exposure**

- "there's an established quality of science, reporting standards & audit framework for studies that use in vivo models"

- "animals are intact biological systems - a **suitable model** for the human system"

- "not the ideal model of uncertainty but it is pragmatic"

- "we've done it this way for decades and it seems to work"

- "there's a broad level of scientific **acceptance** in the approach"

- "there's a broad level of scientific acceptance in the approach"
NEXT GENERATION RISK ASSESSMENT (NGRA)

Using new tools and approaches to build risk assessments without animal testing.

- Exposure led
- Mechanistic
- Hypothesis driven

Base decisions on exposure and biological pathway indicated hazard concerns (using human in-vitro cell models).
DEVELOPING MODELS WITHIN A TIERED STRATEGY

Tier I
- Hazard Identification
  - Publications
  - In-silico alerts
  - MIE atlas
  - AOP wiki

Tier II
- Pathway identification
  - Transcriptomics
  - Proteomics
  - Receptor screens
  - Stress Panel

Tier III
- Pathway characterisation
  - Live cell imaging
  - Systems toxicology models
  - Repeat dose
  - Organotypic models

Regression (SAR/QSAR) or Docking Models

Dose-Response Modelling

Systems Modelling

Mechanistic understanding

Uncertainty
Core requirement to utilise biological data to support read across arguments
Utilise approaches to determine Mode of Action of relevance for Risk assessment decisions.

Using gene expression profiles/signatures to represent different biological states, and then to establish the connections among these states based on their gene expression patterns
Primary aim can defining similarity across gene signatures aid in reducing uncertainty in a read-across argument
• Support similarity in mode of action
• Challenge – applicability to non drug like (specific) acting molecules
• Application of information within a dose response curve to strengthen signal
Availability of Data sets

CMAP 1 – 560 gene sets – Affymetrix arrays

CMAP, build 02, [18] data, which comprises 6,100 gene expression instances (treatment vs. vehicle control pairs) from primarily three human cultured cell lines (MCF7, PC3, and HL60) treated with 1,309 bioactive small chemical molecules at varying concentrations
Many relevant compounds will likely have a non-specific toxicity leading to cellular stress.
APPLICATION SCENARIO
- STILL IN DEVELOPMENT

Exposure due to consumer use mg/kg/day

In vitro threshold concentration (uM) measured as free media concentration

Free plasma concentration (uM) corresponding to consumer use, from PBPK modelling

In vitro to in vivo extrapolation and POD comparison
• Predicting systemic exposure

• Enabling us to select and test relevant doses

• Increased role for clinical work to confirm systemic exposure levels
BIOLOGICAL SPACE – IS THE MODEL SUFFICIENT/SUITABLE

- Is technology sensitive or too conservative
- Breadth of coverage of biological pathways
- Biological sample coverage. Complexity of surrogate test system
- Acute vs chronic responses

CCLE

HPA
APPROACHES POINT OF DEPARTURE

Category

5017

6897

6427

non parametric fit

HepaRG Gene changes vs Dose

NOAEL/LOAEL

NOEL

Category

5017

6897

6427

non parametric fit

BMDexpress 2
MARGIN OF EXPOSURE AND SAFETY DECISIONS

Exposure

10^x fold difference?

Tipping point

10 fold difference?

Safety decision

Refine uncertainties

Safety decision
In vitro cell culture

Characterise stress response

- Which cell model? 2D or 3D?
- Primary or cell line?
- Which pathways/biomarkers (coverage)?
- How many time points/dose points?

- How do we calculate the tipping point?
- Number of pathways?
- Duration of response?

Low-risk exposure

Characterise uncertainties to facilitate decision making

- Cells in media vs tissue?
- Chronic vs acute exposure
CELL STRESS PATHWAYS AND TIPPING POINTS

APPLICATION FROM DNA DAMAGE

Use of Model to provide biological relevant mechanistic insight into dose response behaviour – threshold vs linear

*Data from Neumaier et al.*
SCOPE OF LIVER OXIDATIVE STRESS SYSTEMS MODEL
HIGH CONTENT *IN VITRO* ASSAY DATA

**Chemicals:**
- Sulforaphane
- DEM
- tBHQ
- CDDO-Me

**SRXN1, NRF2 & KEAP1**

- **Increased expression of fluorescently tagged Srxn1 (green) with increased dose of Diethyl maleate**

  Prof B. van de Water, U. Leiden

**Mitochondrial ROS**

**ROS/GSH**

**Prof Peng, AMMS**
MODEL FITS TO TBHQ DATA: NUCLEAR NRF2 & SRXN1

31 µM | 56 µM | 100 µM
---|---|---

31 µM | 56 µM | 100 µM

Electrophile

NRF2

SRXN1
EVALUATING THE MODEL: KNOCKDOWN DATA

Prediction based on NRF2 data fits

Knockdown data

[Graphs showing concentration over time for different concentrations and knockdown data]
CHALLENGE OF TEMPORAL EFFECTS
COUPLED PBPK SYSTEMS BIOLOGY MODEL

Intracellular kinetics

(Model predictions)
Response

Given exposure (e.g. tBHQ)

~2 fold difference

100µM tBHQ
RL34 hepatocytes

(Model predictions vs. time and CsA dose for Nrf2 pathway)


Yoshimasa Nakamura et al. (2003). Pivotal Role of Electrophilicity in Glutathione S-Transferase Induction by tert-Butylhydroquinone
APPLICATION OF MODEL FROM EXPOSURE TO POPULATION EFFECT

SKIN ALLERGY RISK ASSESSMENT (SARA) MODEL-BASED FRAMEWORK

Expert judgement on whether product exposure to skin is significant

In silico evaluation & expert judgement on whether ingredient is directly or indirectly protein reactive

product use info.

applied dose & skin toxicokinetic models

skin absorption & peptide kinetics data

exposure to skin

protein reactive

product exposure

clinical potency

allergy risk

Weight of Evidence clinical potency model

DEREK-NEXUS prediction & in vitro data

Human repeat insult patch test (HRIPT) data
### SARA WOE MODEL: PROBABILITY OF EXCEEDING REFERENCE SENSITISATION RATES

**Key:**
- $p < 0.05$
- $0.95 < p < 0.05$
- $p > 0.95$

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**Exposure to skin:***
- **Protein reactive:***
- **Product exposure:***
- **Clinical potency:***
- **Allergy risk:***
HOW SIGNIFICANT ARE YOUR UNCERTAINTIES?

Parameter Uncertainty
- Direct estimates from data
- Expert Knowledge Elicitation
- MC Simulation & Bayesian stats
- Quantitative uncertainty analysis

Model Uncertainty
- Empirical corroboration
- Assess model assumptions

Risk communication with probabilistic outcomes!
BUILDING CONFIDENCE IN USING MODELS AS EVIDENCE IN SAFETY ASSESSMENT
APPLICATION OF MODELS IN RISK ASSESSMENT

Justify deviation from methods...

THE SCCS NOTES OF GUIDANCE
FOR THE TESTING OF COSMETIC INGREDIENTS
AND THEIR SAFETY EVALUATION
9th revision

Pre-validated methods and other in vitro data (non pre-validated) can be used to gather information to provide additional data for the evaluation and interpretation of in vivo or in vitro data, as part of the mechanism of action (e.g. kinetic in vitro data, toxicogenomics, metabolomics),

The safety evaluation of cosmetic substances and finished products remains a scientific exercise that can only be performed on a case-by-case basis.

When major deviations from standardised protocols/procedures in the safety evaluation process occur, a scientific justification is essential.

The "Notes of Guidance" will be revised as scientifically required as the science of toxicology advances, validated alternative methods are adopted and legislative changes are introduced.

...and provide all details for reproducibility...

build the model, preferably in the form of a peer-reviewed publication. Further, all equations, input parameters, information of software used should be provided – preferably in a tabular form.

SCCS will use data from PBPK models for quantitative risk assessment only if sufficient details are provided so that the calculations can be evaluated. Otherwise, the data may only be used as supporting information.
GOVERNANCE AND EXTERNAL DATA STANDARDS – CASE STUDY OMICS

Provision of Guidance has been and is ongoing to ensure recognised standards are met for use in risk assessment.

A study can be truly reproducible when it satisfies at least the following three criteria.

1. For every result, keep track of how it was produced.
2. Avoid manual data manipulation steps.
3. Archive the exact versions of all external programs used.
4. Version control all custom scripts.
5. Record all intermediate results, when possible in standardized formats.
6. For analyses that include randomness, note underlying random seeds.
7. Always store raw data behind plots.
8. Generate hierarchical analysis output, allowing layers of increasing detail to be inspected.
9. Connect textual statements to underlying results.
10. Provide access to scripts, runs, and results.

– All methods are fully reported.
– All data and files used for the analysis are available.
– The process of analyzing raw data is well reported and preserved.

• FDA - MAQC
• MIAME GUIDELINES
• FAIR PRINCIPLES
• Transparency/ Accessibility/ Provenance

Modified from https://www.r-bloggers.com/what-is-reproducible-research/
http://www.reproducible-bioinformatics.org/
HAVE YOU USED STANDARDS IN REPORTING?

**Sources of Models:**
- Developed internally or
- Academic collaboration
- Commercial software
- Literature models
Do you have a suitable model?

Using argument notation to engineer biological simulations with increased confidence

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Arguing ‘fit for purpose’ using Goal Structuring Notation
DISCUSSION

• All the models we develop are meant as tool in the risk assessment process but the specific purpose and therefore biological relevant extrapolation needed may be different

• A whole series of available tools and standards that have been defined and are still be defined within the community – determine which of these are useful to build confidence in the data, models and assumptions

• However, no model (even animal or in-vitro cell based) is ‘perfect’ when do we know the models we use are ‘good’ enough?

• A challenge and need is to capture the uncertainties and explain them

• More available data to utilise and aid understanding of uncertainties and variability in in vitro systems
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