Data Requirements for Developing IVIVE Models

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I disclose that I have no conflicts of interest.
Overview

What data is needed for IVIVE models?

- *In vitro* assays in risk assessment
- QIVIVE: an integrated approach
- Parameters in PBPK models
- Toxicokinetics and toxicodynamics assays
- Challenges translating *in vitro* effect concentrations
In Vitro Assays

- Toxicodynamics
- Toxicokinetics

![Diagram of in vitro assays showing concentration vs. % of living cells with EC50 marker.]

- Apical compartment
- Cell monolayer
- Porous membrane
- Basolateral compartment

Chemical (or metabolite)
In Vitro Assays in Risk Assessment

- Toxicity Testing in 21st Century

- From hazard identification to hazard characterization

- Quantitative *In Vitro-In Vivo* Extrapolation (QIVIVE)
  - Estimating environmental chemical exposures producing target tissue exposures in humans equivalent to those associated with effects in *in vitro* toxicity tests
In vitro toxicodynamics
Mouse embryonic stem cell test

In vitro toxicokinetics
Biotransformation kinetics in hepatocytes

Input: BMC values metabolite alkoxyacetic acid
Output: predicted oral embryotoxic dose parent glycol ethers

Evaluation of IVIVE model with in vivo embryotoxic doses
Integrated Approach

- PBPK, reverse dosimetry
- Exposure assessment
- Sensitivity analysis
- *In silico* tools (e.g. read-across, QSAR, QSPR)
- AOP
- *In vitro* tools, KE / TK
- Margin of exposure
- Tiered/iterative process
Steps in IVIVE Model Development

Bottom-up approach

1. QSAR QSPR
   - Metabolite ID

2. In Vitro Kinetics
   - Hepatic clearance
   - Intestinal uptake / metabolism
   - Renal clearance
   - Partitioning

3. Reverse Dosimetry

4. In vivo Human Toxicity Estimate
   - In vitro exposure profiles
   - Target tissue response

Potential Target Tissue

In vivo Dynamics

Nature of Toxicity

From Miyoung Yoon
Parameterization PBPK for IVIVE

- Physiological/Anatomical
  - Tissue blood flow or respiration rate (Q, e.g. L/hr)
  - Tissue volumes (V)
  - Glomerular filtration rate


\[
dA_t/dt = V_t \cdot dC/dt = Qt \cdot (C_{in} - C/P) \\
C_{out} = C_{free} = C_{tissue}/P \\
dA_m/dt = R = C_{free} \cdot V_t \cdot K = (V_{max} \cdot C_{free})/(K_M + C_{free})
\]
Parameterization PBPK for IVIVE

Physicochemical

- Tissue-blood partition coefficients ($P = \frac{C_{\text{tissue}}}{C_{\text{free}}}$)
- Protein binding constants
- Tissue clearance/elimination rates ($K, V_{\text{max}}, K_M$)
- Absorption (bioavailability)

In silico tools

- QSPR estimating partitioning/protein binding
- Challenge: metabolic clearance parameter (QSAR limited, mostly qualitative, METEOR, OECD Toolbox, Multicase)

\[
\begin{align*}
\frac{dA_t}{dt} &= V_t \cdot \frac{dC}{dt} = Qt \cdot (C_{\text{in}} - C/P) \\
C_{\text{out}} &= C_{\text{free}} = \frac{C_{\text{tissue}}}{P} \\
\frac{dA_m}{dt} &= R = C_{\text{free}} \cdot V_t \cdot K = \left(\frac{V_{\text{max}} \cdot C_{\text{free}}}{K_M + C_{\text{free}}}\right) \\
\end{align*}
\]

\[\Rightarrow \quad \text{In vitro tools}\]
Toxicokinetics Input

- In silico tools
- Tier 1

Passive diffusion down C gradient

\[ J = P_{\text{app}} \times SA \times C \]

Diffusion into tissue
Non-saturable protein binding
Well-stirred tissue
Whole-body D perfusion-limited

Liao et al. (2007) Risk Anal. 27, 1223
Wetmore (2015) Toxicol. 332, 94
Toxicokinetics Input

- **In vitro tools**
  Bessems et al. (2014) Reg. Toxicol. Pharmacol. 68, 199

Absorption
- Oral (Caco-2, PAMPA)
- Dermal (OECD TG 428, Skin PAMPA)
- Inhalation (Head space model for VOS, Calu-3)

Distribution
- Partition coefficients
- Protein binding
- Permeation coefficients

Metabolism
- Human microsomes, (cryopreserved) primary hepatocytes, HepaRG, HepG2

Excretion
- See distribution & human physiology, headspace model, e.g. cell lines RPTEC/TERT1
## In Vitro Tools for Distribution Parameters


<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibrium Dialysis</td>
<td>Widely used</td>
<td>Time consuming, membrane binding</td>
</tr>
<tr>
<td>Ultracentrifugation</td>
<td>No binding to apparatus</td>
<td>Expensive apparatus</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Fast and inexpensive</td>
<td>Filter binding, equilibrium shift</td>
</tr>
</tbody>
</table>
Solid Phase Microextraction

- Measuring $K_{\text{compartment}}$
- SPME

- Measuring free conc.
- Nd-SPME

Amount fiber $<<$ Amount medium

$$K_{f} = \frac{C_{f(eq)}}{C_{\text{free(eq)}}}$$

$$F_{f} = \frac{1}{1 + \frac{V_{m}}{V_{f} \cdot K_{f}}(1 + K_{a} \cdot [A])}$$

Increasing serum concentration
In Vitro Tools for Metabolism Parameters

- Tissue slices
- Primary hepatocytes
- Hepatic cell lines
- Microsomes, cytosol
- Recombinant CYPs

- Parent chemical disappearance vs. metabolite generation
- Dose-response (Vmax, Km) vs. activity
Toxicodynamics Input

- Concentration-effect relationships
- AOP
- ‘Omics’
Parameterization Challenges

- Variability in *in vitro* readout
- Poorly defined / Uncertainties
  - Chemical applicability domain
  - Biological applicability domain/link effect with adversity
  - *In vitro* effective dose
- Good Modeling Practice
  - Formal verification process useful
- Good Cell Culture Practice
  - maximise reproducibility, reliability, credibility, acceptance and proper application of *in vitro* results
  - minimum standards in cell and tissue culture

Loizou et al. (2008) Reg Toxicol. Pharmacol. 50, 400
In Vitro Dose Metrics

- Plastic binding
- Protein binding
- Evaporation
- Free in medium
- Plastic binding
- Cell binding
- MEDIUM
- CELL
- Target
- Metabolism
- Free in cell

(Schematic diagram showing the different dose metrics and their interactions.)
Cell Assay Components ...

... determining target concentration *in vitro*

- Medium components (e.g. serum, 3D matrix)
- Headspace/air flow
- Well plate dimensions
- Cell density
- Exposure time
- Temperature
- pH
- Metabolic capacity
- Transporter expression
Physicochemical Properties …

…determining target concentration in vitro

- $K_d / \text{LogP}/\text{LogD}_{7.4}/ K_{OW}$
- $H$
- $pKa$
- Solubility
- Reactivity
- Transporter affinity
- Metabolic enzyme affinity
Medium Constituents

Medium Components


Serum: 1.25%  2.5%  5%
Nominal EC$_{50}$: 73 µM  105 µM  187 µM
Free: 7%  5%  3%
Free EC$_{50}$: 5 µM  5 µM  6 µM
### Medium Components


<table>
<thead>
<tr>
<th>Serum (%)</th>
<th>1.25%</th>
<th>2.5%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal EC$_{50}$ (µM)</td>
<td>73</td>
<td>105</td>
<td>187</td>
</tr>
<tr>
<td>Free (%)</td>
<td>7%</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>Free EC$_{50}$ (µM)</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

- Proteins vs. lipids
- Neutral vs. ionized

Polycyclic aromatic hydrocarbons
Well Plate Dimensions

Polycyclic aromatic hydrocarbons

Dependent on:
- Chemical (ionization)
- Concentration
- Time

EU FP7 Predict-IV
- Kramer et al. 2015 Toxicol. In Vitro 30, 217

### Recovery from medium after 48h
- Conventional dosing: 11%
- Continuous dosing: 105%

### EC$_{50}$ (µM)
- Conventional: 135
- Continuous total: 38
- Continuous free: 11
- Fathead Minnow: 16

### Bound to Serum Constituents
- 70%

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**Graph:**
- **Y-axis:** % of control
- **X-axis:** Log 1,2,4-trichlorobenzene (concentration in µM)
- **Legend:**
  - Alamar Blue
  - CFDA-AM
  - Neutral Red
  - Cont. free
  - Cont. total
  - Nominal
Exposure Time

- 1800 uM*min = EC_{50}
(see also Gulden et al. (2015) Toxicology 335, 35, time to incipient EC_{50} is chemical dependent)
Cell Density


\[
\text{% of viable cells} \quad \text{Dieldrin (µM)}
\]

\[
\text{Log } K_{\text{OW}} \quad \text{Log } K_{\text{lipid}}
\]
Cell-Associated Concentration

Groothuis et al., manuscript in preparation

![Graph showing concentration vs. nominal concentration](image)

- **Nominal concentration (µM)**
- **Concentration in cell lipid (mmol/kg)**

- EC50: Various concentrations for BAC 10, 12, 14, 16, 18
Cell-Associated Concentration

Groothuis et al., manuscript in preparation

- Chemicals acting by narcosis (basal cytotoxicity, accumulation in cell membranes)
- Passive vs. active uptake/efflux
- Time

![Graph showing concentration in cell lipid vs. nominal concentration](image)
Repeated Dosing

Wilmes et al. (2013) J Proteomics 79: 180


Area under the curve or Cumulative dose (irreversible mechanism?)
Modeling Kinetics *In Vitro*

Modeling Kinetics *In Vitro*

\[ F = \frac{1}{1 + 10^{0.37 \log K_{ow} - 0.29}[S] + 10^{0.97 \log K_{ow} - 6.94}[P] + 10^{1.25 \log K_{ow} - 3.70}[C] + \frac{H}{8.3144T} \cdot \frac{V_a}{V_m}} \]

\[ C_W = \frac{1}{K_{AW}V_A + V_W + K_{SAW}V_{SA} + K_{SW}V_{SI} + K_{DW}V_D + K_{CW}V_C} \]
RTgill-W1 exposed to phenanthrene

<table>
<thead>
<tr>
<th>Serum</th>
<th>0%</th>
<th>2%</th>
<th>5%</th>
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<tbody>
<tr>
<td>Measured</td>
<td>21%</td>
<td>8%</td>
<td>5%</td>
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<tr>
<td>free</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modeled</td>
<td>32%</td>
<td>9%</td>
<td>5%</td>
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<tr>
<td>free</td>
<td></td>
<td></td>
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<tr>
<td>Measured</td>
<td>10%</td>
<td>4%</td>
<td>2%</td>
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<tr>
<td>in cells</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Modeled</td>
<td>14%</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>in cells</td>
<td></td>
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</tr>
</tbody>
</table>

Viability (% of control) vs. Log phenanthrene (μM)
Conclusions

- Data needed for IVIVE model dependent on
  - Required precision
  - Chemical
  - Exposure
- Physiological vs. chemical-specific parameters
- Tiered, integrated approach
- Partitioning parameters from *in silico* tools
- Clearance parameters from *in vitro* tools
- Define applicability domains tools for parameterization
- Consider *in vitro* kinetics as well as *in vivo* kinetics
Thank you

- Questions?
  - At this time, please limit questions to those specific to this presentation, and save general questions for the Roundtable Discussion with all the presenters.
References

- Groothuis et al. (2015) Toxicol. 332, 30-40
Acknowledgements

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