Exposure and Dosimetry Considerations for Adverse Outcome Pathways

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Adverse Outcome Pathways: From Research to Regulation
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Introduction

- Risk is a function of both hazard and exposure.
- Toxicokinetic (TK) models can determine whether chemical exposures produce potentially hazardous tissue concentrations.
- Whether or not an AOP initial molecular event (MIE) occurs depends on both exposure and TK.
- As high throughput screening (HTS) identifies putative MIEs and key events, chemical-specific TK and exposure data will be needed to make prioritizations based on risk.
Context for Adverse Outcome Pathways

Activation or Deactivation of Receptor by Binding or Displacement of Endogenous Compound

- Peroxisome Proliferators
- Dioxin-like Compounds (AHR)

Cascade of Proliferative Signals
- PPARs
- C-fos, jun-B, c-jun, jun-D

Cell Cycle Progression (G1 Chckpt.)

Inappropriate Proliferation

Hepatic Lesions

Inappropriate Apoptosis

Apoptosis
- DNA Damage Sensing
- TGFB1
- Bcl2

Homeostasis

Putative AOP derived from:
Roberts et al. (1997)
Guyton et al. (2009)
Initial Molecular Event

Cascade of Proliferative Signals

- PPARs
- C-fos, jun-B, c-jun, jun-D

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Homeostasis

Activation or Deactivation of Receptor by Binding or Displacement of Endogenous Compound

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Exposure: How Many Molecules Are There?

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Cascade of Apoptotic Signals
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Apoptosis

DNA Damage Sensing

C-fos, jun-B, c-jun, jun-D

Cell Cycle Progression (G1 Checkpt.)

Inappropriate Proliferation

Inappropriate Apoptosis

Hepatic Lesions
Toxicokinetics: How Many Molecules Get to Site of Action?

Cascade of Proliferative Signals
- PPARs
- C-fos, jun-B, c-jun, jun-D

Cell Cycle Progression (G1 Checkpoint)
- Inappropriate Proliferation
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Cascade of Apoptotic Signals
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Homeostasis

Sequestered in Tissue

Excreted
Differences in species and dosing regimen can create apparent differences in doses needed to produce adverse effects.

Figure from Wambaugh et al. Tox. Sci. (2013)
PK Modeling of tissue concentrations can reconcile these differences.

Figure from Wambaugh et al. Tox. Sci. (2013)
There are thousands of chemicals, most without enough data for evaluation.

High throughput *in vitro* methods (e.g., ToxCast) beginning to bear fruit on potential hazard for many of these chemicals.

High throughput toxicokinetic methods (HTTK) approximately convert these *in vitro* results to daily doses needed to produce similar levels in a human (IVIVE).

High throughput exposure forecasting (ExpoCast) can bound mean human exposures for key populations.

e.g. Judson *et al.*, (2011)
Concordance of *In Vitro* Bioactivity, *In Vivo* Toxicity, and Exposure

Estimated or measured average serum or plasma concentrations associated with the

- **LOAEL** (solid) or
- **NOAEL** (open)
dose rates in animal studies underlying existing toxicity reference values

Estimated average serum or plasma concentrations in humans consistent with chronic exposure reference values

Biomonitored serum or plasma concentrations in:

- occupational populations
- in volunteers using products containing the chemical
- the general population

Aylward and Hays (2011)
Journal of Applied Toxicology 31 741-751
Data Availability for *In Vitro* Bioactivity, *In Vivo* Toxicity, and Exposure

- As in Egeghy et al. (2012), there is a paucity of data for providing context to HTS data.
**Tox21**: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)

**ToxCast**: For a subset (>1000) of Tox21 chemicals ran >500 additional assays (Judson et al., 2010)

Most assays conducted in dose-response format (identify 50% activity concentration – AC50)

All data is public: [http://actor.epa.gov/](http://actor.epa.gov/)
Putative Molecular Initiating Events

HepaRG cells treated by ThermoFisher (formerly Cellzdirect)

Gene expression conducted by Expression Analysis

93 assay genes + 3 house keeping genes (for normalization) on a Fluidign Chip

Number of Assayed Genes Downstream of Nuclear Receptor

ToxCast HepaRG analysis not yet complete
ToxCast *in vitro* AC50s

- One point for each chemical-*in vitro* assay combination with a systematic (Hill function) concentration response curve

*Results from Wetmore et al. (2012)*
Successful methods have been developed for pharmaceutical compounds to determine high throughput TK (HTTK) from limited in vitro measurements and chemical structure-derived property predictions.

In vitro plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated.

At steady state this allows conversion from concentration to administered dose.

No oral absorption/bioavailability included.

Steady-State Plasma Concentration

\[ C_{ss} = \frac{\text{oral dose rate}}{(GFR \times F_{ub}) + \left( \frac{Q_{l} \times F_{ub} \times CL_{int}}{Q_{l} + F_{ub} \times CL_{int}} \right)} \]

Oral dose in (mg/kg/day) \[\rightarrow\] Sum of hepatic and renal clearance (mg/kg/day)
Steady-State Model is Linear

Steady-state Concentration ($\mu$M) vs. Daily Dose (mg/kg/day)

**Prediction**

Slope = $C_{ss}$ for 1 mg/kg/day

$C_{ss} = \frac{\text{oral dose rate}}{(GFR \times F_{ub}) + \left(Q_{int} \times F_{ub} \times \frac{Cl_{int}}{Q_{int} + F_{ub} \times Cl_{int}}\right)}$

- Can calculate predicted steady-state concentration ($C_{ss}$) for a 1 mg/kg/day dose and multiply to get concentrations for other doses
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)

- Swap the axes
- Can divide bioactive concentration by $C_{ss}$ for a 1 mg/kg/day dose to get oral equivalent dose

**Prediction**

Slope = mg/kg/day per $C_{ss}^{1 \text{mg/kg/day}}$
- It appears harder to prioritize on bioactive *in vitro* concentration without *in vivo* context

*Results from Wetmore et al. (2012)*
Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies.

*Results from Wetmore et al. (2012)*
Physiologically-based Toxicokinetic (PBPK) Model

- Out of 239 ToxCast chemicals examined by Wetmore et al. (2012), only 11 had some sort of human-relevant TK data or model
- HTTK predictions of steady-state behaviors were generated in Wetmore et al. (2012) using *in vitro* TK methods
- Can build generic, high throughput PBPK (HTPBPK) models parameterized with
  - the same *in vitro* HTTK data used for steady-state work, **plus**
  - QSARs for tissue-specific properties
  - Assumptions about unknown dynamic processes, such as absorption
- These HTPBPK models can provide a simulated in vivo context for tissue simulations
Predicted PK Metrics

- Human hepatic concentration of various chemicals as a function of 28 daily doses (10 mg/kg/day)

- Can predict mean and peak concentration and time integrated area under the curve (AUC) for various tissues
Evaluating HTPBPK Predictions from *In Vitro* Data

- HTPBPK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~3.6x higher than intravenous dose AUC (p-Value 0.021)
Evaluating HTPBPK Predictions from *In Vitro* Data

- $C_{\text{max}}$ predictions relatively decent ($R^2 \sim 0.69$)
The Exposure Component of Risk

- Ultimately hope to do a rapid risk prioritization of chemicals with minimal information
- Identify chemicals most in need of additional resources and traditional methodologies
- Risk is the product of hazard and exposure
- High throughput exposure forecasting (ExpoCast) can bound mean human exposures for key populations

\[ \text{Risk} = \text{Hazard} \times \text{Exposure} \]

Potential Exposure from ExpoCast

Potential Hazard from ToxCast with Reverse Toxicokinetics

mg/kg BW/day

Lower Risk  Medium Risk  Higher Risk

e.g. Judson et al., (2011)
Systematic Empirical Evaluation of Models (SEEM)

Data and Models

- Chemical Manufacture
- Environment al Release
- Food
- Air, Soil, Water

Direct Use (e.g., lotion)
Residential Use (e.g., flooring)

Air, Dust, Surfaces
Waste

Near-Field Direct
Near-Field Indirect

Dietary
Far-Field
Ecological

Human

Biomarkers of Exposure
Media Samples
Biomarkers of Exposure

Ecological Flora and Fauna

EXPOSURE PATHWAY (MEDIA + RECEPTOR)

Data and Models

- Monitoring Data
- Receptors

DATA and MODELS
Illustration of the SEEM Framework

Apply calibration and uncertainty to other chemicals

EDSP Chemicals
QSARs and HTE Data
Biomonitoring Data
Dataset 1
Dataset 2

Exposure Inference

Inferred (Reverse) Exposure

Evaluate Model Performance and Refine Models

Forward Predictions

Calibrate models

Model 1
Model 2

Evaluate Model Performance and Refine Models

Estimate Uncertainty

Evaluate Model Performance and Refine Models
Five factors can explain roughly 50% of the chemical-to-chemical variance in NHANES chemical exposures across demographics, including women of child-bearing age and children aged 6-11.
We focus on the median and upper 95% predictions because the lower 95% is below the NHANES limits of detection (LoD).

Dotted lines indicate 25%, median, and 75% of the LoD distribution.
Exposure Predictions for 7968 ToxCast Chemicals

- Chemicals currently monitored by NHANES are distributed throughout the predictions
- Chemicals with the first and ninth highest 95% limit are monitored by NHANES
Conclusion

- Using in vitro TK methods developed for pharmaceuticals, we can parameterize HTPBPK models

- We can model the difference between \textit{in vivo} measurements and HTTK predictions (\textit{i.e.}, the residuals or errors)

- We can connect HTPBPK models to tissue simulations to provide simulated \textit{in vivo} context for assessing the impact of chemical perturbations identified by high throughput screening assays
Rapid Exposure and Dosimetry
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NERL
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ToxCast HepaRG Assay
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