NTP
National Toxicology Program

In Vitro to In Vivo Extrapolation for High Throughput Prioritization and Decision Making

Wednesday, February 17 · 8:00 a.m. – 6:00 p.m.
Thursday, February 18 · 8:30 a.m. – 3:00 p.m.
http://ntp.niehs.nih.gov/go/ivive-wksp-2016

EPA United States Environmental Protection Agency

Breakout Group Questions

In Vitro to In Vivo Extrapolation for High Throughput Prioritization and Decision Making

- During the discussion, keep in mind the following global questions:
  - What are the effects/implications when considering human vs. rat values, or non-animal vs. in silico values?
  - How are we defining the "purpose" in fit-for-purpose, and what are the implications for using the approach or assumption in each application (prioritization/screening/risk assessment)?

	Group A: **TK Model Considerations**	**Group B:** **In Silico and Non-Animal Methods for Obtaining TK Parameters**	**Group C:** **Application to Prioritization/ Screening/Risk Assessment**
Session 1 **8:30-10:00 a.m.**	• What needs to be done to determine the state of the science (including current toolbox)? How well are these tools working for understood chemicals / kinetic processes? • What are the pros and cons of a simple (one-compartment) model? How do we assess when models are good enough?	• What experiments/methods are needed for determining oral bioavailability? What about methods for other routes of exposure? • What is best practice for rapidly parameterizing a model? How should confidence in these parameters be evaluated and reported?	• Who are the stakeholders? What are their needs? How do their needs vary? • How do we increase buy-in and what are the training needs? On regulatory and industry side? How do we build capacity and what resources are needed?
Session 2 **10:15-11:45 a.m.**	• How can the in vitro output be related to the in vivo toxicity/adverse outcome? • How do we validate methods and approaches (context, limitations, scope)?	• How do we define the domain of applicability for the in silico models? How should this be evaluated and reported? • How do we store/share models and information/data? What reporting requirements are needed? Do existing reporting formats currently exist, or can existing formats be changed to meet our needs?	• Can IVIVE refine how default uncertainty factors are applied? Can it be used to develop data-driven uncertainty factors (interspecies and inter-individual)? • What are the requirements or implications for use in prioritization/regulation? What areas are ready to incorporate IVIVE in the short term? In the long term?

Breakout group A: Toxicokinetic Model Consideration

Annie Jarabek, EPA
Alicia Paini, EURL ECVAM
Judy Strickland, ILS NICEATM

Questions:

A1:	What needs to be done to determine the state of the science (including current toolbox)?
A2:	How well are these tools working for understood chemicals / kinetic processes?
A3:	What are the pros and cons of a simple (one-compartment) model?
A4:	How do we assess when models are good enough?
Break	
A5:	How can the in vitro output be related to the in vivo toxicity/adverse outcome?
A6:	How do we validate methods and approaches (context, limitations, scope)?

What needs to be done to determine the state of the science (including current toolbox)?

- In vitro data needs context (what does it mean?). Need to communicate this when data are reported.
  - Do we always need to extend to ECSS equation?
    - Replication of biology (e.g. clearance terms). Fecal elimination is missing in equivalent dose equation
    - In vitro data could be organized in domains for type of assay (around mechanism)
    - E.g. httk for prioritization. What assays should be used?
    - Only regulatory context for HTS so far is endocrine disruption – for prioritization
      - Research areas use distribution of AC50s
    - Use HTS to bin by mechanism
- Review with a quantitative evaluation of models
  - How do parameter omissions affect the result?
  - Bin the review to chemicals and biological systems
  - What is distribution of bioavailability and other parameters?
  - State of the science depends on the question to be answered. What is the purpose? May not be relevant to certain routes.

What are the pros and cons of a simple (one-compartment) model?

- ECSS extend with elimination pathways
  - Can compute the parameters needed
- Easy to understand, most freely available (encourages use)
- Education will make models more understandable/accepted
  - Easy to run models may not be well understood (anyone can run them!)
- Open access models enhances transparency
  - SEURAT models provided workflow (can run on website or on desktop with code)

How can the in vitro output be related to the in vivo toxicity/adverse outcome?

- Model is important. Nominal concentrations can be misleading.
  - ECVAM's cell culture model for chemical distribution. Translates dose to oral or dermal exposure.
    - Chemicals could be binned by MOA
- Need methods that use the boxes between exposure and apical endpoint (i.e, key events)
- Focus on AOP **sufficient** key events (assures adverse outcome occurs)
  - NCEA determines these mathematically. Start with a disease and work backward (which nodes are affected).
- What is the in vitro assay a surrogate for?
  - Cell death is different from receptor activation
  - What diseases are expected?
  - Need quantitative work to determine relative contribution of AOP and at what exposures

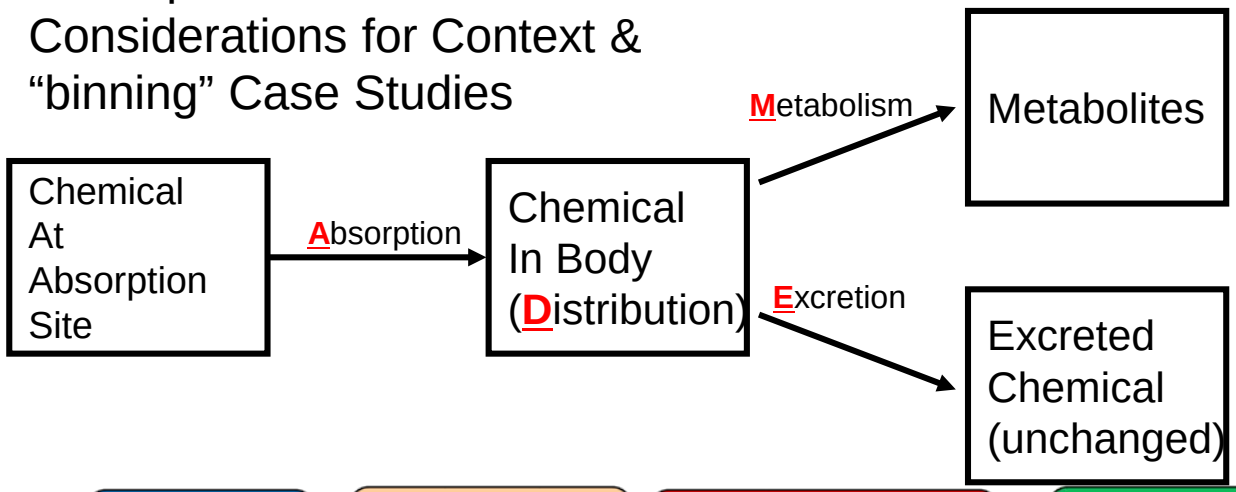
How do we assess when models are good enough?

- Context is everything!
  - Degree of biological fidelity needed
  - Level of confidence needed.
    - Empirical v mechanistic description. Uncertainty in in vivo and in vitro data used
    - What do risk managers consider acceptable in a given context?
    - Can minimal levels of practice be defined for the individual applications?
- Consider whether accuracy and biological fidelity are acceptable for a given application
  - Should results be compared to current procedures (i.e., UF)?
- We need general improvement in the whole process
  - We need better assays, better designs, better data!
- Use level of confidence to determine which purpose a model can be used for.
  - What is improvement in prediction by adding a certain feature?
- Need to understand the variability of individual model parameters – how do influence prediction of in vivo parameters

How do we validate methods and approaches (context, limitations, scope)?

- Considerations: Biological systems, MOA, drug/chemical properties, experimental design
  - Define best practices, modeling your assay.
    - What caused effect observed (i.e., concentration at receptor)? Helps feed AOP.
  - How does it compare to existing method? Existing method may be terrible. So how do we evaluate new methods? Need a new way.
    - Moving from PK to PBPK
    - Compare to orthogonal in vitro assays?
    - Do we need ex vivo assays?
    - Need an animal in vitro suite of assays to replicate the whole animal (the in vitro data are mostly human but we don't have human data to compare it with)
    - Should animal studies be done to better understand rat physiology/toxicology. Animals could be added to existing experiments.
  - When comparing to default assumptions, we need to convey what the default represents
    - Design validation around particular endpoint studies (e.g. 90 day study – for specific toxicity endpoint/adverse perturbations in specific organ systems)?
    - In vitro assays may identify endpoints not noted in an in vivo study (e.g. 90 day study)
  - How can we predict outcomes outside the training set? (e.g., can you change exposure route (or can we scale a rodent model to human) and still get a reasonable answer)
    - Assay design - if we looked at major diseases, what should we target for assay development?
    - NTP's HTS gene array developed by asking for nominations

Conceptual Construct for Mechanistic Considerations for Context & "binning" Case Studies



Systems Data	MOA/Assay Target	Drug / Chemical Data	Experimental Design
Biological parameters for • Respiratory • Dermal • GI	Disease burdens Toxicological endpoints e.g., EDSP	ECSS + PC properties pKa Permeability Solubility Acids/Zwits Bases/Neutrals	Cell free Suspensions Sandwiches Culture conditions

Action Items

- Characterize the differences between drugs and environmental chemicals
  - Drugs are ionizable
  - Industrial chemicals are neutral organics
  - Drugs designed to active in parent form. Environmental chemicals may be metabolized to actives
- Constructing a paradigm with the different considerations for determining a path forward

Breakout group B-***maximum strike force***: In silico and non-animal methods for obtaining TK parameters

John Wambaugh, EPA
Nisha Sipes, NIEHS-NTP
Neepa Choksi, ILS NICEATM

Questions:

B1:	What experiments/methods are needed for determining oral bioavailability?
B2:	What about methods for other routes of exposure?
B3:	What is best practice for rapidly parameterizing a model?
B4:	How should confidence in these parameters be evaluated and reported?
Break	
B5:	How do we define the domain of applicability for in silico models?
B6:	How should domain of applicability be evaluated and reported?
B7:	How do we store/share models and information/data?
B8:	What reporting requirements are needed (for models and data)? Do existing reporting formats currently exist, or can existing formats be changed to meet our needs?

Take Homes

- Need to know what we're looking at: cellular partitioning vs. oral bioavailble
- Need a public database for reporting information
  - Consistent format for collecting and reporting formation
  - Machine readable
- Better communication regarding the scope and limitations of the model
  - Applicability domain (e.g., chemical class)
  - Assumptions and parameters used for model development
- Consistent model used for data collection to inform model development

Human vs. Rat

- PBPK is essential for across-species extrapolation
- Descriptions needed on all parameters
  - Dose route (gavage vs. drinking water)
  - Formulation
- Microsome and/or S9 allows for easier species extrapolation, but you miss biology
- Hepatocytes give you more biology, but need several species
- Caco2 is as useful as it is, regardless of species

Bioavailability

- Mix of solubility and ionization, fraction absorbed in gut, first pass hepatic metabolism, and formulation
- Models available for all parts
  - But should measure hepatic metabolism and fraction absorbed
- Potentially can use QSAR to estimate value of data

Action item: Dream Database

- In vitro and In vivo PK/TK data
- Values from peer-reviewed publications (and the papers/reports themselves)
- Model code (MEGen XML?)
- Provides a MIAME-like standard for reporting with teeth
- Can we make it like StackExchange?
- Machine readable data and models
- Home for negative data – DOI for data

Breakout group C: Application to prioritization/screening/risk assessment

Nicole Kleinstreuer, NIEHS-NTP

Scott Lynn, EPA

Dave Allen, ILS NICEATM

Questions:

C1:	Who are the stakeholders? What are their needs and how do their needs vary?
C2:	How do we increase buy-in and what are the training needs (considering both regulatory and industry sides)?
C3:	How do we build capacity and what resources are needed?
Break	
C4:	Can IVIVE refine how default uncertainty factors are applied?
C5:	Can IVIVE be used to develop data-driven uncertainty factors (interspecies and inter-individual)?
C6:	What are the requirements or implications for use in prioritization/regulation?
C7:	What areas are ready to incorporate IVIVE in the short term? Long term?

Overarching Themes

- Transparent communication (between govt/industry, govt/govt, etc.)
- Scientific confidence framework
- Clear definitions of current regulatory requirements
- International harmonization
- Hazard- vs Risk-based assessments
- Fit-for-purpose validation

Stakeholders/Needs

- Regulatory agencies (US and international)
- Industry
- Communities
- NGOs
- Consumers
- Base knowledge transcends across all groups
- Applicability domain will vary significantly among them (and even within chemical sectors – e.g., EPA – pesticides, industrial chemicals, Superfund issues)
- Application drives shared stakeholder needs (e.g., prioritization, screening, otherwise?)

Stakeholders/Needs

- Legal Implications
  - Daubert standard (5 criteria a judge has to weigh before determining it is OK for testimony)
  - Multi-lab; peer reviewed; error rate; standards/controls; widespread acceptance within scientific community
- Areas where in vitro could be used if there was a clearer understanding of what in vitro data mean
  - Superfund
  - IRIS
  - TSCA
  - FIFRA
  - REACh
- Look at specific endpoints to gain consensus
- Worker safety – different exposure paradigms; need to identify worst case
- PMN process – structure based decisions to order testing (traditionally animal tests)
  - Therefore, how to get test orders directed towards non-animal

Stakeholders/Needs

- Communities
  - How to develop public trust in results (e.g., Elk River spill)
  - Need to contextualize results – bring exposure into the discussion from the beginning is critical
- How to capture concerns associated with bi-products (e.g., HAPs – hazardous air pollutants; VOCs)
  - Currently done ad hoc
  - Data poor chemical issue
  - Most techniques are aqueous based – volatiles not currently within applicability domain
- Priorities vary depending on stakeholder and the specific testing purpose they are fulfilling (prioritization, screening, hazard id, RA)
- Consumer level confidence

Increasing Buy-in

- Transparent communication is key
- HESI project – Framework for Non-animal Methods
  - Apply SCF to methods themselves (list of criteria to rate assays – to allow a common rubric across assays)
  - Model predictive performance (what is needed to have confidence in a model?)
  - Utilization – what is needed to match assay/model to a particular level of decision making
- Industry makes decisions early on (pre-submission) based on non-animal results
  - How to utilize these data to better inform issues described above?
  - "safe harbor" needed? – more transparency in how these data would be used/applied
  - Biomarker qualification process at FDA is a precedent
- Journal editors/review processes important to ensure that publications have proper biological context
  - Control press releases that spread "mis-information"
  - Reporters have associations that can be a centralized resource for communications

Increasing Buy-In/Resources Needed

- How best to “vet” assays/approaches that rise to the top?
  - Will be a fluid process – important to consider as we gain more experience/data
- Fit-for–purpose validation and performance-based test guidelines
- Academic community is at the cutting edge of methodologies
  - Need proper context and association with testing needs
  - Specific guidance to help better inform key principles/needs for adoption/implementation
  - SBIR Phase 2B vehicle for test method validation
- Increasing analytical techniques and ability to detect low levels of compounds
- Developing standards (e.g., GLP) for HTS/in silico with minimal reporting requirements
- Develop techniques for HAPs/VOCs

Refining uncertainty factors

- Need to address discomfort with “unknown uncertainties”
- Current practice – uncertainties associated with: database (i.e., to account for missing information); inter- and intra- species; LOAEL-NOAEL; subchronic to chronic
- Can now apply data-driven uncertainty factors based on ExpoCast predictions
- Critical to determine where the uncertainty factors are best applied (at the end?)
- Need to characterize the uncertainty in the physiological parameters that are being modeled (i.e., don’t use single point estimates, but instead, distributions)
- Need to educate on use and application
- Need to define the target – what are UFs intended to address? (susceptible populations, etc.)
  - Otherwise, there will always be criticism that something isn’t covered (i.e., what is the dose that will be protective to all populations – model elderly, infants, etc.)

Refining uncertainty factors

- Continual issue: Where do I get the data?
  - Need to improve international communication on where reliable data sources can be found/applied
  - And communicated in a biological context
- Use Bayes factors (frequently used in medicine)
  - can establish relative risk
- Monte Carlo sampling methods to characterize variability
- Allows use of consistent data sets on all chemicals

Use in prioritization/regulation?

- Normal exposures vs catastrophic exposures
  - Can IVIVE be used to inform?
- Need to consider international regulations (i.e., EU regs on hazard; US on risk)
  - Lack of harmonization of requirements presents corporate challenges for global companies (i.e., test based on “most extreme” requirements)
  - Until animal tests are rejected, they will be done to fulfill requirements
  - Also must address differences with member states
- Again emphasizes the need for transparent communication

Short and Long Term

- Short term:
  - EDSP provides an example of current use
  - Dose selection (and dose spacing) and acceptability of traditional tox studies (rather than MTD to eliminate high dose phenomenon)
    - also to extrapolate to relevant human dose to support selection of lower, more relevant doses
  - Application to data poor areas (some data better than none)
  - Better risk communication
- Long term:
  - Necessary component of a scientific confidence framework for in vitro assays and to put into proper context of exposure
  - Necessary for dose response assessment of in vitro data for RA applications
    - Should become part of the toxicologist's and risk assessor's lexicon
  - Incorporate into the AOP framework (e.g., BPAD approach – Judson et al.)
  - Defining chemical-specific exposure information to feed into an AOP

What now?

Thank you!!

Please fill out your evaluation ☺