Potential applicability and challenges of using *in vitro* and *in silico* methodologies in food ingredient safety assessment

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Topics of Discussions for this Talk

• Vision of the NRC Report on Toxicity Testing in the 21 Century
• Tox 21 Partnership between FDA, EPA, NIEHS, and NCATS
• Other Emerging “Tox 21” Methods
  – SAR/QSAR
  – Read Across
  – AOPs
  – Organs of a Chip
• New ICCVAM Activities
• New NRC Report

• Sponsored by EPA
• Use cell-based (high throughput) assays to understand how chemicals perturb normal cellular functions (i.e., toxicity pathway)
  – Establish relationships of perturbations with “adverse outcomes”
• Develop *in vitro* to *in vivo* extrapolation methods
• Integrate results to predict hazard/risk

Broader coverage of chemicals & endpoints
Reduce cost & time of testing
Use fewer animals
Vision of the NRC report

- The NRC report laid out a roadmap for revamping toxicity testing.
- Focus should shift away from identification of toxicant-induced apical endpoint effects towards an identification of a sequence of key events/modes of action as the organizing principle for risk assessment.
- The use of mechanistic data will help risk assessors gain a better understanding of how chemicals exert their toxic effects.
Vision of the NRC report

- NRC also advocated the use of adverse outcome pathways (AOPs) as a critical aspect of predictive toxicity testing.
- The AOP Framework was established to systematically collect, organize and evaluate mechanistic data and causally link them to adverse effects.
Memorandum of Understanding for Tox 21

• 5-year Memorandum of Understanding (MoU) on “High-Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings” released on Feb 14, 2008 signed by NHGRI (F.S. Collins), NIEHS/NTP (S.H. Wilson), and EPA (G.M. Gray).

• Revised 5-year MoU to add FDA signed on July 19, 2010 by NHGRI (E.D. Green), NIEHS/NTP (L.S. Birnbaum), EPA (P.T. Anastas), and FDA (J. Woodcock).

• Known informally as Tox21 for Toxicology in the 21st Century

• MOU revised July 2015. Dr. Susan Mayne, Director of the Center for Food Safety & Applied Nutrition, signed for FDA.
Tox21 Goals

• Identify patterns of compound-induced biological response in order to:
  – characterize toxicity/disease pathways
  – facilitate cross-species extrapolation
  – model low-dose extrapolation

• Prioritize compounds for more extensive toxicological evaluation

• Develop predictive models for biological response in humans
Agency Points of Contact

FDA – Suzanne Fitzpatrick Ph.D.
NCGC/NCATS – Anton Simeonov, Ph.D.
EPA/NCCT – Russell Thomas, Ph.D.
NIEHS/NTP – Rick Paules, Ph.D.

Assays & Pathways Working Group
Co-Chairs
Kevin Gaido, Ph.D. (FDA)
Keith Houck, Ph.D. (EPA)
Kristine Witt, M.S. (NTP)
Menghang Xia, Ph.D. (NCGC)

- Identify toxicity pathways & corresponding assays
- Review nominated assays and prioritize for use at the NCGC

Chemical Selection Working Group
Co-Chairs
William Leister, Ph.D. (NCGC)
Donna Mendrick, Ph.D. (FDA)
Ann Richard, Ph.D. (EPA)
Suramya Waidanatha, Ph.D. (NTP)

- Establish compound libraries for qHTS (10K, mixtures, water-soluble)
- Establish QC procedures for compound identity, purity, concentration, and stability

Informatics Working Group
Co-Chairs
Ruili Huang, Ph.D. (NCGC)
Richard Judson, Ph.D. (EPA)
Nisha Sipes, Ph.D. (NIEHS)
Weida Tong, Ph.D. (FDA)

- Evaluate assay performance
- Develop prioritization schemes and prediction models
- Make all data publicly accessible

Targeted Testing Working Group
Co-Chairs
Michael DeVito, Ph.D. (NTP)
David Gerhold, Ph.D. (NCGC)
Timothy Shafer, Ph.D. (EPA)
Robert Sprando, Ph.D. (FDA)

- Evaluate relevance of prioritization schemes & prediction models
- Extrapolate in vitro concentration to in vivo dose
Exposure

Uptake-Delivery to Target Tissues

Perturbation

Cellular response pathway

Biologic inputs

“Normal” Biological Function

Adaptive Responses

Early cellular changes

Cell injury, Inability to regulate

Molecular initiating event

Perturbed cellular response pathway

Adverse outcome relevant to risk assessment

Toxicity Pathway

Adverse Outcome Pathway

NAS, 2007
Tox Cast Inventories

• ToxCast Phase I (293 unique cmpds)
  – EPA pesticidal actives w/ rich in vivo data
  – PFOAs, BPA, approx 12 metabolite/parent pairs
• ToxCast Phase II (767 unique new cmpds)
  – EPA pesticides, high interest EPA and stakeholder inventories, data rich chemicals (EDSP, OPPT, antimicrobials, inerts, green alternatives, fragrances, water ...)
  – FDA CFSAN data rich, NCTR LTKB Priority 1 drugs
  – Toxicity reference chemicals, data-rich chemicals, NTP immunotox
  – 135 Donated pharma cmpds -- failed drugs w/ pre-clinical or clinical tox data
• ToxCast E1K (800 unique new cmpds)
  – Endocrine active reference cmpds, SAR predicted ER-active/inactives, EDSP cmpds
• EPA’s Tox21 library (3727 unique cmpds out of current 8599 total)
  – Complete on-hand EPA sample library used to build ToxCast inventories
Tox21 Robot System

NCGC screened 1408 compounds (1353 unique) from NTP and 1462 compounds (1384 unique) from EPA in 140 qHTS assays representing 77 predominantly cell-based reporter gene endpoints.

http://www.youtube.com/watch?v=ECloTsdD-xo
Current Limitations of Data for Regulatory Use

- Lack of xenobiotic metabolism
- Inability to screen volatile or highly hydrophillic chemicals
- Limited coverage of biological targets
- Lack of a pragmatic path forward for validation
- Inability to confidently translate perturbations at molecular level to likely tissue and organ-level effects
- These are all the challenges/goals for all four agencies going forward with this program
SAR/QSAR

- Structure-Activity Relationships (SAR) are relationships between a compound’s chemical structure and physiochemical properties and biological effects on living systems.
- Complex computer software modeling programs have been and are being developed to predict carcinogenic and mutagenic potential using quantitative SAR or QSAR.
- QSAR analysis – could be a useful tool for complementing and possibly reducing the battery of genetic toxicity testing requested for food contact substances.
Read-Across

• Read-across is when the already available data of a data-rich substance (the source) is used for a data-poor substance (the target), which is considered similar enough to the source substance to use the same data as a basis for the safety assessment

• Uses of read Across
  – To avoid additional animal testing
  – To save time and cost
  – To use human data, if available for one compound but not possible to produce for another
  – To cover more substances with one safety assessment
Opportunities for incorporating *in vitro/in silico* data into read-across

Reducing uncertainty in a read-across argument in a regulatory submission:
- Using *in vitro/in silico* data to confirm the similarity in the mechanism of action within a category and/or between the “target” and “source” compounds
- Confirming or refuting a hypothesis that proposed analogues may have “other” effects
- Assessing the relative “potency” of the analogues
Understanding an AOP Provides A Basis to Inform The Use of Data for Risk Assessment & Decision Making

**Structure Activity Relationships**

- **Toxicant**
  - Chemical reactivity profiles
  - Chemical category

- **Molecular Interactions**
  - Receptor
  - DNA
  - Protein interactions

- **Cellular Responses**
  - Gene activation
  - Protein production
  - Signal alteration

- **Organ**
  - Altered function
  - Altered development

- **Organism**
  - Lethality
  - Sensitization
  - Reproduction
  - Cancer

- **Population**
  - Structure
  - Extinction

**Key events or predictive relationships spanning levels of biological organization**

**Adverse outcome relevant to risk assessment**

**Greater Toxicological Understanding** (Qualitative AOP)

**Greater Risk Relevance** (Quantitative AOP)
FDA-DARPA-NIH Microphysiological Systems Program

• Started in 2011 to support the development of human microsystems, or organ “chips,” to screen for safe and effective drugs swiftly and efficiently (before human testing)

• Collaboration through coordination of independent programs
  - Engineering platforms and biological proof-of-concept (DARPA-BAA-11-73: Microphysiological Systems)
  - Underlying biology/pathology and mechanistic understanding (RFA-RM-12-001 and RFA RM-11-022)
  - Advise on regulatory requirements, validation and qualification
U18 generated cell resources
UH2 generated organ systems

24 months

Base period

Period 1

Period 2

Period 3

DARPA bioengineering Platform + 2 systems

UH3 phase:
- Incorporation of differentiated stem- and progenitor-derived cells
- Integration of various organ systems

24 months

Integration & validation

4 systems

7 systems

10 systems

- Multicellular architecture
- Vascularization, innervation, hormonal, humoral and immunological signaling
- Genetic diversity and pharmacogenomic capacity
- Representation of normal and disease phenotypes

60 months

• Cell viability for 4 weeks
• Integrated system predicts human in vivo efficacy, toxicity, and pharmacokinetics:
  o safe and effective
  o safe and ineffective
  o unsafe, but effective
  o unsafe and ineffective

Period 3
The Tissue Chip Program

**GOAL:** Develop an *in vitro* platform that uses human tissues to evaluate the efficacy, safety and toxicity of promising therapies.

**Current Goals:**
- Integration
- Compound testing
- Validation
- Partnerships
- Adoptions of the tech to the community
The Tissue Chip...Diversity a Good Thing
ICCVAM Activities

• Developing a Strategy and Roadmap to Replace the Use of Animals for Toxicity Testing
• Very similar to the EU ToxRisk Plan
• Focus on the following:
  – Repeat Dose Toxicity
  – Pharmacokinetics and Metabolism
  – Carcinogenicity
  – Reproductive and Developmental Toxicity
  – Neurotoxicity
Combining the recommendations of the two reports:
- Toxicity Testing in the 21st Century
- Exposure Testing in the 21st Century
This new report will focus on 21st Century Science-based Risk Strategies into Risk Assessment
FDA was not part of committee for either of these two previous reports
But FDA recognizes the importance of this new vision for toxicology
FDA has made two presentations to the committee on FDA’s Risk Assessment Challenges
Report due end of 2016
Thank You for Inviting Me to Talk

• Questions

• You can contact me at:
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