New Approaches in Chemical Risk Assessment

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The changing landscape of toxicology

- Large # of chemicals with limited toxicological information
- Novel material and processes (e.g. nanomaterials, biotech products)
- Combined exposure
- New technologies (e.g. high throughput assays, 'omics, bioinformatics, systems biology, computational toxicology)
- Human relevance testing laboratory species is being questioned
- Increasing demand non-animal approaches
Paradigm shift in toxicology

Paradigm shift in toxicology

old/present way:

Compounds

All: animal testing

Mechanism

in vivo/
in vitro

Cellular/
molecular
mode of action

few toxic compounds
with known mode of action

new vision:

few toxic compounds
requiring animal testing

animal testing

Definition of
toxicity pathways

All: in silico/in vitro testing

Compounds

Leist et al., ALTEX 25(2), 103-114
**Definition of adverse outcome pathways**

21st century toxicity evaluation = “Bottom up”
defining adverse outcome pathways

- Chemical structure & Properties
- Molecular initiating event
- Cellular responses
- Organ Response
- Organism Response

Conventional toxicity testing = “Top down”
High throughput-screening e.g. ToxCast

EPA’s Toxicity Forecaster (ToxCast)
> 1,800 chemicals
> 700 high-throughput assays ~ 300 signaling pathways

<table>
<thead>
<tr>
<th>Assay Provider</th>
<th>Biological Response</th>
<th>Target Family</th>
<th>Assay Design</th>
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</thead>
<tbody>
<tr>
<td>ACEA</td>
<td>cell proliferation and death</td>
<td>Response Element</td>
<td>viability reporter</td>
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<tr>
<td>Aprecda</td>
<td>cell differentiation</td>
<td>Transporter</td>
<td>morphology reporter</td>
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<tr>
<td>Attogene</td>
<td>mitochondrial depolarization</td>
<td>Cytokines</td>
<td>conformation reporter</td>
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<td>BioSeek</td>
<td>protein stabilization</td>
<td>Kinases</td>
<td>enzyme reporter</td>
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<td>CellzDirect</td>
<td>oxidative phosphorylation</td>
<td>Nuclear Receptor</td>
<td>membrane potential reporter</td>
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<td>NCGC/Tox21</td>
<td>reporter gene activation</td>
<td>CYP450 / ADME</td>
<td>binding reporter</td>
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<td>NHEERL MESC</td>
<td>gene expression (qNPA)</td>
<td>Cholinesterase</td>
<td>inducible reporter</td>
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<td>NHEERL NeuroTox</td>
<td>receptor activity</td>
<td>Phosphatases</td>
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<td>NHEERL Zebrafish</td>
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<td>Proteases</td>
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<td>NovaScreen</td>
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<td>XME metabolism</td>
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<td>Odyssey Thera</td>
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<td>GPCRs</td>
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<td>Ion Channels</td>
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<table>
<thead>
<tr>
<th>Readout Type</th>
<th>Species</th>
<th>Tissue Source</th>
<th>Detection Technology</th>
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<tbody>
<tr>
<td>Single</td>
<td>Human</td>
<td>Lung</td>
<td>qNPA and ELISA</td>
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<td>Multiplexed</td>
<td>Rat</td>
<td>Breast</td>
<td>Fluorescence &amp; Luminescence</td>
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<td>Multiparametric</td>
<td>Mouse</td>
<td>Vascular</td>
<td>Alamar Blue Reduction</td>
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<td>Zebralish</td>
<td>Skin</td>
<td>Arrayscan / Microscopy</td>
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<td>Sheep</td>
<td>Kidney</td>
<td>Reporter gene activation</td>
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<td>Boar</td>
<td>Cervix</td>
<td>Spectrophotometry</td>
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<td>Radioactivity</td>
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<td>Uterus</td>
<td>HPLC and HPEC</td>
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<td>Guinea pig</td>
<td>Brain</td>
<td>TR-FRET</td>
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http://www.tera.org/Peer/EDSP/presentations/Dix_HTP_Assays.pdf
High throughput screening e.g. ToxCast

Sipes et al, 2013, Reif et al., 2010
Advanced in vitro models

e.g. body-on-a-chip, (human) stem cell derived assays

D.E. Ingber, Trends in Cell Biology, (21) 2011
Reverse dosimetry

In vitro concentration-response curve

In vivo dose-response curve

Reverse Dosimetry

PBPK/other kinetic models

Reverse dosimetry of ToxCast data by Wetmore et al., 2012

Example Bisphenol A

Bioactivity Data for Compound Bisphenol A [CID: 6623], Active in 23 of 507 Targets AC50 1.1- 44.7 μM (Pubchem); Wetmore et al. 0.016- 181.5 μM

Reverse dosimetry

\[ C_{ss} = \frac{\text{oral dose rate}}{\left( GFR \times F_{ub} \right) + \left( Q_1 \times F_{ub} \times \frac{C_l\text{int}}{Q_1 + F_{ub} \times C_l\text{int}} \right)} \]

Renal clearance

Hepatic clearance based on in vitro measurements

0.86 μM (Css)  
1.1 μM (AC50)

1 mg/kg bw (oral dose)  
? mg/kg bw

Reverse dosimetry of ToxCast data by Wetmore et al., 2012

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Renal clearance

Hepatic clearance based on in vitro measurements

0.86 μM (Css) 1 mg/kg bw (oral dose)

1.1 μM (AC50) ~1.3 mg/kg bw

Reverse dosimetry of ToxCast data by Wetmore et al., 2012

Estimated daily intake of each chemical

Human in vivo equivalent doses

FIG. 3. Continued.
Some challenges in achieving a paradigm shift in toxicity testing

- Toxicological/biological space adequately covered?
- Reliability of extrapolation from in vitro toxicity pathways to biologically relevant hazards? (e.g. adequate cell models, exposure duration)
- Establishing fitness-for-purpose of new methods (who and how)? (e.g. anchoring against data from laboratory species?)
- Quantitative accuracy of in vitro – in vivo extrapolations?
- Domain of applicability?
New approaches for Food & Food ingredients

ILSI Task Force: New Approaches to Chemical Risk Assessment for Food & Food Ingredients

Objectives:
- Examine the scientific opportunities of novel approaches for food chemical risk assessment.
- Improve and implement these new technologies in risk assessment for toxicity testing in food industry.

ILSI EU expert group:
**Exploitation of ToxCast data** on opportunities for their use in the safety risk assessment of food chemicals.
Possibilities and challenges ToxCast data for food related chemicals.

False positive (fluorescence quenching)?

Lack of metabolic activation?

Summary

- A marked change is taking place in toxicology

- New approaches for safety assessment, based on a **mechanistic understanding** of biological action and **exploiting an array of new technologies** (eg high throughput assays, 'omics, bioinformatics, systems biology, computational toxicology)

- These new approaches need to be further evaluated with respect to opportunities and challenges for food related chemicals
Acknowledgements

- Members of the ILSI Task Force on new approaches on chemical risk assessment and the ILSI expert group on the Exploitation of ToxCast data
- IAFP Organization for Speaker Support Travel Grant