The Process of Transformation in Toxicity Testing

NAS Systems Biology-Informed Risk Assessment Workshop

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My Charge

1) Give a visionary talk
   • Be inspiring about the new approaches

2) Define “systems biology”

3) Go beyond a nice science review
   • Give guidance on breaking the bonds of the traditional framework
The Big Picture

How Endocrinology and Toxicology have differing perceptions of endocrine disruption, and both are right

Bisphenol A

HO-\text{CH}_3-\text{CH}_3-\text{CH}_3-\text{OH}
Example: Bisphenol A

Bisphenol A is an endocrine disruptor that highlights differences between endocrinological and toxicological perceptions of effect

– binds ERα with $K_d$ of $10^{-5} – 10^{-4}$ M
– in vitro effects in the nM range
– non-GLP studies report animal effects at ng/kg
– GLP studies report animal effects at mg/kg
– controversy over [bisphenol A] at target tissue
Example: Bisphenol A

Dose

Response

Endocrinology

Physiologic adversity?

Molecular adversity?

Toxicology
“Exogenous estrogens are a serious confounding variable in rodent studies designed to assess the effects of endocrine-disrupting chemicals, and, because estrogenic contaminants can be present in food, bedding materials, water, and caging materials, controlling for their presence is a daunting task.” Muhlhauser et al. Biol Reprod 2009.
Can we all agree on this?
Can we all agree on this?

Yes…..except:

• My normal is different than your normal
  – In fact, how do we define normal?
• My adverse is different from your adverse
  – In fact, how do we define adverse?
• And what about everything we don’t know…..
  – Epigenetic effects are a good example of an
    adaptive response that, when extreme, is adverse
“The conceptual creations of science... become accepted as fact through a complex process of social consolidation. These thought products... are never finalized but can undergo transformation.... The older way of looking at things may become incomprehensible under the new thought style, and the process of transformation.... may be a rapid gestalt switch or a slow process of differentiation....”

From the Preface to Genesis and Development of a Scientific Fact, Ludwik Fleck (1935)
The Ideal Toxicity Test System

Chemical X

Safe
Not Safe
The Ideal Toxicity Test System

NO!
Ignored biology
Linear
Simplistic
Goal is to find one number
The Ideal Toxicity Test System

**NO!**
- Ignores biology
- Linear
- Simplistic
- Goal is to find one number

**YES!**
- Biology-based
- Interactive
- Complex
- Probabilistic
Ignores Biology → Biology-Based

NOW

THE FUTURE
Too Linear → Interactive
“Simplicity often lies on the other side of complexity”

Simplicity (of ignorance)

Complexity

Complication = obscuring of the details

With toxicity pathways (and biology), the details may at first seem overwhelming and unconnected, but ultimately it will be possible to identify those details that matter.

Eric Berlow, an ecologist and network theorist, in a recent TED Talk (see www.ericberlow.net)
Goal Is to Find One Number →
Probabilistic

“....a complete and accessible account of the theoretical foundations and computational methods that underlie plausible reasoning under uncertainty....”
Probabilistic Reasoning in Intelligent Systems: Networks of Plausible Inference Judea Pearl 1988
The Ideal Toxicity Test System

Chemical X

Plausibly Safe

Plausibly Not Safe
Systems Biology – Definition

an interconnected network of events predictive of emergent properties

Response

Time

seconds minutes hours days

ligand-receptor activation
kinase activation
transcriptional activation
nuclear receptor translocation
metabolic activation
physiologic endpoint
Challenges........

- Assay Design/Development for Toxicity Pathways
- Improved methods to identify (predict) and test metabolites
- Co-ordinated development of ‘functional genomic tools’ to map and model pathways and use results to establish safe levels of exposures
- Train toxicologists and regulators about need for new approach and then in the tools and methods that will be involved in the transformation
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Systems biology-based toxicity pathway model

Genotoxicant → DNA damage

DNA repair pathway ← p53 ↔ Mdm2

Cell cycle pathway → pAND → Cell death

Apoptosis pathway → Mutagenesis

Proliferation
In Vitro Based Risk Evaluation Approach

**IVIVE**
In vitro - in vivo extrapolation

**Consideration of variability and sensitivity of subpopulations**

**Risk Evaluation**

- Determination of appropriate POD (point of departure)
- Exposure assessment
- Literature review
- Structural physicochemical reactivity characterization
- Determination of biokinetic behaviour

**In vitro - in vivo extrapolation**

- Concentration-response modeling
- In vitro concentration response
- In vitro effects battery

**In vitro risk assessment**

- In silico/literature evolution
- Identification of appropriate tests and conditions

**Reconsideration**
Go beyond a nice science review
“Give guidance on breaking the bonds of the traditional framework”

1) Screening/ranking of chemicals – determining whether chemicals are more or less likely to be hazardous to human health
2) Hazard identification – determining whether a compound may cause a particular type of effect
3) Mode of action analysis – testing a hypothesis about how a compound causes a particular effect
4) Adversity – the third rail of the new toxicity testing
Screening/ranking of chemicals – determining whether chemicals are more or less likely to be hazardous to human health

Of course this already happens based upon:
public concern politics economics
QSAR exposure TOXCAST
Ames tests animal tests production volume
etcetera…….

The BIG question: Do we create an official screening/ranking/prioritization protocol, and is it distinct from our “real” testing protocol?
Is our screen in vitro/in silico testing?
Is our “real” testing animal testing? I hope we are beyond this idea…….
Screening/ranking of chemicals – Two Models

Different conceptual and technical approaches

Real Test

Same conceptual and technical approach

Test System

Screening Elements
Systems Biology –
an interconnected network of events
predictive of emergent properties

- ligand-receptor activation
- kinase activation
- transcriptional activation
- nuclear receptor translocation
- metabolic activation

physiologic endpoint
Screening/ranking of chemicals – Reasons for a unified approach

- Parsimonious
- Continued test system development improves the screening process
- More likely that the screen and test will have the same order
- A common system is easier to defend

Same conceptual and technical approach
Hazard identification – determining whether a compound may cause a particular type of effect

What is meant by “a particular type of effect?”
Does our in vitro test system need to predict in vivo endpoints? (or just give us a probability estimate of safe or not-safe)

Put another way, do we have to have models (actual or conceptual) for each tissue and its responses? Or……do we just have to be sure that the full range of potential biological responses is covered?
Targeted testing

Tissue-specific testing

Development of the Toxicity Pathway Portfolio

Start

Agnostic Approach

Directed Approach

Parallel Approach

Repro/Dev
Neuro
Immuno
Cancer
Cardiac
Renal
Hepatic

Finish
Systems Biology –
an interconnected network of events
predictive of emergent properties

For risk assessment, the physiologic endpoints of concern are cellular perturbations. We should regulate on these cellular endpoints. Extrapolation of these cellular endpoints to effects at the organ/tissue level is important and interesting, but is not necessary for risk assessment. We should avoid this unnecessary complication.
Let's do our risk assessment here
systems toxicology

epidemiology & medicine

systems risk assessment

molecular epidemiology & medicine

systems level understanding of health and disease
Mode of action analysis – testing a hypothesis about how a compound causes a particular effect

chemical properties ➔ molecular event ➔ cellular response ➔ tissue response ➔ organism response

Mechanism of action
Toxicity pathway
Mode of action
Adverse outcome pathway
Adversity– the third rail of the new toxicity testing

The industry fear: that every little molecular blip in a cell will be considered adverse, and nothing will be considered safe

The endocrinologist’s fear: that if we ignore low dose phenomena, we are missing biologically important effects

The toxicologist’s fear: that by failing to consider the integrated physiological response (like with a whole animal), that we are underestimating the capacity for compensation (missing the forest for the trees)

Everyone’s fear: that what we don’t know might kill us
Autumn 1944 – Western Holland was blockaded by the Nazis, who turned away all shipments. This led to a food shortage during one of Europe’s coldest winters.

The Dutch Hunger Winter

- The Dutch survived on < 500 calories per day (¼ of pre-war consumption)
- Food restriction during pregnancy had dramatic consequences for the offspring
- Higher rates of obesity, diabetes, heart disease, higher blood pressure, hypercholesterolemia, and glucose intolerance

Studies by Tessa Roseboom and colleagues established many of these relationships during the 1990’s when the children of the Hunger Winter were in their 50’s.
Exogenous estrogens are a serious confounding variable in rodent studies designed to assess the effects of endocrine-disrupting chemicals, and, because estrogenic contaminants can be present in food, bedding materials, water, and caging materials, controlling for their presence is a daunting task.” Muhlhauser et al. Biol Reprod 2009.
Estimate acceptable concentration *in vitro*
Implementation challenges.....

• It is important not to over-promise and fail to deliver, because this is a long-term effort

• Experience is important and cumulative... this will take time, patience, and accepting wrong turns

• A centralized-enough effort is very important

• More focus on pathways and commonality of responses

• Taking a ‘systems’ approach to human biology that relies on mechanisms and pathways has inherent value, contributing both to improved toxicity testing and to the fundamental molecular elucidation of human disease
Lessons Learned – Human Genome Project

• Build the best teams
• Process must be science-driven
• Meet managerial challenges
• International participation important
• Explicit milestones and quality assessment are valuable
• Technology matters

Borrowed from Chris Austin and Collins et al., Science 300:286, 2003