Identification and Characterization of Adverse Effects in 21st Century Toxicology and Risk Assessment

Daland R. Juberg, PhD
Dow AgroSciences

Douglas A. Keller, PhD
Sanofi US

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Outline

• Defining the issue
• Defining “Adverse” and “Adaptive”
• Toxicity testing in the 21\textsuperscript{st} Century
• New data types and how to use them
  – Dimethylarsinate example
  – ToxCast example
• Risk assessment implications
• HESI advancements/conclusions
The Issue

- Advances in technology are changing approaches to toxicology testing
  - Molecular mechanisms
  - Biomarkers
  - ‘Omics
- The NRC has suggested a new vision and strategy for toxicology testing
  - Shift from whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin

The data from these studies do not fit neatly into the analysis paradigm of “adverse” or “not adverse” developed over many years from in vivo studies.
Why Is This Issue Important?

- In the absence of compelling human data (rarely available), doses that cause adverse effects in animals are used to regulate:
  - Allowable concentrations in air, water, soil, crops
  - Doses used in clinical trials of drugs
  - Exposure limits in occupational settings

- Setting an adverse effect level lower than scientifically justified can have a high economic impact:
  - Expensive emission controls
  - Protracted, expensive remediation
  - Longer, more expensive pharmaceutical development
  - Discontinued development of potentially useful chemicals and pharmaceuticals
  - Etc.

- Setting an adverse effect level higher than scientifically justified can lead to unwarranted risk.

- The use of mechanistic and molecular information in risk assessment is not well defined.
A Little History…
Adverse vs. Adaptive


TABLE 3.—Characteristics of adaptive effects.

- Do not compromise viability at all levels of tissue organization
- Constitute potentially beneficial effect on function or structure
- Result in enhanced capacity to respond to stress
- Are reversible

TABLE 4.—Characteristics of toxic effects.

- Can be lethal at the cellular or organismal level
- Impair function or structure
- Diminish capacity to respond to stress
- Can be irreversible
A Little More History...


The Classical View

Fig. 5. Approach to classifying toxicology study results as adverse or non-adverse (modified from Lewis et al., 2002).
Definitions agreed on (but subject to development)

**Adverse Effect:** A change in morphology, physiology, growth, development, reproduction, or life span of a cell or organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.

**Adaptive Effect:** In the context of toxicology, the process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function.

Not perfect, but not really a topic of discussion here.
And Then Along Came …

The 2007 NRC Report

TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY

Committee on Toxicity Testing and Assessment of Environmental Agents
Board on Environmental Studies and Toxicology
Institute for Laboratory Animal Research
Division on Earth and Life Studies

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And now…
NRC’s 21\textsuperscript{st} Century Vision for Toxicity Testing

Adapted from NRC (2007)
The Vision Is to Look at “Toxicity Pathways”

- Evaluation of perturbations in toxicity pathways rather than apical end points.
- Emphasis on high-throughput approaches using cells or cell lines, preferably of human origin.
- Use of medium-throughput assays of more integrated cellular responses.

- Testing conducted to evaluate metabolites, assess target tissues, and develop understanding of affected cellular processes at genomics level.
- Limited types and duration of in vivo studies, focusing on up to 14-day exposures.
- More extensive testing for representative compounds in novel chemical classes.
Applying a Systems Toxicology Approach

Modified from NRC, 2007

Toxicity pathways: cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects.
Applying a Systems Toxicology Approach

Source
Fate/Transport
Exposure
Tissue Dose
Biologic Interaction
Perturbation

Biologic Inputs

Alternative outcome:
Adaptive Stress Responses
Early Cellular Changes
Normal Biologic Function
Cell Injury
New Normal State
Morbidity and Mortality

Normal Biologic Function
Modified from NRC, 2007
The NRC acknowledged the potential issues, but little attention has been focused here "... because virtually all environmental agents will perturb signaling pathways to some degree, a key challenge will be to determine when such perturbations are likely to lead to toxic effects and when they are not."
Meek and Doull, Pragmatic challenges for the vision of Toxicity Testing in the 21\textsuperscript{st} Century in a regulatory context: \ldots Toxicol Sci 108, 19-21, 2009.

“A pragmatic and seemingly essential first step in addressing this re-evaluation of adversity would be a recommendation to relate early perturbations to apical endpoints in frameworks designed to systematically address consideration of key events in modes of action and their subsequent implication for dose-response risk assessment.”

Eight other commentaries on the NRC vision and its implications (summed up by Andersen and Krewski, Toxicol Sci 117, 17-24, 2010)
Future state of toxicity testing based on knowledge of key toxicity pathways and the critical nodes in the pathways. Boxes in red indicate the areas for research where the most emphasis is needed to allow use of this paradigm.
Present and future testing paradigms for understanding mechanisms of toxicity. Currently, MOAs are postulated followed by determination of intermediate networks and pathways, culminating in screening assays to detect compounds that present this MOA. In the future, screening assays will be used to postulate MOAs by prior understanding of the links between the screen, targets, pathways, networks and MOAs.
Dimethylarsinic Acid: Key Events in Mode of Action

$\text{DMA}^V \rightarrow \text{DMA}^{III}$

(sustained) Cytotoxicity

(sustained) Enhanced Cell Proliferation

Hyperplasia

Tumors

Urinary bladder from a female F344 treated with 100 ppm $\text{DMA}^V$.

BrdU Labeling

Urinary bladder tumors
Dimethylarsinonic Acid: Key Events in Mode of Action

What happens here?

DMA\(^{V}\) $\rightarrow$ DMA\(^{III}\)

(sustained) Cytotoxicity

(sustained) Enhanced Cell Proliferation

Hyperplasia

Tumors

Urinary bladder from a female F344 treated with 100 ppm DMA\(^{V}\)

BrdU Labeling

Urinary bladder tumors
ToxCast

Combined Approaches

QSAR

In vitro/HTS

Structure + Biological profile

In Vivo
ToxRefDB

Predictive Models Prioritization

Courtesy of US EPA
ToxCast and Bioinformatics
In Vitro Model for Rat Liver Tumor Risk

Model Targets:
- PPARA/HMGCS2/PPARG
- AR – endocrine disruption
- MCP1 – angiogenesis
- Oxidative Stress
- IL8 – Inflammatory response
- SLC01B1 – xenobiotic removal
- ADORA2 – Adenosine receptor

Model is being validated with targeted animal studies:

1. Does in vitro activity predict in vivo?
2. At what doses do in vivo target effects start?
3. Does target activity occur at doses below the cancer dose?
BPAC – Is ANY Activation Adverse?

Pharmacodynamics
- MOA
- Key Events
- Toxicity Pathway
- HTS Assays

Pharmacokinetics
- Dose-to-Concentration Scaling Function ($C_{ss}/DR$)
- Probability Distribution
  - Probability Distribution for Dose that Activates Biological Pathway
  - PK Model
  - Intrinsic Clearance
  - Plasma Protein Binding

Biological Pathway Activating Concentration (BPAC) Probability Distribution

Courtesy of US EPA
Common structure of cellular stress pathways based on negative feedback design and ‘high loop gain’ in control pathways from multiple embedded signaling components.
**EC XX**

- **a.** $\rightarrow$ conc 1
- **b.** $\rightarrow$ conc 2
- **c.** $\rightarrow$ conc 3
- **d.** $\rightarrow$ conc 4

**Adversity_{xx} = f(conc_i)**

**In Vitro – In Vivo Extrapolation**

**Systems model for DNA repair**

**EC XX= effective concentration for a xx % response; exp_c = predicted human exposure giving rise to conc_i in vivo; exp_o = observed exposure in human populations; MOE (y) = margin of safety from results of testing with toxicity pathway “y”.

**Compare to exp_o and estimate MOE**

**Courtesy of Kim Boekelheide**
Traditional View of Risk Assessment Paradigm

Exposure

Dose

Response
A Modern View of the Risk Assessment Paradigm

Exposure → Dose → Response → Adverse Health Effect

Response

Genome

Transcriptome

Proteome

Metabolome
HESI Workgroup Objectives

Develop criteria to facilitate the determination of adverse from other types of changes (adaptive or homeostatic). Criteria should include biologically relevant information; temporality, genomic and tissue response, and identification of target organ or system.

Develop a framework or approach that integrates and prioritizes information (including new technologies) to characterize the change in a biological system that evaluates the continuum of effects for which the characterization of an effect may vary depending on the context.
The HESI Framework – First Pass

- Characterization of the effect
  - What is the observation? Reversible? Dose-response? etc.

- Relative placement of the effect to other levels of biological organization
  - Key event in known or postulated MOA? Known to be associated with altered organ/tissue/system function? Depleted physiological reserve? Precursor to another effect? etc.

- Human relevance
  - Does or can the effect occur in humans? Relevance of dose levels/exposure to humans? MOA known in humans?
Adversity Re-defined…
Some Questions

- What about dose response for pathways?
- What about duration of exposure?
- Is adversity defined by the testing system itself, or by extrapolation to a higher level of organization?
- Is a change of state sufficient, or does the change need to be irreversible?
Placement of the Effect within a Biological System: RPTC

- Relevant Pathways of Toxicological Concern (RPTCs)
- Concept of toxicological pathway important in NRC Report
- Number of RPTCs is not known
  - At low doses, changes in RPTC may reflect adaptation
  - At higher doses, changes in RPTC may be adverse
- Need to determine which pathways are RPTCs and to identify key nodes and dose-transition points
Relevant Response for Regulation (RRR)

- Another term developed at workshop
- An endpoint which forms the basis for risk assessment
- An RPTC could be a RRR, mitigating need for \textit{in vivo} studies
- Much more needs to be known about the structure and dose-response of pathways
Conclusions

- Leverage *in vitro* and in silico data to predict later-occurring apical endpoints from precursor dose transitions in RPTCs
- All toxicological responses should be viewed and considered within a time- and dose-response continuum
- Presently, there is a lack of a qualitative distinction between the toxicogenomic profile (and other *in vitro*/in silico biomarkers) associated with a low-dose exposure (not linked to an adverse apical endpoint) and later or higher-dose exposure (potentially or more often linked to an adverse apical endpoint)
- Need for determination where transition to adversity occurs
Conclusions, cont’d.

- Research priority given to defining and characterizing RTPCs
  - Dose transition(s)
  - Critical node identification
  - Threshold (presence or absence) identification

- Risk assessment frameworks should promote effective use of rigorous, validated, and standardized *in vitro* and/or *in silico* data that have established relevance to human biology
Conclusions, cont’d.

- Weight of evidence approach should be considered for evaluation of all data
- Consideration of context (cellular, tissue, organism) is critical for determining point of concern or point of departure
- Consideration of relevant exposure and human exposure to putative toxicant is important
- Most appropriate use presently of high data-content information is for prioritizing chemicals for additional evaluation
- Not yet ready for determining a specific MOA, but with appropriate research, in the future this information can inform risk assessment and regulatory decisions
2012 HESI Project Committee

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