Eminent Toxicologist Lecture Series

Society of Toxicology
Eminent Toxicologist Lecture Series

Humane Science in Risk Assessment and Beyond

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Conflict of Interest Declaration

• I have no conflicts of Interest. I am not a paid consultant to any organization.
Scientific Revolutions—T. Kuhn, ‘62

• Scientific Revolutions “necessitated the community’s rejection of one time-honored theory in favor of another incompatible with it.”

• Animal Testing to *In Vitro* Approaches
Today’s Presentation

1. Defining the Problem—The Quality of Animal Data
2. Johns Hopkins Center for Alternatives to Animal Testing (CAAT) to Toxicity Testing in the 21st Century
3. Ways to Think about In Vitro—In Vitro Available Today Some Specific Examples
4. In Vitro and Risk Assessment
5. Validation and Regulatory Acceptance
6. Toxic Ignorance to Pathways of Toxicity
7. Summary and Conclusions
Defining the Problem

The systems we are using were not designed for what we are requesting.
The Questionable Quality of Animal Data

Examples:
Draize Eye Test
Carcinogenicity
Ocular Irritancy Draize Score

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Cornea</td>
<td>0-80</td>
</tr>
<tr>
<td>Iris</td>
<td>0-10</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>0-20</td>
</tr>
<tr>
<td>Edema</td>
<td>0-10</td>
</tr>
</tbody>
</table>

The larger the number, the greater the damage.
Minimum-Maximum Score for Eyes of Individual Rabbits

24 HR Reference Procedure
Results are for 1 chemical from 3 different laboratories

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>4-100</td>
</tr>
<tr>
<td>25</td>
<td>35-39</td>
</tr>
<tr>
<td>30</td>
<td>2-6</td>
</tr>
</tbody>
</table>

Weil and Scala 1971, page 291
# Cancer Bioassay Results

Ames and Gold, 2000 and Gold et al., 2005

<table>
<thead>
<tr>
<th></th>
<th>Proportion</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chem. tested in rats and mice</td>
<td>379 / 648</td>
<td>58 %</td>
</tr>
<tr>
<td>- natural</td>
<td>86 / 165</td>
<td>55 %</td>
</tr>
<tr>
<td>- synthetic</td>
<td>293 / 493</td>
<td>59 %</td>
</tr>
<tr>
<td>Chem. tested in rat or mice</td>
<td>751 / 1456</td>
<td>52 %</td>
</tr>
<tr>
<td>- Natural pesticides</td>
<td>41 / 75</td>
<td>52 %</td>
</tr>
<tr>
<td>- Commercial pesticides</td>
<td>79 / 198</td>
<td>55%</td>
</tr>
<tr>
<td>- Chemicals in roasted coffee</td>
<td>23 / 32</td>
<td>72 %</td>
</tr>
<tr>
<td>- Mold toxins</td>
<td>15 / 25</td>
<td>60 %</td>
</tr>
<tr>
<td>Drugs (PDR)</td>
<td>117 / 241</td>
<td>49 %</td>
</tr>
<tr>
<td>Drugs (FDA)</td>
<td>125 / 282</td>
<td>44 %</td>
</tr>
</tbody>
</table>
LLNA—Caution
Local Lymph Node Assay

Although once favored, for pharmaceuticals, at this point in time we do not recommend that the LLNA or any other animal study be conducted for contact sensitization. A study will nearly always be conducted in humans. The LLNA gives so many false positive results with dermatologic vehicles, that its value is questionable and drug sponsors follow up the positive LLNA results with guinea pigs- thus using extra animals unnecessarily. We are awaiting the in vitro battery currently being assessed by ECVAM. The LLNA is definitely not the gold standard.

—A. Jacobs, 2012 (FDA)
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CAAT to Toxicity Testing in the 21st Century

Society of Toxicology
Russell and Burch

- Replacement
- Reduction
- Refinement

Hypothesis: The most humane science is the best science.
Henry Spira

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Johns Hopkins Center for Alternatives to Animal Testing

Working with academia, industry and governments since 1981 to understand how the latest technologies and the most humane science (3Rs of Alternatives—replacement, reduction, and refinement) can be used for decision making/risk assessment.
February, 1982

Leon Golberg—President of CIIT and member of the CAAT Board:

“We must use human cells in culture if we are to develop a new way to study toxicology.”
The Tipping Point

- Malcolm Gladwell
- Science writer from the *New Yorker*
- Tipping point: when an event leads to sudden change
- How do we think about events and why do we remember what we do about events?
- The tipping point has occurred in *in vitro* toxicology.
National Academy of Sciences

- Animal studies—time consuming and expensive
- Lack of predictability of animal tests
- Use of human cells in culture
- Systems Biology, Pathways and Mechanisms
Animal Testing

“It was expensive, time-consuming, used animals in large numbers, and it didn’t always work.”

—Francis Collins
Director, NIH National Human Genome Research Institute
February, 2008
*Currently Director of NIH
“With an advanced field of regulatory science, new tools, … we can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects.”

—M.A. Hamburg, FDA 2011
EU Cosmetic Directive

The Cosmetics Directive provides the regulatory framework for the elimination of animal testing for cosmetics purposes. Specifically, it establishes:

Testing ban—prohibition to test finished cosmetic products and cosmetic ingredients on animals;

Marketing ban—prohibition to market finished cosmetic products and ingredients in the EU which were tested on animals.
Ways to Think About *In Vitro*
Ways to Think About New Methods Development

- Endpoint
- Mechanisms
- Organ-Specific Functions
- Modeling
Replacement Examples

• Pregnancy testing
  ▪ Rabbit to monoclonal antibodies
• Pyrogen testing
  ▪ Rabbit to
  ▪ LAL (Limulus) to
  ▪ Cytokine Release (RBC)
In Vitro Assays Available Today

- Dermal irritation, corrosion and sensitization, ocular irritation and corrosion
- Percutaneous absorption
- Phototoxicity and photosensitization
- Acute toxicity and systemic toxicity
In Vitro Assays Available Today

- Endocrine disruption screens
- Specific tox screen: liver, kidney, lung, cardiac
- ADME
- Mutagenicity and carcinogenicity
Sensitization (ACD)—Current Methods

Chemical Reactivity
  Direct Chemical Reactivity Assay—OECD

Keratinocyte Activation
  KeratinoSens—OECD
  Sens-IS

Dendritic Cell Activation
  h-CLAT
  MUSST
  GARD
*In vitro* toxicology has become a full-fledged industrial activity, with many products and services supporting it.
Next Steps

• Multi-organ Technologies
• Organs-on-a-Chip
• Organoid Models
Improved Cell Models, New In Vitro Methods and the Ability to Apply In Vitro Approaches Opened the Doors for New and Existing Companies

• The TOXEXPO catalogue is only a partial listing—From cell and tissue manufactures to contract laboratories
In Vitro and Risk Assessment: Validation and Regulatory Acceptance
The Big Question:

How do we use *in vitro* data to achieve *in vivo* human risk assessment?
Current Approaches to Regulatory Toxicology

- Using animal studies when required by regulation
- Initiating the use of human cells in culture for product development and hazard assessment
- Learning to do *in vitro*/*in vivo* extrapolation
Regulatory Acceptance

Validation
A formal study, and not possible for many of the 21st century approaches

Scientifically Valid
Where one can convince by publication and repetition by others that the assay works-or assays work together

Evidence-Based Toxicology
Systematic reviews of the literature to defined criteria. Allows one to eliminate methods that do not meet the criteria.
“The difficulty lies, not in the new ideas, but in escaping from the old ones.”

John Maynard Keynes (1883-1946)
Toxic Ignorance

• 1984: National Academy of Sciences study
  ▪ Only 22% of chemicals have enough information in the public literature to make risk-based decisions

• 1997: Environmental Defense Fund (Ellen Silbergeld)
  ▪ 71% of High Production Volume (HPV) chemicals lack minimum toxicity data in the literature
  ▪ We live in an age of “toxic ignorance”

• EPA and American Chemistry Council independently confirmed results (1998)
  ▪ Only about 10-20% of the chemicals that we interact with on a daily basis have basic hazard data available
Animal studies—time consuming and expensive
Lack of predictability of animal tests
Use of human cells in culture
Systems Biology, Pathways and Mechanisms

National Academy of Sciences, 2007
EPA ToxCast Program

Thousands of chemicals → Hundreds of biological assays → Bioinformatics/Machine Learning → Chemical Toxicity Prioritization or Prediction
HUMANENESS

BETTER SCIENCE
Journals

• There are several journals devoted to *in vitro* approaches

• This is one more piece of data that *in vitro* toxicology has its own place in the discipline
Summary and Conclusions: Results of the Ongoing Scientific Revolution

• US and EU are encouraging *in vitro* approaches for regulatory use
• Validation to evidence-based approaches
• Skin/eye irritation and sensitization—several assays available, some provide potency
• Systemic toxicity—*in vitro* for almost all organs
• Numerous and interpretable *in vitro* assay are in use throughout industry and are readily available from CROs
• *In vitro* toxicology is the path forward
Nothing is more powerful than an idea whose time has come.

—Victor Hugo