

Eminent Toxicologist Lecture Series

Society of Toxicology

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Humane Science in Risk Assessment and Beyond

Alan M. Goldberg

Professor of Toxicology

Founding Director (Emeritus) Johns Hopkins

Center for Alternatives to Animal Testing (CAAT)

Johns Hopkins University Bloomberg School of Public Health

Society of Toxicology

Conflict of Interest Declaration

- I have no conflicts of Interest. I am not a paid consultant to any organization.

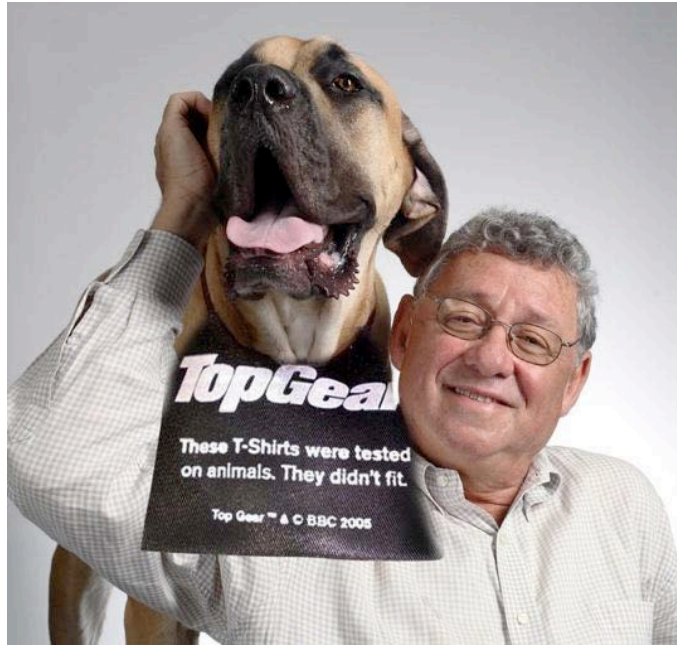
Scientific Revolutions—T. Kuhn, '62

- 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



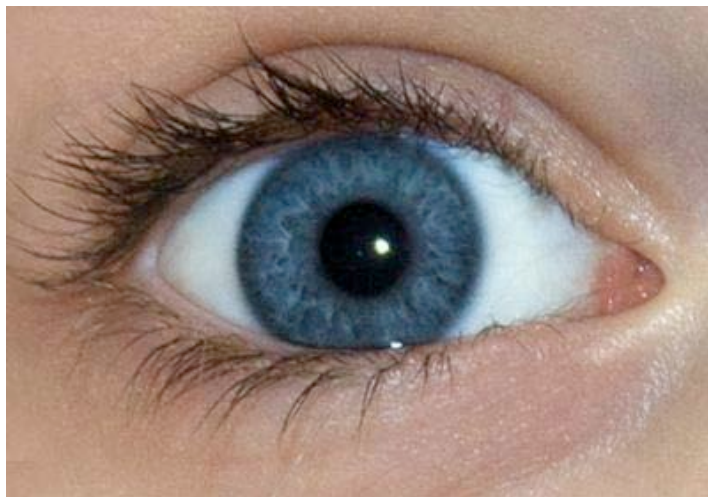
The systems we are using were not designed for what we are requesting.

The Questionable Quality of Animal Data



Examples:
Draize Eye Test
Carcinogenicity

Ocular Irritancy Draize Score



Cornea	0-80
Iris	0-10
Conjunctiva	0-20
Edema	0-10

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

Laboratory	Score
2	4-100
25	35-39
30	2-6

*Weil and Scala 1971,
page 291*



Cancer Bioassay Results

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



	Proportion	%
Chem. tested in rats and mice	379 / 648	58 %
- natural	86 / 165	55 %
- synthetic	293 / 493	59 %
Chem. tested in rat or mice	751 / 1456	52 %
- Natural pesticides	41 / 75	52 %
- Commercial pesticides	79 / 198	55 %
- Chemicals in roasted coffee	23 / 32	72 %
- Mold toxins	15 / 25	60 %
Drugs (PDR)	117 / 241	49 %
Drugs (FDA)	125 / 282	44 %

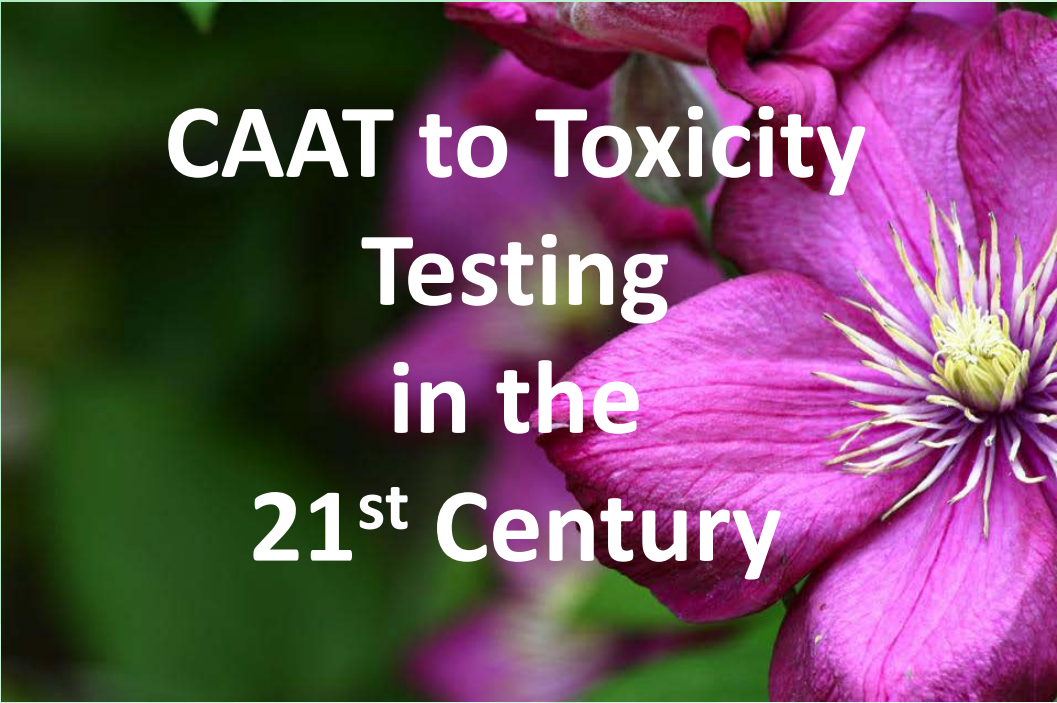
LLNA—Caution

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)

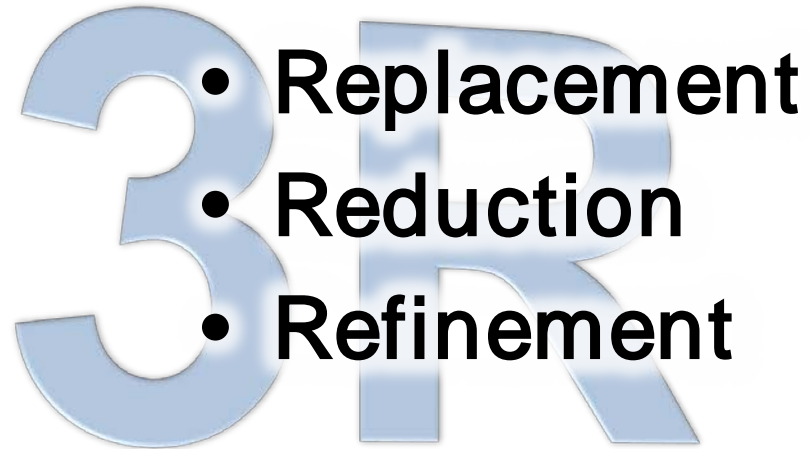
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**CAAT to Toxicity
Testing
in the
21st Century**

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Russell and Burch



Hypothesis: The most humane science is the best science.

How many rabbits does Revlon blind for beauty's sake?



WALD IN THE MIRROR
SABOTAGE TEST

YOU HARBOR
THE ONLY REASON TO... ANIMALS

THE ECONOMIC SHOW
THE SECURITY OF THE MARKET
IS IN QUESTION!

IN THE NAME OF SAFETY
STOP THE SHOW!

THE ACCIDENTAL
MILKMAKER TESTS!

ANALYSIS & REPLY
THE LAST ONE

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.

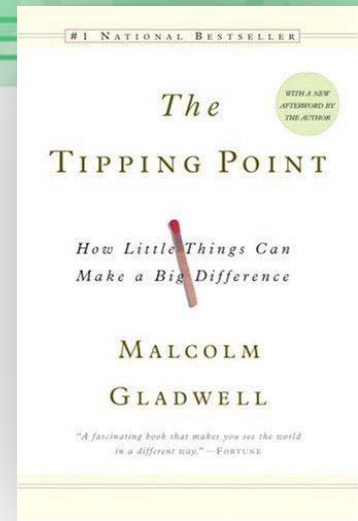
February, 1982

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.”*

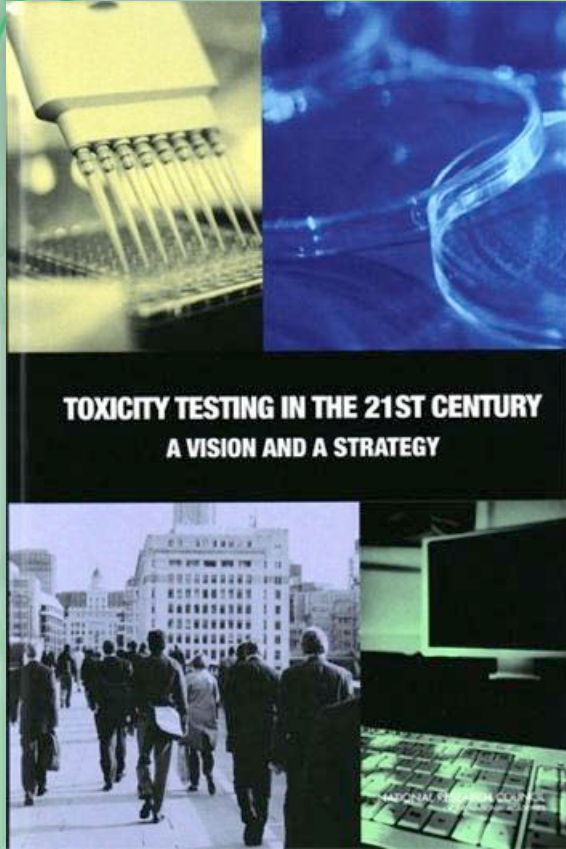
The Tipping Point

- 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

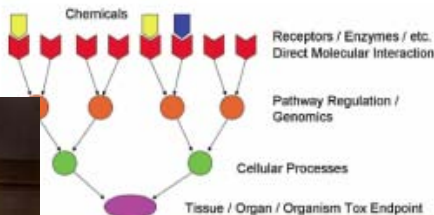
“It was expensive, time-consuming, used animals in large numbers, and it didn’t always work.”

—Francis Collins
*Director, NIH National Human
Genome Research Institute*

February, 2008

**Currently Director of NIH*

The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals



T21C Report a Game-Changer



*“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

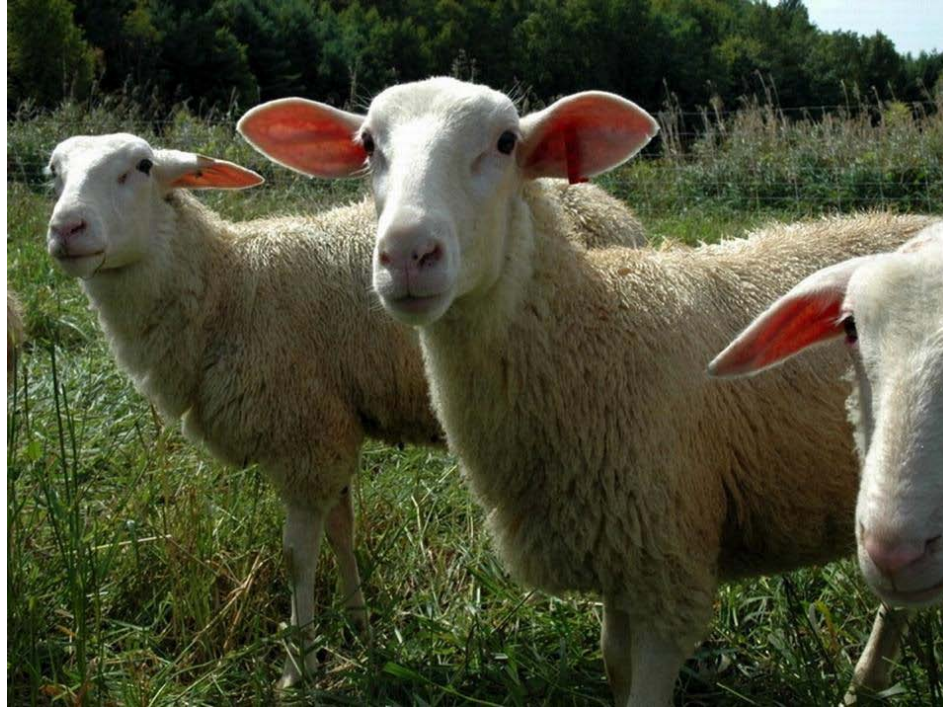
The Cosmetics Directive provides the regulatory framework for the elimination of animal testing for cosmetics purposes.

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*



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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

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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.

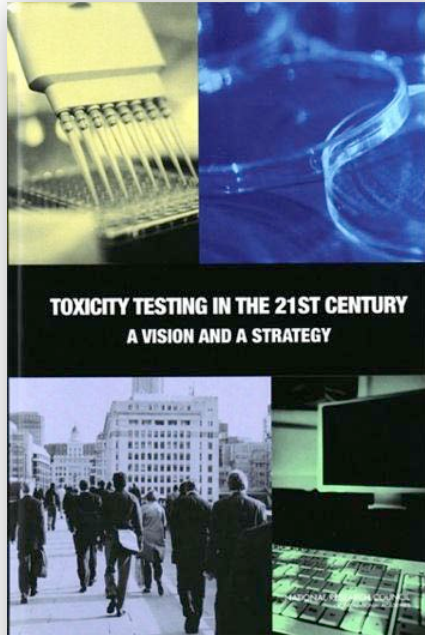
**“The difficulty lies, not in the
new ideas, but in escaping
from the old ones.”**

John Maynard Keynes (1883-1946)

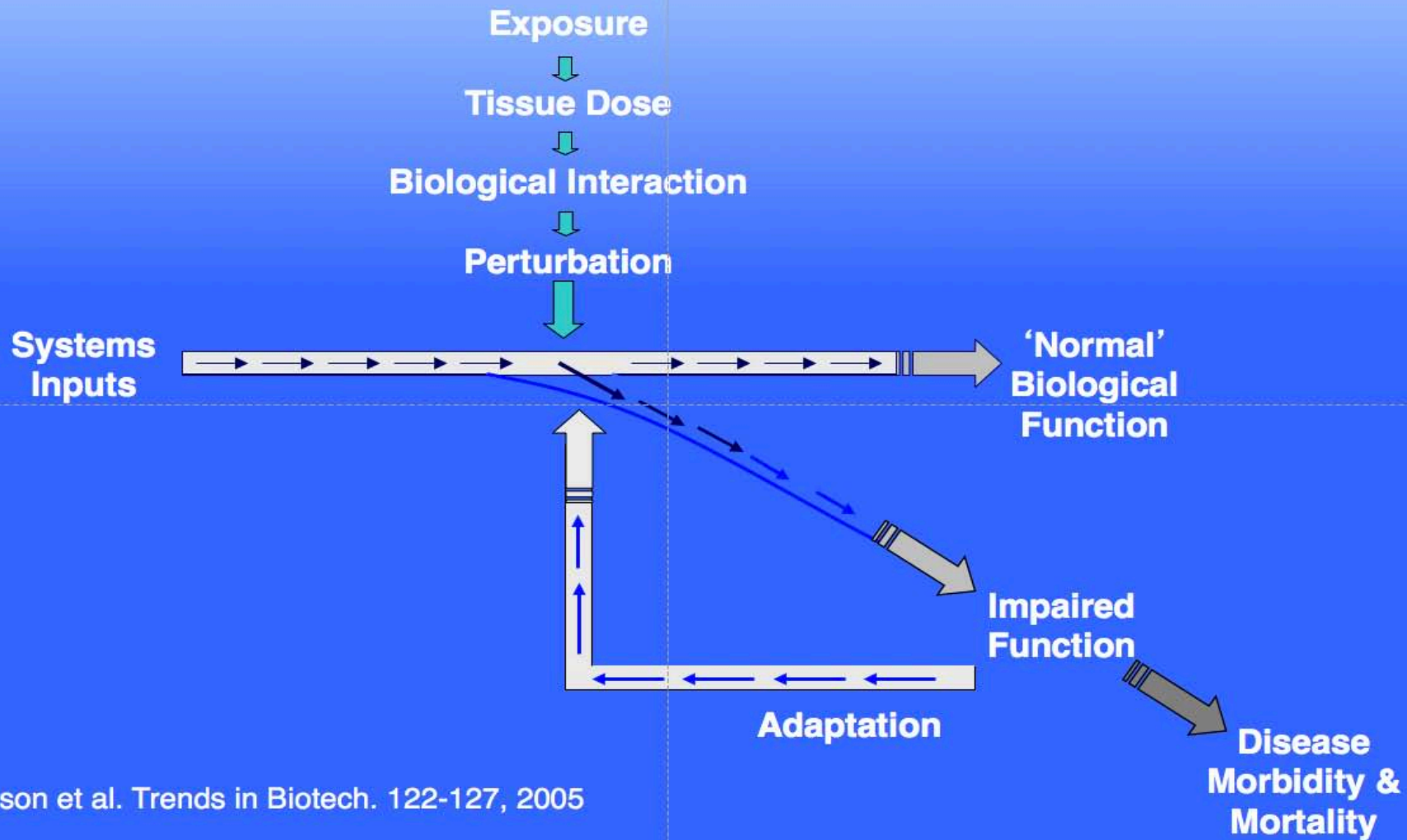
Toxic Ignorance

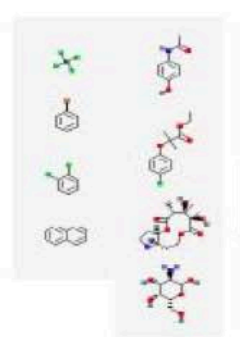
- 1984: National Academy of Sciences study
 - Only 22% of chemicals have enough information in the public literature to make risk-based decisions
- 1997: Environmental Defense Fund (Ellen Silbergeld)
 - 71% of High Production Volume (HPV) chemicals lack minimum toxicity data in the literature
 - We live 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

National Academy of Sciences, 2007

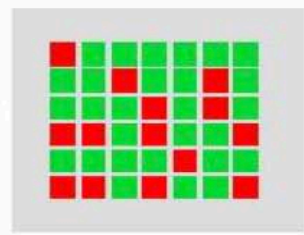


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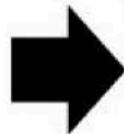




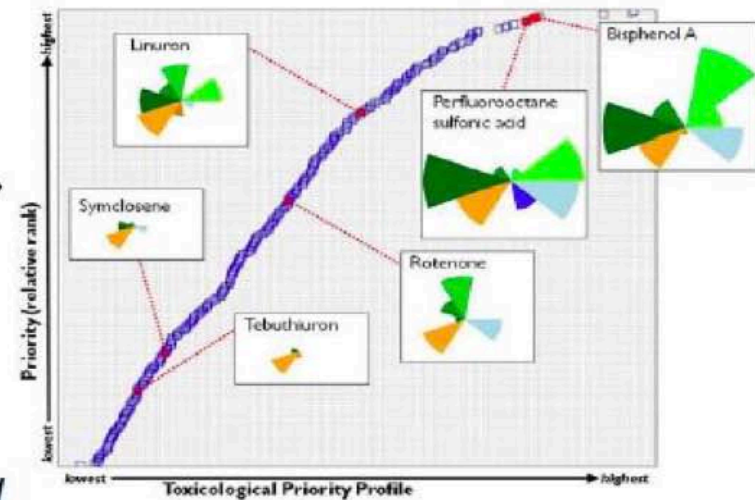
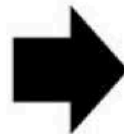
Thousands of
chemicals



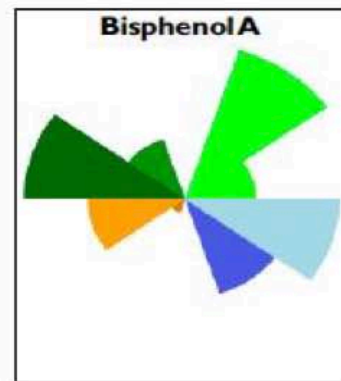
Hundreds of
biological assays



Bioinformatics/
Machine Learning



EPA ToxCast Program



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
and idea whose time has come.**

—Victor Hugo