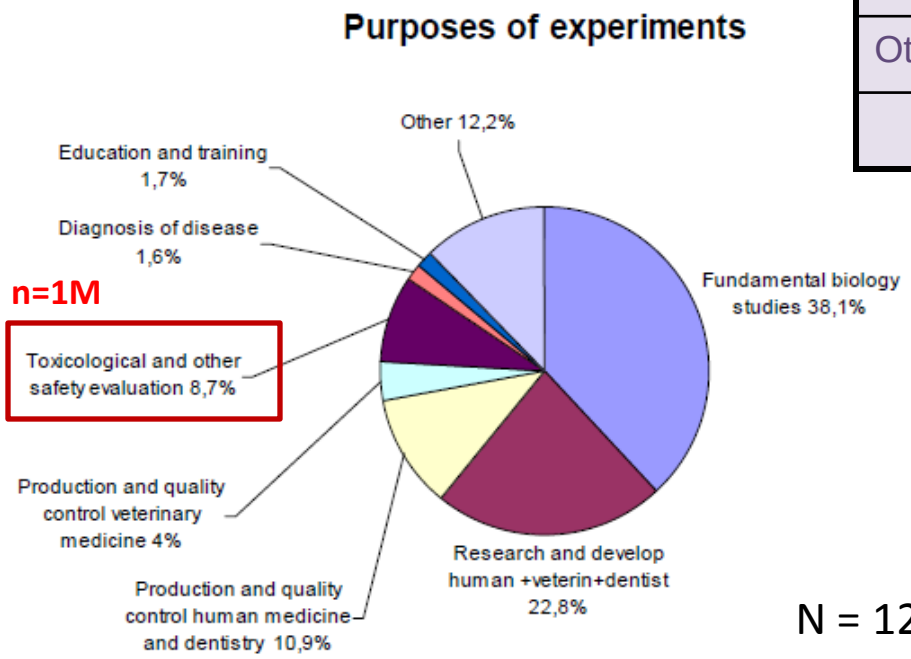
An NGO Perspective on Regulatory Acceptance of Non-animal Data and Related Issues

Martin Stephens, Ph.D.

The Humane Society of the United States

Animal Use Numbers

EU (2008)



Rabbits	222,167
Guinea pigs	203,098
Hamsters	150,051
Farm animals	101,137
Nonhuman primates	70,444
Dogs	67,337
Cats	20,160
Other counted animals	145,378
Subtotal	979,772

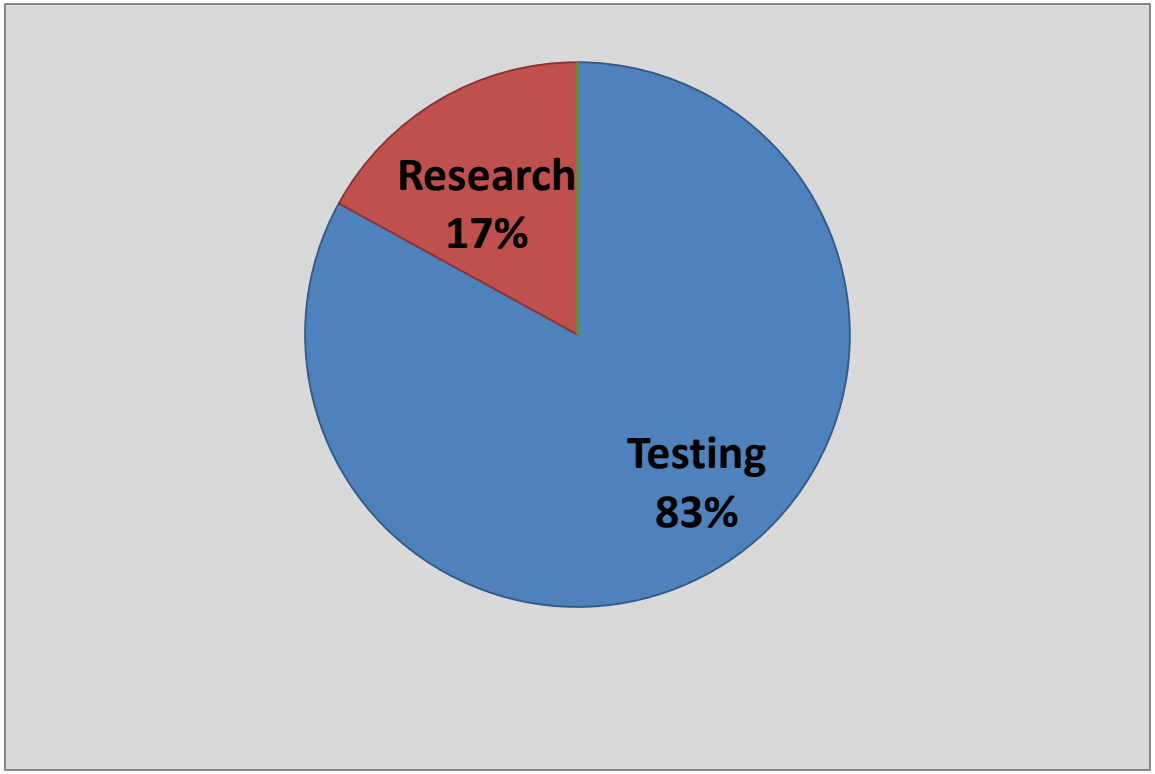
US (USDA, 2009)

N = 12 M

Number of animals experiencing moderate to severe suffering (2009)

Country	# of animals
Canada	899,430
Netherlands	27,040
New Zealand	65,201
Switzerland	128,804

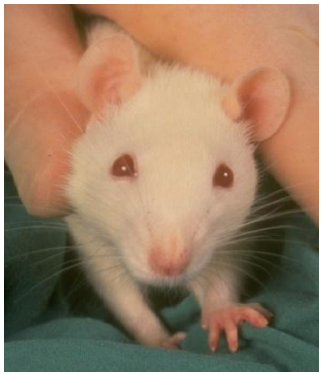
Category of Experimentation Causing "Unrelieved Pain and Distress" (US)



Notes: USDA-regulated animals only (N=88,643) during 1992.
1 animal was used in education (not shown).
From Stephens et al., JAAWS, 1998.

General frustration with animal-based approaches in toxicology

- Not just over animal use & suffering
- Date to 1930s, with modest changes over time
- Little use of modern biology
- Low throughput; expensive
- Questionable relevance to human risk
- Conservative extrapolation tools



Humane endpoints

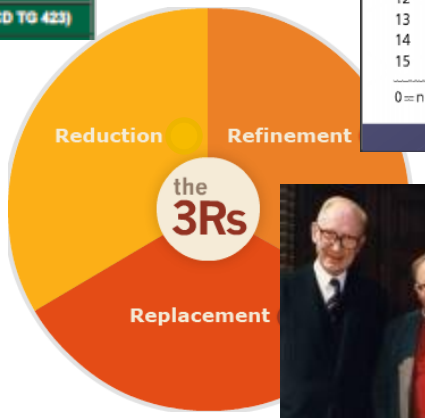
Acute Oral Systemic Toxicity		
Test Method	U.S. Acceptance	International Acceptance
Up-and-Down Procedure (oral)	2002	2002 (OECD TG 425)
Fixed Dose Procedure (oral)	2002	2002 (OECD TG 420)
Acute Toxic Class Method (oral)	2002	2002 (OECD TG 423)

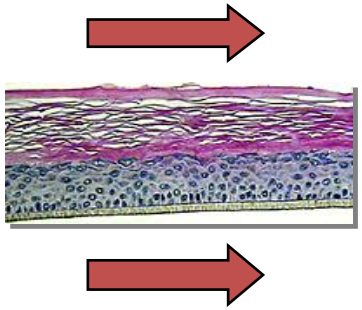
Table 2 Score of clinical signs of rabies in a group of mice

Day p.inf.	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	1	1	1	2	1	0	0
7	2	0	0	1	2	0	2	3	1	0
8	5	0	0	1	4	0	4	4	1	0
9	–	0	0	2	5	0	5	5	1	0
10	–	0	0	4	–	0	–	–	3	0
11	–	0	0	5	–	0	–	–	–	0
12	–	0	0	–	–	0	–	–	–	0
13	–	0	0	–	–	0	–	–	–	0
14	–	0	0	–	–	0	–	–	–	0
15	–	0	0	–	–	0	–	–	–	0

0 = normal; – = animal died

Cussler et al. 1999



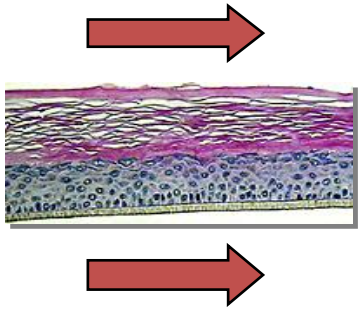


The Classical Road to Replacement

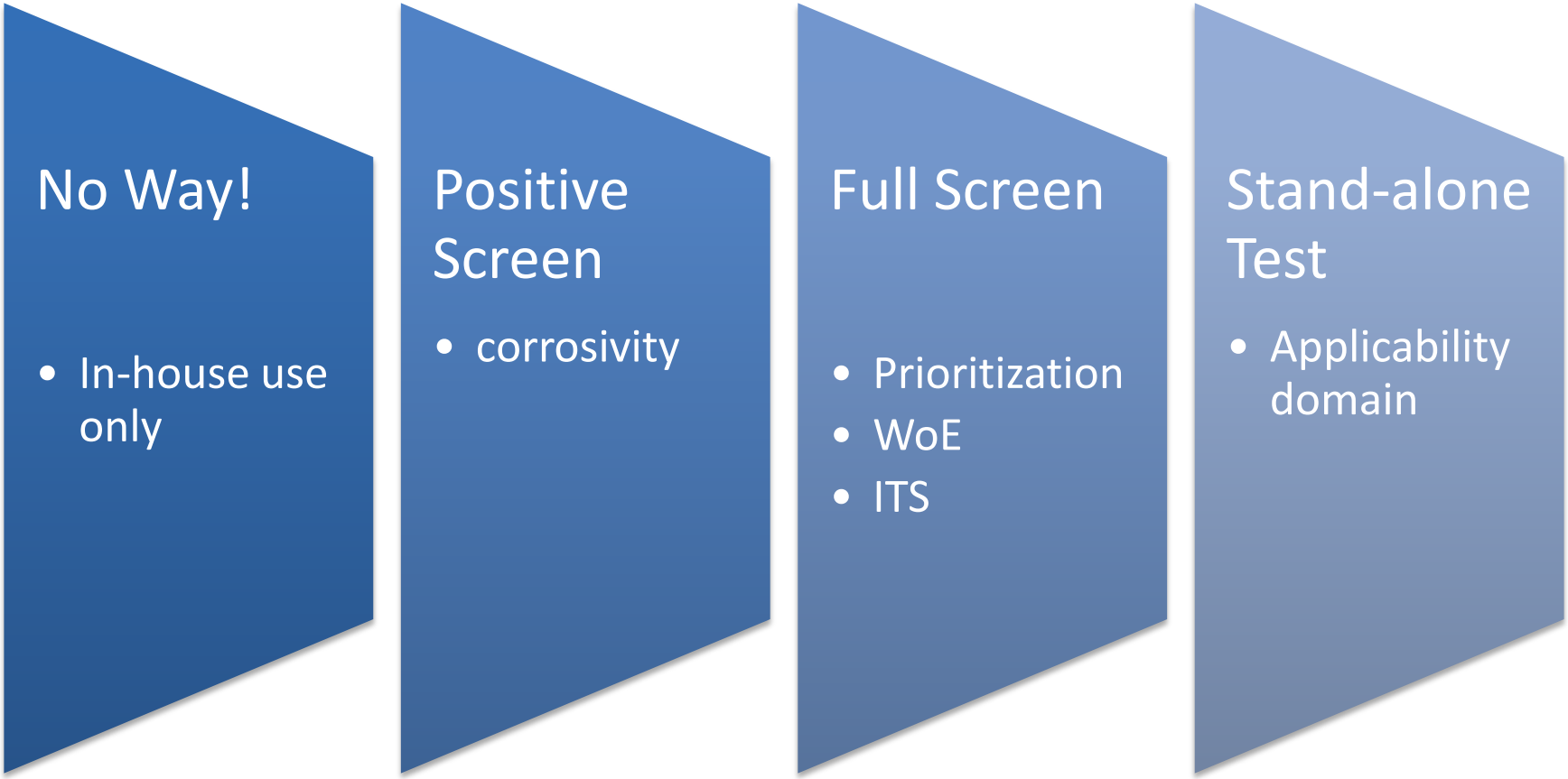
3yrs	2yrs	1yr	1yr	2yrs	1-3yrs	
R&D	Pre-Validation	Validation	Peer Review	Reg. Acceptance (Regional)	Reg. Acceptance (Global)	Usage

∑ 11yrs

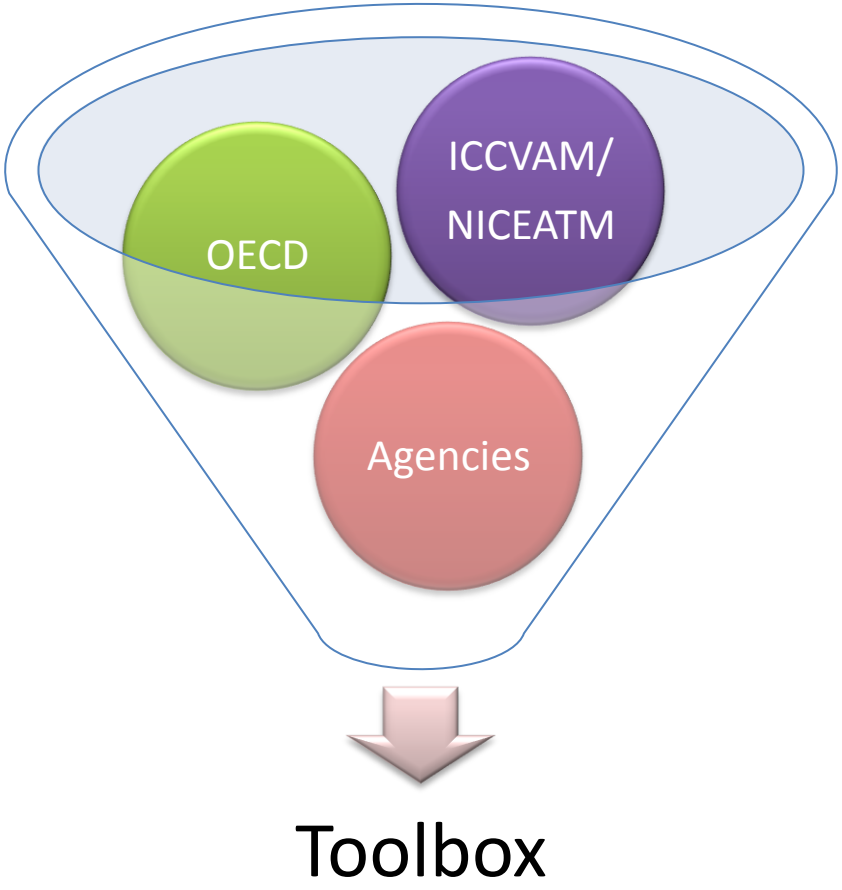
"Alternatives 1.0"



Evolution of Regulatory Acceptance



Multiple Routes to Acceptance



Alternative Methods Accepted by US Agencies

= in vitro

Acute Oral Systemic Toxicity		
Test Method	**U.S. Acceptance**	**International Acceptance**
Up-and-Down Procedure (oral)	2002	2002 (OECD TG 425)
Fixed Dose Procedure (oral)	2002	2002 (OECD TG 420)
Acute Toxic Class Method (oral)	2002	2002 (OECD TG 423)
In Vitro Cytotoxicity Test Methods: 3T3 Cells	2006	2010 (OECD GD 129)
In Vitro Cytotoxicity Test Methods: NHK Cells	2006	2010 (OECD GD 129)

Dermal Corrosivity		
Test Method	**U.S. Acceptance**	**International Acceptance**
Corrositex® Assay	2000	2006 (OECD TG 435)
EpiDerm™ Assay	2004	2004 (OECD TG 431)
EPISKIN™ Assay	2004	2004 (OECD TG 431)
Rat Transcutaneal Electrical Resistance Assay	2004	2004 (OECD TG 430)
SkinEthic RHE Assay	2004	2004 (OECD TG 431)

Dermal Irritation		
Test Method	**U.S. Acceptance**	**International Acceptance**
SkinEthic RHE Assay	via OECD Test Guideline)	2010 (OECD TG 439)

Ocular Toxicity		
Test Method	**U.S. Acceptance**	**International Acceptance**
Bovine Corneal Opacity and Permeability (BCOP) - Ocular Corrosivity/Severe Irritation	2006	2009 (OECD TG 437)
Isolated Chicken Eye (ICE) - Ocular Corrosivity/Severe Irritation	2006	2009 (OECD TG 438)
Integrated Non-animal Testing Strategy for Eye Irritation Potential of Antimicrobial Cleaning Products	*Anticipated 2011*	
BCOP - Nonsevere Ocular Irritation	*Anticipated 2011*	
HET-CAM - Nonsevere Ocular Irritation	*Anticipated 2011*	
ICE - Nonsevere Ocular Irritation	*Anticipated 2011*	
IRE - Nonsevere Ocular Irritation	*Anticipated 2011*	
Cytosensor Microphysiometer Test Method	*Anticipated 2011*	
Routine use of topical anesthetics, systemic analgesics, and humane endpoints in *in vivo* testing	*Anticipated 2011*	

Immunotoxicity		
Test Method	**U.S. Acceptance**	**International Acceptance**
Murine Local Lymph Node Assay (LLNA)	1999	2002 (OECD TG 429)
Updated LLNA Protocol (20% animal reduction)	2010	2010 (Updated OECD TG 429)
Reduced LLNA Test Method	2010	2010 (Updated OECD TG 429)
Development of LLNA Performance Standards	2010	2010 (Updated OECD TG 429)
Nonradioactive LLNA Method: LLNA:DA	*Anticipated 2010*	2010 (OECD TG 442A)
Nonradioactive LLNA Method: LLNA:BrdU-ELISA	*Anticipated 2010*	2010 (OECD TG 442B)
Use of the LLNA for Testing Pesticide Formulations, Metals, Substances in Aqueous Solutions, and Other Products	*Anticipated 2010*	2010 (Updated OECD TG 429)

Genetic Toxicity		
Test Method	**U.S. Acceptance**	**International Acceptance**
In Vitro Micronucleus Assay	via OECD Test Guideline)	2010 (OECD TG 487)

Pyrogenicity		
Test Method	**U.S. Acceptance**	**International Acceptance**
The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test	2009	
The Human Whole Blood/ IL-1 *In Vitro* Pyrogen Test Using Cryopreserved Human Whole Blood	2009	
The Human Whole Blood/IL-6 *In Vitro* Pyrogen Test (WB/IL-6)	2009	
In Vitro Pyrogen Test Using Human Peripheral Blood Mononuclear Cells (PBMC/IL-6)	2009	
An Alternative *In Vitro* Pyrogen Test Using the Human Monocytoid Cell Line MONO MAC 6 (MM6/IL-6)	2009	

Biologics and Vaccines		
Test Method	**U.S. Acceptance**	**International Acceptance**
Use of Humane Endpoints in Animal Testing of Veterinary Products	2004	
Use of Humane Endpoints in Animal Testing of Rabies Vaccines	2004	

http://iccvam.niehs.nih.gov/methods/milestones-US.htm

Endpoint	Method Name	Endorsement of Scientific Validity		Regulatory Acceptance	
		Lead Authority	Subsequent Endorsement(s)	International	National/ Regional (for methods not yet accepted int'ly)
Acute mammalian toxicity (oral)	Normal human keratinocyte neutral red uptake (NHK NRU) assay	ICCVAM (2006)		Draft OECD TG	US agencies (2008)
Dermal penetration	In vitro skin absorption methods	OECD Expert Group (2002)		OECD TG 428 (2004)	
Endocrine mechanistic screens	Estrogen receptor binding assay				OPPTS TG 890.1250 (EPA, 2009)
Genotoxicity	*In vitro* micronucleus test	ESAC (2006)		Draft OECD TG 487	REACH Regulation
Hematotoxicity: acute neutropenia	Colony forming unit granulocyte macrophage (CFU-GM) assay	ESAC (2006)			
Eye irritation	Cytosensor Microphysiometer modified (cytotoxicity/cell-function based in vitro assay)	ESAC (2009)			
Eye corrosion	Bovine corneal opacity permeability (BCOP) test	ICCVAM (2007)	ESAC (2007); JaCVAM (2009)	OECD TG 437 (2009)	
Dermal penetration	In vitro skin absorption methods	OECD Expert Group (2002)		OECD TG 428 (2004)	
Phototoxicity	3T3 Neutral Red Uptake Phototoxicity Test	ESAC (1997)		OECD TG 432 (2004)	
Pyrogenicity	Human whole blood IL-1	ESAC (2006)	ICCVAM (2008)		European Pharmacopeia; US agencies
Reproductive & developmental tox	Embryonic stem cell test	ESAC (2002)			
Skin corrosion	EpiSkin® human skin model	ESAC (1998)	ICCVAM (2002)	OECD TG 431 (2004); Draft Rev. TG 431 ('09)	
Skin irritation	EpiDerm™ SIT model	ESAC (2008)		Draft OECD TG	EU test method B.46 in COM regulation 440/2008/EC
Vaccine potency	Toxin binding inhibition test for human tetanus vaccines batch potency testing	ESAC (2000)			EDQM/European Pharmacopeia

Past Sore Points: Example

- Dermal Corrosivity Testing (ICCVAM)
  - In vitro human skin models as positive screens only
  - Despite OECD acceptance as stand-alone
  - Given low prevalence…
    - Negatives >> considerable confirmatory animal testing

Informal Acceptance Examples from Industry Contacts

- *"We were able to convince the **EPA** to accept BCOP data (with histology) for registration of topical formulations which contained an already approved and tested insect repellant. We did not have to submit new in vivo eye data for the formulations, even though BCOP was and is currently not validated. I believe this first occurred in [early 2000s]."*

- *"An EFPIA* member … has successfully used in vitro phototoxicity data in a regulatory submission. The drug was weakly positive in an in vitro 3T3 phototoxicity assay. Due to the large safety margin of anticipated human therapeutic plasma levels versus the in vitro 3T3 IC50 value, … **FDA** accepted the in vitro data, considered the clinical trial design to be acceptable and did not request further phototoxicity assessment."*

* European Federation of the Pharmaceutical Industries and Associations

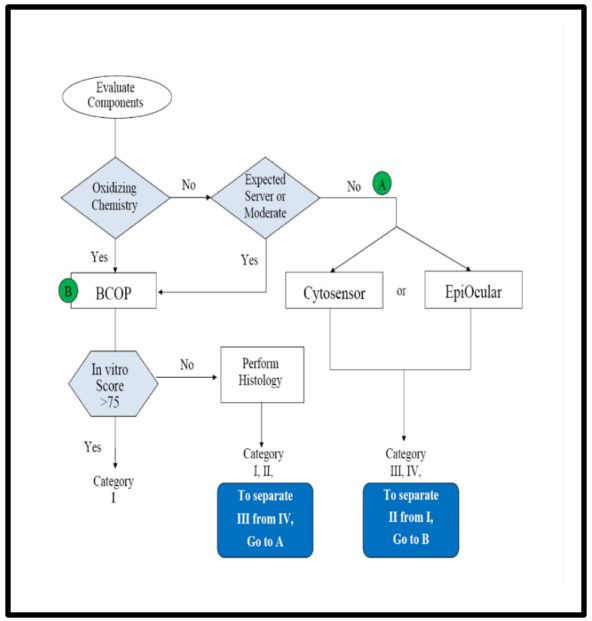
“ICCVAM Validated” Tests Submitted to EPA

Endpoint	Test Method	# Submissions
Skin sensitization	LLNA	241
Acute systemic tox	Up and Down	1139
Corrosivity	Corrositex	0
Skin irritation	Episkin/Epiderm/Skinethic	2
Eye irritation	BCOP	14
	Cytosensor	0
	EpiOcular	3
Pyrogenicity	in vitro pyrogen tests	0
Estrogenic activity	LUMI CELL	0
	CerticChem	0
Data courtesy J. Fowle, EPA		1399

in vitro

EPA/OPP Antimicrobial Cleaning Product Testing Strategy

- Pro-active!
- Voluntary Pilot Program ’09
- Outcome of work with stakeholders since ‘04
- BCOP, CM, EpiOcular + incident data & available Draize eye data
- 6 submissions?
  - 3 accepted, 3 pending
- 1.5 yr program; made permanent soon?



http://www.epa.gov/oppfead1/cb/csb_page/updates/2009/eye-study.html

Frustrations with “Alternatives 1.0”

- Process: time-consuming & expensive
- New tests: medium throughput at best
- Sometimes: limited applicability or are components of cumbersome ITS
- Limited capacity to address chronic endpoints
- Performance assessed WRT existing animal tests
- Fed into same framework of identifying hazards

Signs of the Times, I.

Advancing Regulatory Science
Margaret Hamburg, FDA Commissioner
Editorial, Science, Feb. 25, 2011

We must bring 21st-century approaches to 21st-century products and problems.

Most of the toxicology tools used for regulatory assessment rely on high-dose animal studies and default extrapolation procedures and have remained relatively unchanged for decades, despite the scientific revolutions of the past half-century.

With an advanced field of regulatory science, new tools … can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects.

The FDA is … working to eventually replace animal testing with a combination of in silico and in vitro approaches. The inherent complexity of the vertebrate reproductive system represents a major challenge to developing such technologies that replace whole-animal tests, and advanced regulatory science is needed to address this challenge.

Policy-makers, industry leaders, and the scientific community have the opportunity and the power to answer this call to action. It cannot wait any longer.

Signs of the Times

President's FY2012 EPA Budget

EPA will begin a multi-year transition from the ... EDSP to validate and more efficiently use computational toxicology methods and high throughput screens that will allow the Agency to more quickly and cost-effectively assess potential chemical toxicity.

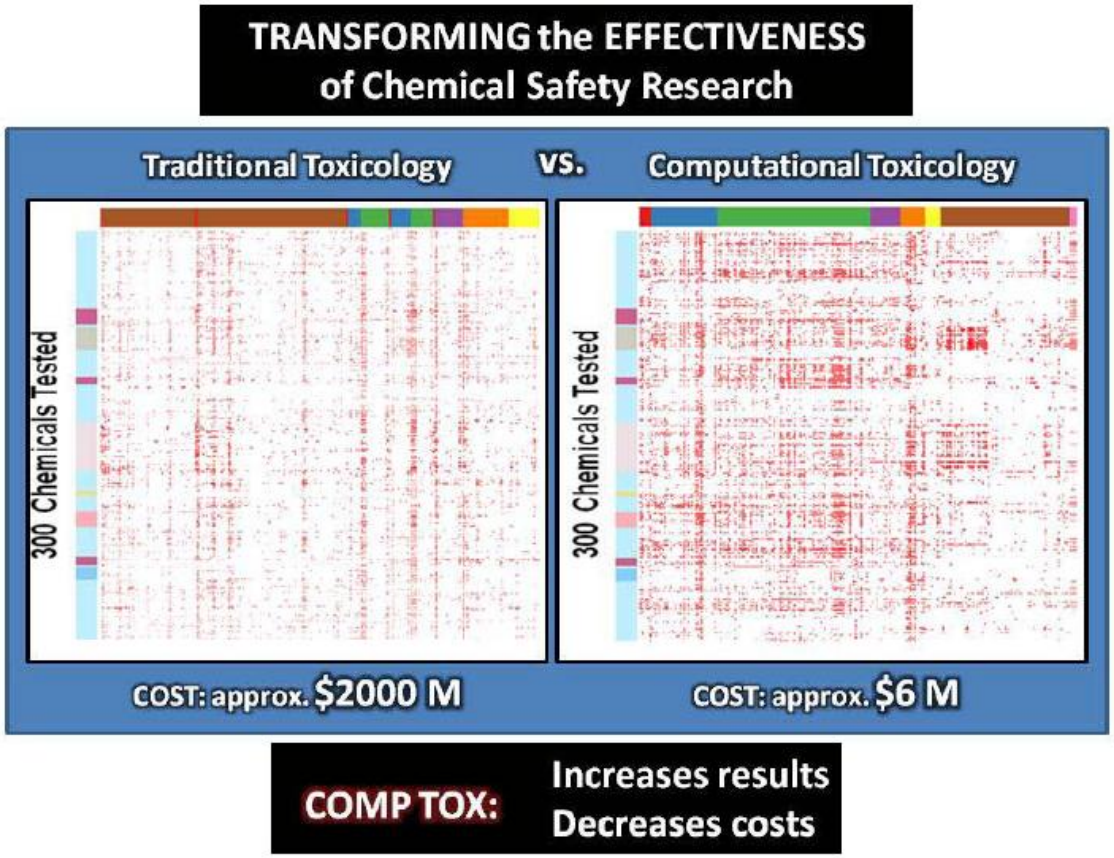


Figure V: EPA research is developing computational toxicology tools that are faster, more efficient, and have the capacity to test thousands of chemicals at a fraction of the cost for traditional animal-based testing (e.g., $2 billion versus $6 million for 300 chemicals). This innovative research is critical to catalyzing sustainable solutions that inform decisions on chemical safety.

…on the heels of last year's disappointment

June 16, 2010

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Revised ORD Statement on the Use of ToxCast Data in EDSP

FROM: Robert J. Kavlock, Director
National Center for Computational Toxicology (B205-01)

Hal Zenick, Director
National Health and Environmental Effects Research Laboratory (B305-01)

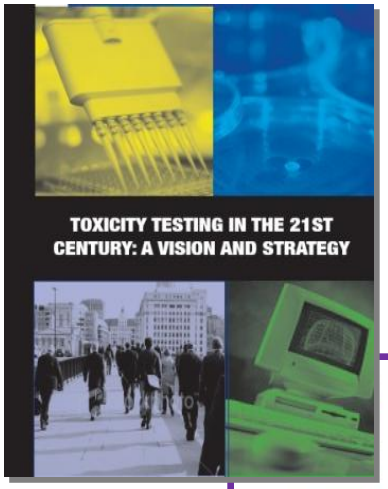
THRU: Elaine Z. Francis
National Program Director (8101R)

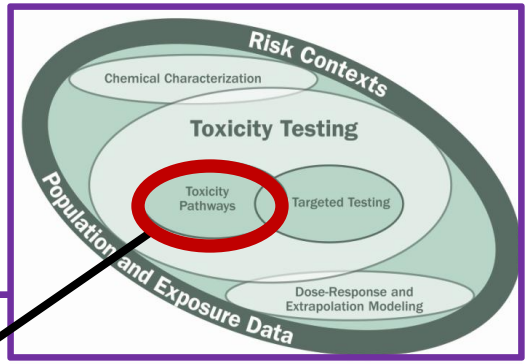
TO: Gary Timm, Co-Chair
Endocrine Disruptor Review Team
Office of Science Coordination and Policy

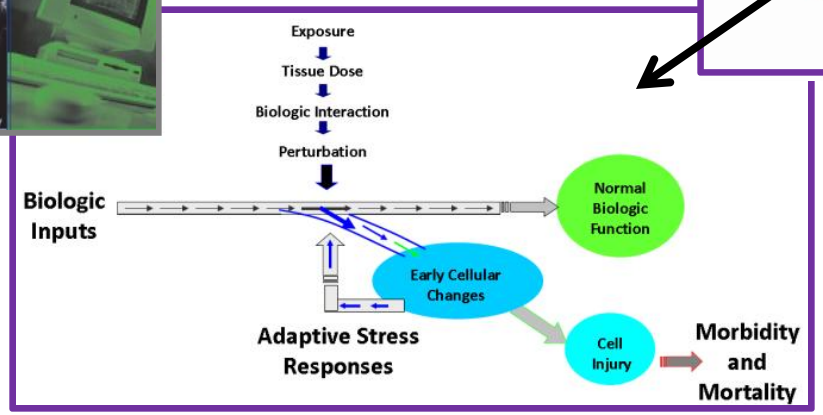
Karen Whitby, Ph.D, Co-Chair
Endocrine Disruptor Review Team
Office of Pesticide Programs

"it is our position that the ToxCast in vitro assays cannot at this time be considered as an acceptable alternative to the EDSP T1 S in vivo or in vitro assays"

NAS/NRC report, 2007







"adverse effects" ("adversity")

"perturbations" to "toxicity pathways"

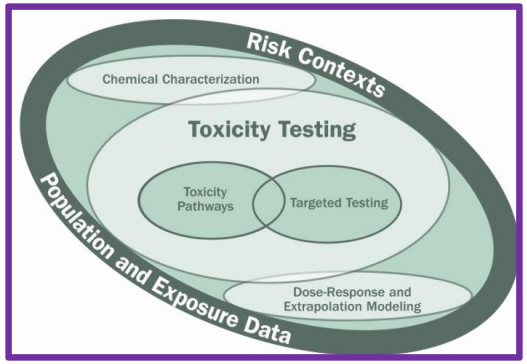
Consequences

I. High-dose animal studies
   >>> *in vitro* methods

… a not-so-distant future where all routine toxicity testing will be conducted in human cells or cell lines *in vitro* by evaluating perturbations of cellular responses in a suite of toxicity pathway assays.

Andersen and Krewski (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life. Tox. Sci., 107, 324-330.

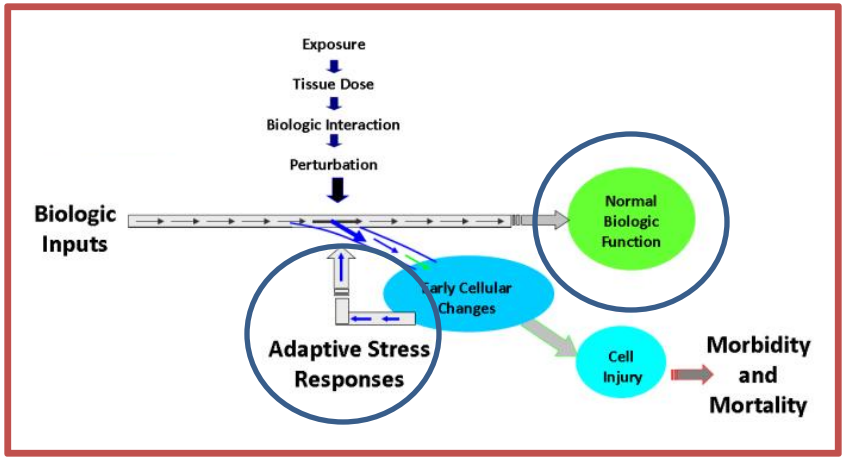
II. Hazard ID
   >>> safety assessment



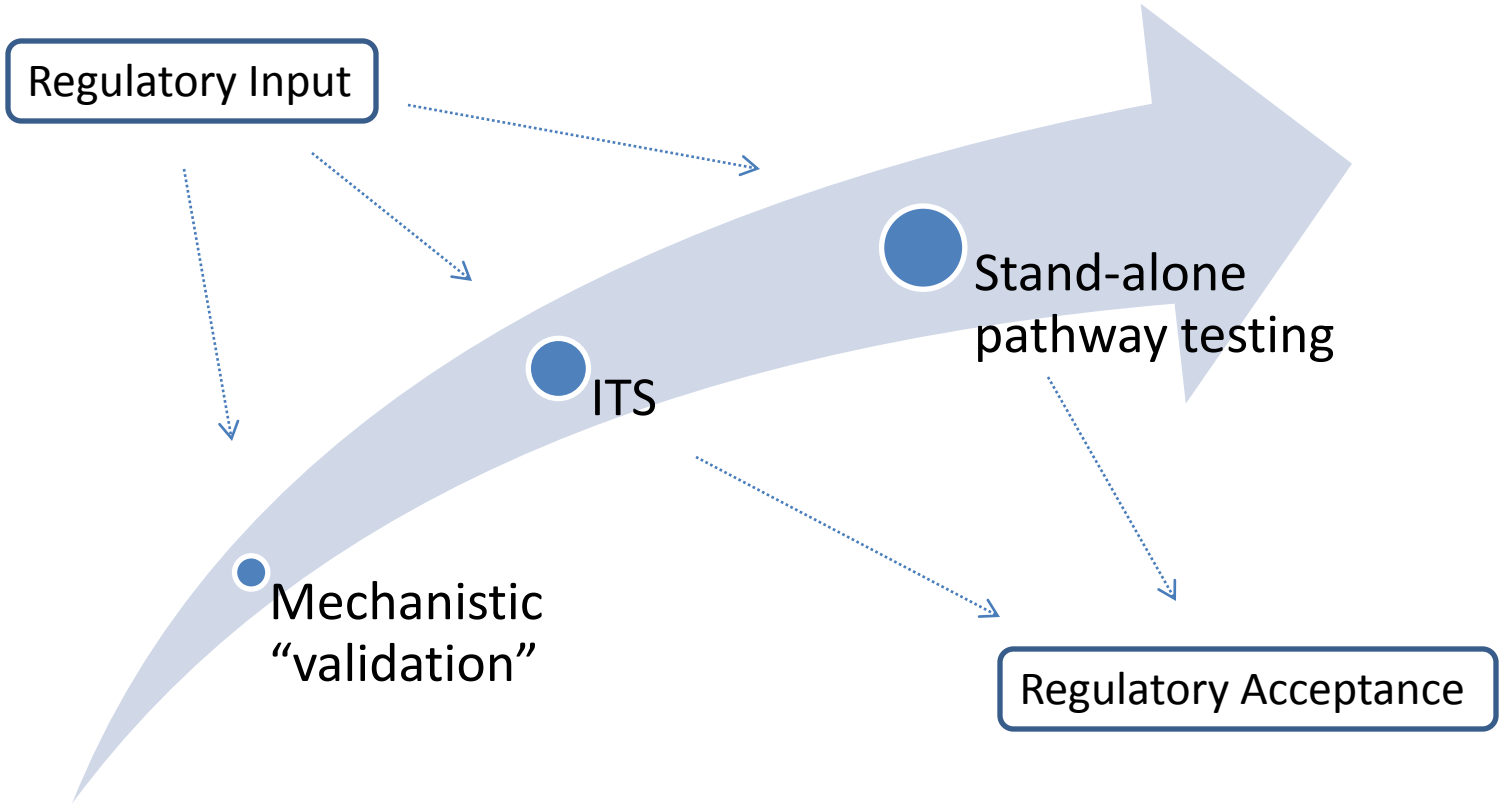
Hazard ID

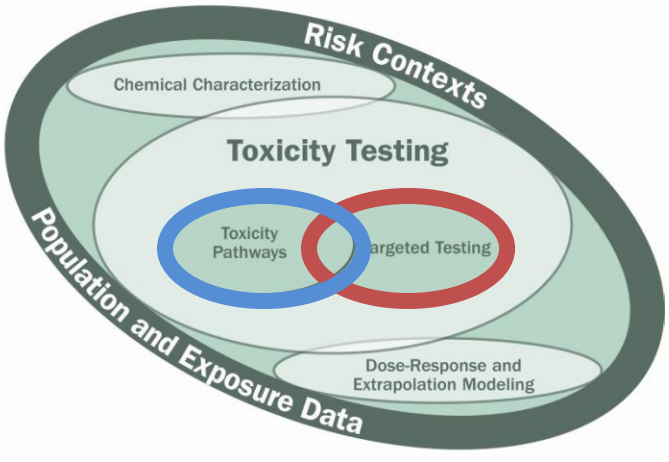
Zones of Safety

- High-dose animal studies
- Apical effects

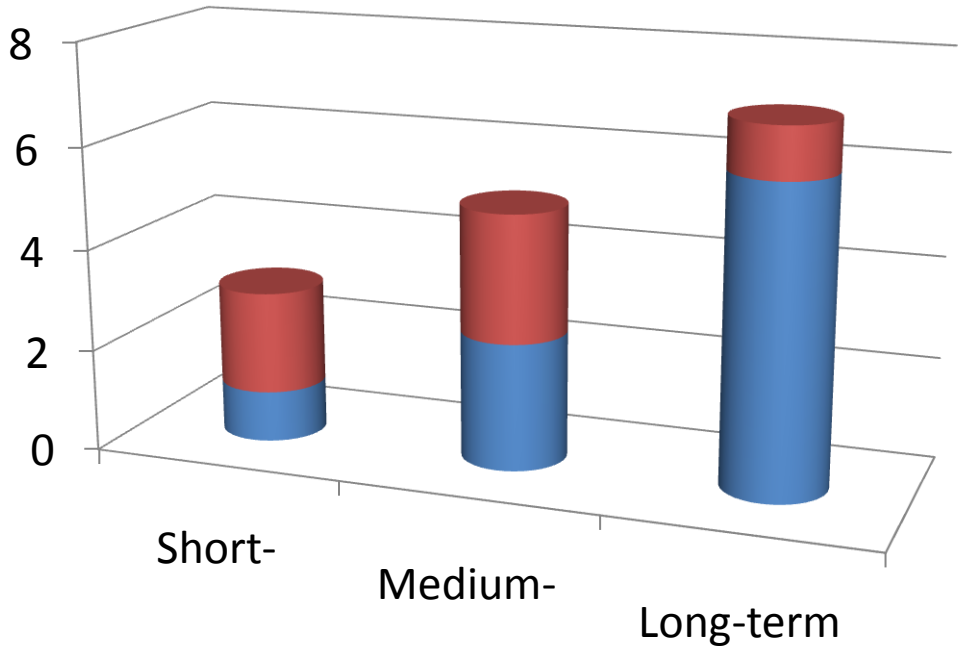


The Road to Replacement (Alternatives 2.0)

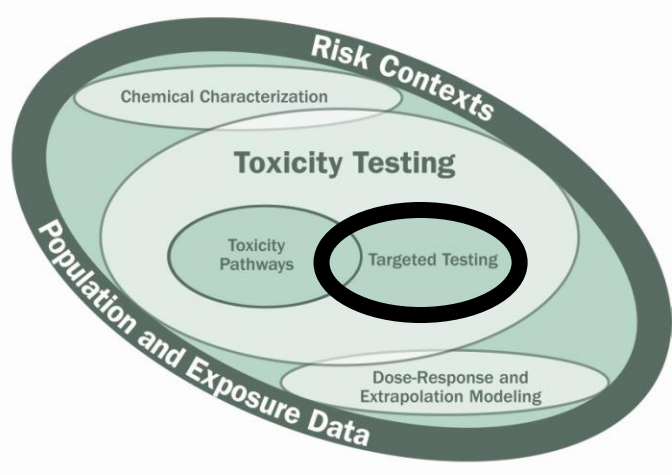


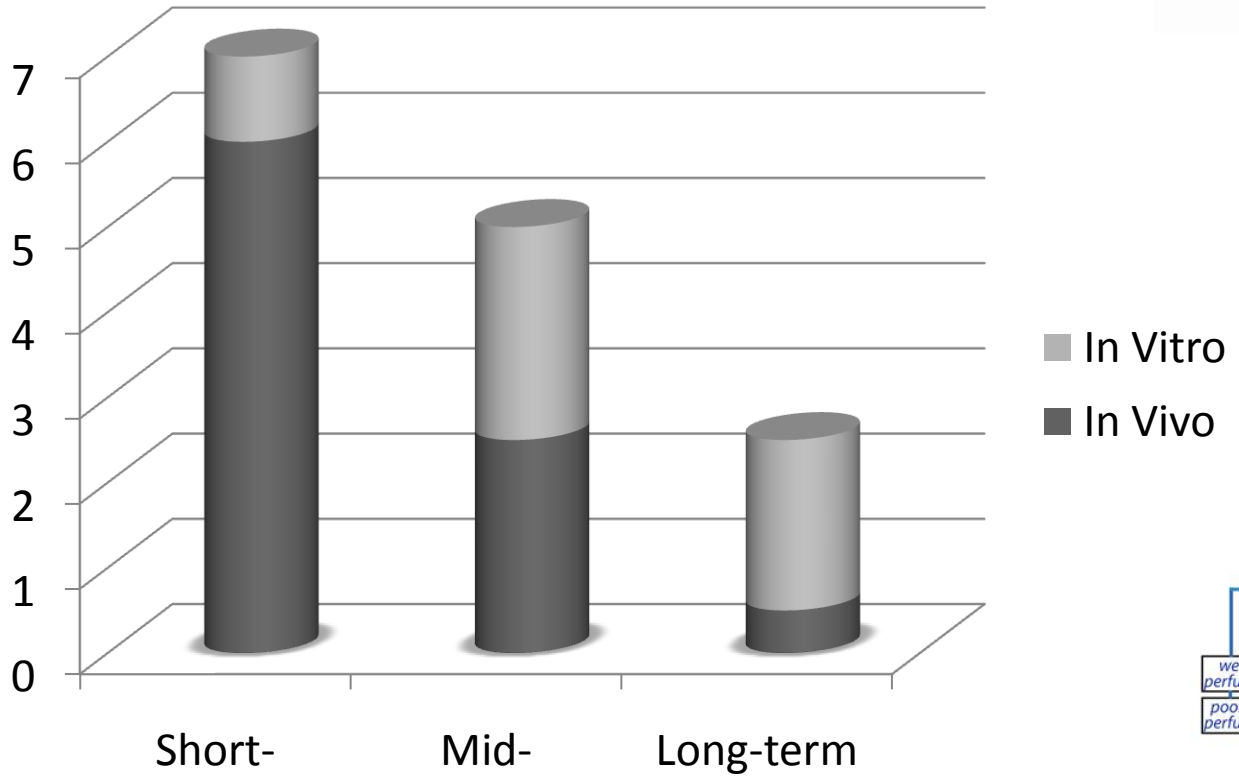


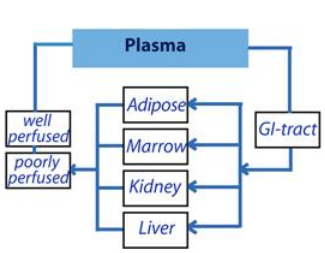
The mix of pathway vs. targeted testing

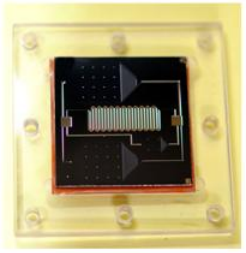


Evolving Nature of Targeted Testing Itself



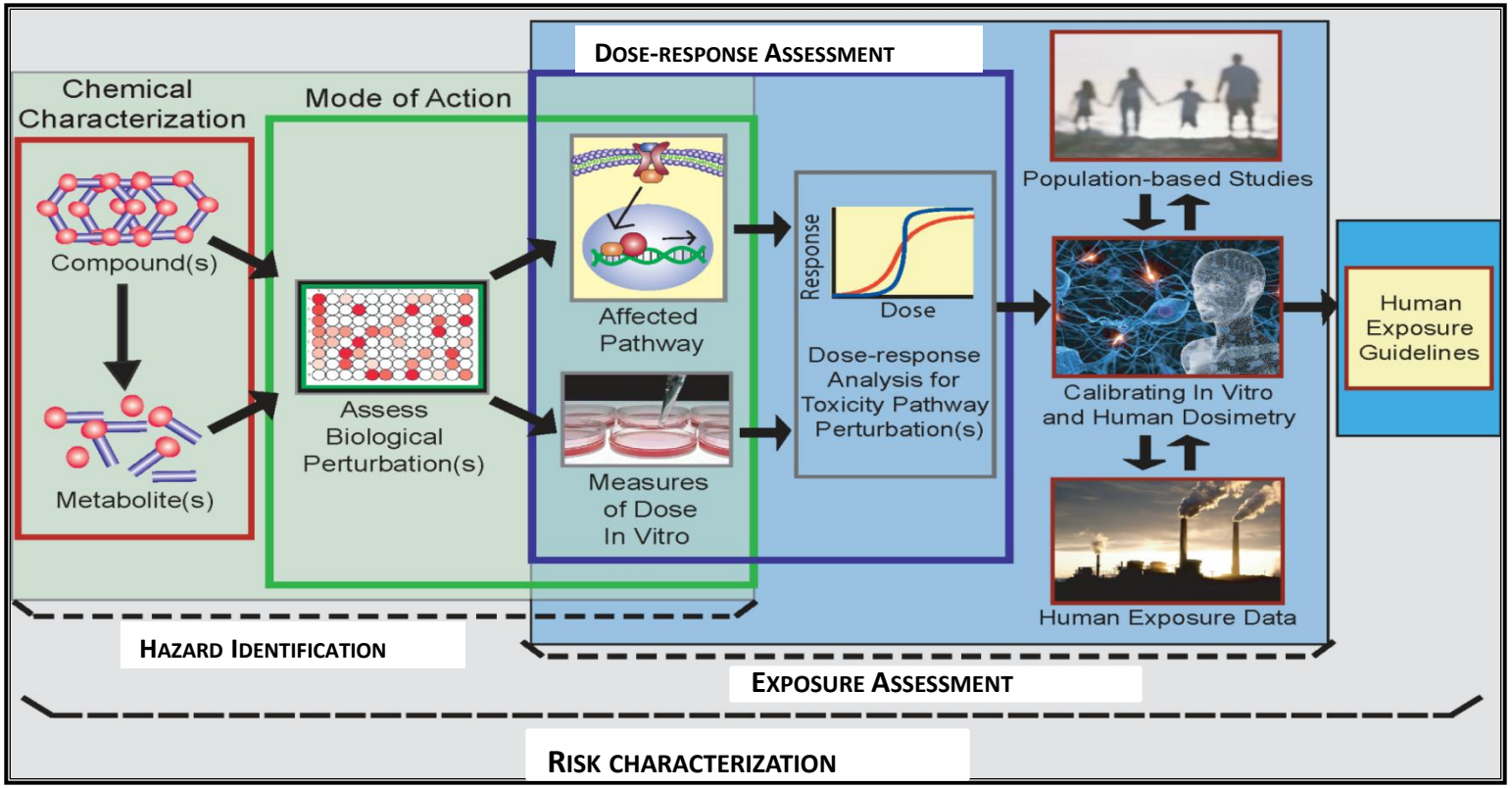






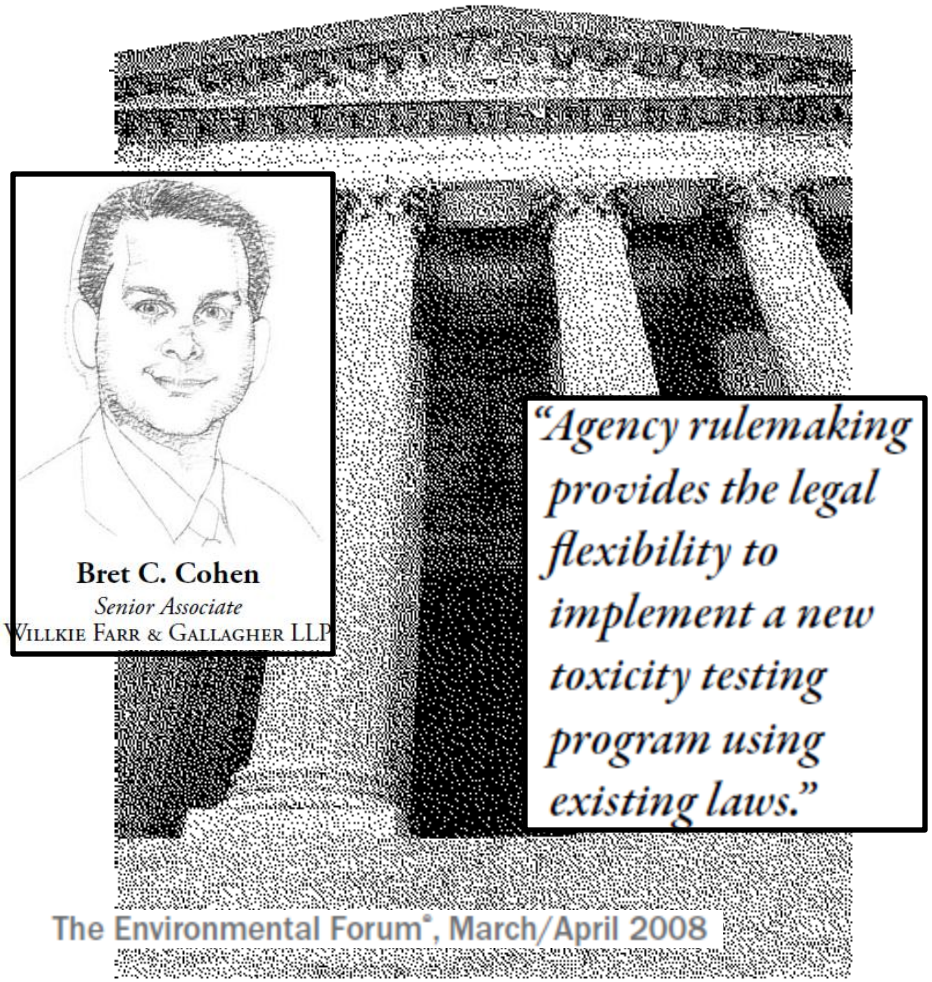
Schuler & Esch, 2009
http://alttox.org/ttrc/way-forward/

Mapping New Approach to RA



Modified from Krewski et al. (2011)

Statutory Readiness



Implementing the National Academy's Vision and Strategy for Toxicity Testing: Opportunities and Challenges Under the U.S. Toxic Substances Control Act

Journal of Toxicology and Environmental Health, Part B: Critical Reviews

Volume 13, Issue 2 & 4, 2010, Pages 376 - 384

Authors: Paul A. Locke[a]; D. Bruce Myers Jr.[b]

Other Issues

- Agencies cannot accept what they don't receive
- They cannot review what is not submitted
- Acceptance vs. decisive use (OECD)
- Acceptance: in silico, read across, etc., as well as in vitro
- Acceptance of chemical categories in large-scale testing programs (e.g., HPV, REACH)

Acknowledgements

- Dawn McPherson, HSUS
- Jack Fowle, EPA
- Industry contacts
- Mel Andersen, Hamner

Thank you!

Martin Stephens

mstephens@hsus.org