Animal Alternatives – Current Status of Validation and Regulatory Acceptance

Thomas Hartung & CAAT team

Toward humane science

CAAT

Russell and Burch

ECVAM

In vitro testing

Validation

Tox-21c

My “welcome present” at ECVAM

The 7th amendment of the Cosmetics Directive 2003

• Marketing ban if testing finished products or not using ECVAM-validated methods since 2004
• Phasing out ingredient testing with test and marketing bans in 2009 and 2013
• Critical need for alternatives
**3R Success stories**

OECD acceptance of validated methods:

- **1999-2001**
  - **Refine:** Painless test for skin sensitisation
  - **Reduce:** Animal numbers for acute tox. from 45 to 8

- **2004 - 2010**
  - **Replace:**
    - phototoxicity
    - skin / eye corrosion / irritation

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**Toxicological Endpoints**

<table>
<thead>
<tr>
<th>Toxicological Endpoints</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin Corrosion, acute phototoxicity, sensitization (LLNA)</td>
<td>OECD accepted before 2002</td>
</tr>
<tr>
<td>Aquatic ecotoxicity</td>
<td>2010 OECD accepted</td>
</tr>
<tr>
<td>Skin Absorption / Penetration</td>
<td>2004 OECD accepted</td>
</tr>
<tr>
<td>Skin Irritation</td>
<td>2010 OECD accepted</td>
</tr>
<tr>
<td>Genotoxicity (MNT)</td>
<td>2010 OECD accepted</td>
</tr>
<tr>
<td>Eye Irritation (BCOP, ICE)</td>
<td>2010 OECD accepted</td>
</tr>
<tr>
<td>Sensitization (rLLNA)</td>
<td>2010 OECD accepted</td>
</tr>
<tr>
<td>Carcinogenicity (CTA)</td>
<td>Under discussion</td>
</tr>
</tbody>
</table>

Source: ECEAE

Pipeline: sensitization in vitro, embryotoxicity, endocrine disrupters, photogenotox, eye irritation, acute tox dose estimate

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**Traditional 3Rs methods will not be the solution to the problem**

- Little perspective for complex endpoints
- 2/3 fail validation
- hardly solved the cosmetics 7th amendment challenge for 2009, no way for 2013
Limitations of in vitro models

- Mycoplasma
- Dedifferentiation favored by growth conditions and cell selection
- Cells are bored to death
- Lack of oxygen
- Lack of metabolism and defense
- Unknown fate of test compounds in culture
- Tumor origin of many cells
- Cell identity

Cell models have many limitations

- Better science
- Less animals
- Human relevance
- Faster and cheaper results
- Refinement
- Information, Grants
- Think tank
- New tools, quality control
- EU branch, policy program
- Stakeholder consensus

Henry Spira and the start of CAAT

Scientific American 2005

PROTECTING MORE THAN ANIMALS
Better science, Less animals, Human relevance, Faster and cheaper results, Refinement, Information, Grants, Think tank, New tools, quality control, EU branch, policy program, Stakeholder consensus

Henry Spira and the start of CAAT
Funding from industry, philanthropy and research funding agencies

The Bernice Barbour Foundation

...and individuals

US Policy program 2006
EU center of excellence

EU Policy program 2012

transatlantic cooperation

32 articles / reports published
2 commissioned articles in preparation
5 workshop reports pending
In vitro publication standards
5+ workshops planned

Financing DZF ends 2015
Scientific roadmap for the future of animal-free systemic toxicity testing

May 2011: EC report on status of alternatives
Sep 2011: Independent review by 19 international experts
Oct 2011: Five white paper on the way forward
Consensus workshop with 35 experts
Feb 2012: Roadmap published
Mar 2012: Stakeholder Forum in Brussels (150 experts)
May 2013: Stakeholder Forum in Washington (200 experts)

30-31 May 2013
“With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, 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

“We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments”

F. Collins, NIH, 2008
Endocrine disruption
• Use "omics" to map PoT for endocrine disruption
• Develop software tools
• Identify PoT
• Develop a process for PoT annotation, validation
• Establish public database on PoT.

Use for PoT identification:
• Homeostasis under stress, i.e. signatures of tox
• Critical cell infrastructures
• Network knowledge
• Reference models
• Reference toxicants
**Level of resolution**

- Perturbed Molecular Network
- Molecular Pathway of Toxicity
- Toxicity Pathway / AOP
- Mode of Action
- Phenomenologic

**Tox-20c**

Evidence-based Tox.

**Tox-21c**

Pathways of Tox (PoT)  
Human Toxome

**Evidence-based Tox.**

Pathways of Tox (PoT)

**Human Toxome**

Evidence-based Tox.

**Tox-20c**

Omics, high-content, HTS  
Bio-informatics & -engineering

**Tox-21c**

Integrated Testing Strategies  
**ITS**

**Organo-typic cultures**

**Human-on-Chip**

**Food for Thought ...**

Integrated Testing Strategies for Safety Assessments

1st Workshop Report  
Integrated Testing Strategies (ITS) for Safety Assessment

- Joint Symposium with ESTIV / IVTIP
The future of ITS

- Interim decision points
- Probabilistic / Bayesian approaches
- Modeling and Machine Learning


Integrating Non-Animal Test Information into an Adaptive Testing Strategy - Skin Sensitization Proof of Concept Case

Joanna Jaworska1, Artiom Hanol1, Petra S. Kern1, and G. Frank Gerberick2

Test Case Skin Sensitization
- Defined AOP
- Ca. 20 tests plus non-test information
- Human and LNNA data

Research Article

Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice

Joanna Jaworska1, Yuri Dancik*, Petra Kern*, Frank Gerberick* and Andreas Natsch*
ITS appear to be able to overcome even the potency assessment problem

Assays were never developed for ITS and potency assessment

Improvement by complementary assays and *in silico* methods

New validation concepts

**Evidence-based Tox.**

**Tox-20c**

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**Human Toxome**

**ITS**

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2006-7: Publication / 1st conference

Mar 2011: US EBTC

Oct 2011: Secretariat at CAAT

www.ebtox.com

Jan 2012: First conference hosted by EPA

Jun 2012: EU EBTC

Diverse working groups

Jul 2013: IUTOX, Seoul, Korea

Sep 2013: EuroTox, Interlaken, Switzerland

Systematic reviews increasingly embraced by EPA/IRIS, NTP and EFSA

21 Nov 2014: Forum Systematic Reviews, Baltimore

**EBT.**

1st International Forum towards Evidence-Based Toxicology (EBT)

October 15-18, 2007, Como, Italy
New concepts for validation from EBT

Evidence-Based Toxicology – the Toolbox of Validation for the 21st Century?

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Food for Thought ...
Mechanistic Validation

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ALTEX 30 (2013) 119-130

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InfoDays
Green Toxicology
Connecticut, Dec 2012
Baltimore, Nov 2013
Zurich, Switzerland 23 Oct 2014
SoT 2015, San Diego
CAAT Read-across Initiative

Food for Thought ...
Read-Across Approaches – Misconceptions, Promises and Challenges Ahead
Grace Patzwitz¹, Nicholas Bailey¹, Richard A. Becker⁴, Evan D. Booth⁴, Mark T. D. Crean⁵, Dinant Krewer⁴, David Stoup⁴, Ben van Rensenberg⁴ and Thomas Hartung⁴

International Steering Group & Whitepaper Workshop in Baltimore 12-14 Jan 2015 “Good Read-across Practice” Stakeholder Fora in Brussels & Washington end 2015

Read-across-21c
• Negative vs. positive read-across
• Support by biological data not only structure
• Expression of uncertainty
• Local validity
• Application to complex mixtures

“Test-across”, 2007
The difficulty lies, not in the new ideas, but in escaping from the old ones.

John Maynard Keynes
(1883 - 1946)