Symposium
20th Anniversary of ZEBET at BfR
and 50 Years of the 3Rs Principle
October 26-27, 2009
Federal Institute for Risk Assessment (BfR), Berlin,

International Pre-validation and Validation Studies

Horst Spielmann
Professor for Regulatory Toxicology
FU Berlin und BfR, Berlin
ZEBET's contribution to the concept of experimental validation

1989 no validation concept existed
1990 CAAT/ ERGATT AMDEN (CH) validation workshop
1990 ERGATT Vouliagmeni (GR) workshop on regulatory acceptance
1992 ECHO eye irritation study ➔ not successful
1993-98 EU/ECVAM/COLIPA/ZEBET validation of in vitro phototoxicity tests ➔ successful
1994 Amden II ECVAM workshop on practical aspects of validation
1995 Prevalidation concept (ECVAM, IIVS & ZEBET)
1996 Solna OECD validation WS & OECD acceptance
1997-2000 ECVAM validation of prediction models ➔ proof of principle successful
1997-2000 ECVAM catch-up validation ➔ proof of principle successful
1998-2002 ECVAM validation of 3 in vitro embryotoxicity tests ➔ successful
2003 ECVAM proposes “modular approach to validation”
2004 ECVAM WS on “Weight of Evidence (WoE) validation”
2005 OECD GD 34 on the “validation of new an updated methods for hazard assessment”
2004-2007 ECVAM validation study on in vitro tests for skin irritation ➔ successful
1990 the first workshops on validation & acceptance

Report and Recommendations of the CAAI/ERGATT Workshop on the Validation of Toxicity Test Procedures.

Michael Bolls, Bas Blaauwboer, David Brusick, John Frazier, Denise Lamb, Mark Pemberton, Christoph Reinhardt, Marcel Roberfroid, Herbert Rosenkranz, Beat Schmid, Horst Spielmann, Anna-Laura Stammati & Erik Wolum.

Reprinted from ATLA 18, 1990.
The Concept of Experimental Validation

Developed at the CAAT/ERGATT validation workshop in AMDEN (CH) 1990 (ATLA 18, 313-337, 1990)

DEFINITION
Validation is the process by which the reliability and relevance of a procedure are established for a particular purpose.

Experimental procedure

TEST-DEVELOPMENT

1. intra-laboratory evaluation
2. inter-laboratory evaluation
3. data base development
4. Independent evaluation (peer review)

VALIDATION

REGULATORY ACCEPTENCE
Practical Aspects of the Validation of Toxicity Test Procedures
The Report and Recommendations of ECVAM Workshop 5\textsuperscript{1,2}

Michael Balls\textsuperscript{3}, Bas J. Blaauwoer\textsuperscript{4}, Julia H. Fentem\textsuperscript{3}, Leon Bruner\textsuperscript{5}, Robert D. Combes\textsuperscript{6}, Björn Ekwall\textsuperscript{7}, Robin J. Fielder\textsuperscript{8}, André Guillouzo\textsuperscript{9}, Richard W. Lewis\textsuperscript{10}, David P. Lovell\textsuperscript{11}, Christoph A. Reinhardt\textsuperscript{12}, Guillermo Repetto\textsuperscript{13}, Pariusz Sladowski\textsuperscript{14}, Horst Spielmann\textsuperscript{15} and Flavia Zucco\textsuperscript{16}
The Role of Prevalidation in the Development, Validation and Acceptance of Alternative Methods

Rodger D. Curren\textsuperscript{1}, Jacqueline A. Southee\textsuperscript{2}, Horst Spielberg\textsuperscript{3}, Manfred Liebsch\textsuperscript{3}, Julia H. Fentem\textsuperscript{4} and Michael Balls\textsuperscript{4}

Prevalidation scheme
proposal for a prevalidation study (laboratory 1)
1. protocol refinement (laboratory 2)
2. protocol transfer (laboratory 3)
3. protocol performance. (laboratories 1-3)
Biostatistically based PREDICTION MODELS may be simple, e.g. linear in vitro/in vivo correlation...or more complex.
Harmonised OECD Validation Concept 1996
& ECVAM, ICCVAM (USA)

Test Development
  basis
  • need
  • protocol
  • prediction model

Scheme for Prevalidation
  • optimisation protocol
  • interlaboratory transferability
  • optimisation

Validation
  • blind trial
  • relevance

Independent Evaluation
Regulatory Acceptance
In vitro phototoxicity

Validation of the 3T3NRU-PT

*in vitro* Phototoxicity-Test

1992-1998
The ECVAM International Validation Study of three in vitro embryotoxicity tests

1998-2002

Embryonic Stem Cell Test (EST) (mouse = mEST)

Micromass (MM) Test (rat)

Whole Embryo Culture (WEC) Test (rat)
Management and Organisation of the ECVAM Embryotoxicity Validation Study 1998-2002

Sponsor: ECVAM, JRC, I

Subcontract
Biostatistics
BfR, D

Subcontract
Selection: chemicals
SGHMS, UK

Subcontract
Shipment: chemicals
RCC-CCR, D

Contract
ZEBET, BfR, D

Subcontract: MM-Test
1. lead laboratory
   SGHMS, UK
2. RIVM, NL
3. Synthelabo Recherche, F
4. KTL-Finland, FIN

Subcontract: WEC-Test
1. lead laboratory
   RIVM, NL
2. SGHMS, UK
3. Syngenta, UK
4. Novartis Pharma, CH

Subcontract: EST-Test
1. lead laboratory
   ZEBET BfR, D
2. ECVAM, I
3. Novartis Pharma, CH
4. Schering AG, D
The ECVAM Scientific Advisory Committee ESAC concluded at the meeting in June of 2002 - published in ATLA - that the

» the 3 in vitro embryotoxicity tests WEC, MM and EST have successfully been validated according to the ECVAM validation criteria.

» Early in 2003 an ECVAM WORKSHOP will evaluate how the 3 in vitro embryotoxicity tests may be used by the industry and/or for regulatory purposes!

» Today the EST is established in several laboratories of the international drug industry.
The Validation of Toxicological Prediction Models

Graeme Archer, Michael Balls, Leon H. Bruner, Rodger D. Curren, Julia H. Fentem, Hermann-Georg Holzhütter, Manfred Liebsch, David P. Lovell and Jacqueline A. Southey

1ECVAM, JRC Environment Institute, 21020 Ispra (VA), Italy; 2The Procter & Gamble Company, Health and Beauty Care Europe, Egham, Surrey TW20 9NW, UK; 3Institute for In Vitro Sciences Inc., Suite 220, 21 Firstfield Road, Gaithersburg, MD 20878, USA; 4Humboldt-Universität zu Berlin, Bereich Medizin (Charité), Institut für Biochemie, Mon Bijou Strasse 2a, 10117 Berlin, Germany; 5ZEBET, Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV), Diederdsorfer Weg 1, 12277 Berlin, Germany; 6BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK; 7Microbiological Associates Ltd, Stirling University Innovation Park, Stirling FK9 4NF, UK
Figure 1: A schematic representation of the role of the prediction model in an alternative method

- Chemicals in the validation study
- Universe of chemicals
- Endpoint value E (in vitro)
- Prediction model
- Endpoint value E (in vivo)
The Importance of the Prediction Model in the Validation of Alternative Tests

Andrew P. Worth and Michael Balls

Figure 1: A schematic representation of an alternative test and its performance properties
Editorial

Michael Balls

Defined Structural and Performance Criteria would Facilitate the Validation and Acceptance of Alternative Test Procedures

It is for this reason that ECVAM and ZEBET are supporting studies on the applicability for *in vitro* corrosivity and photoirritancy testing of another human reconstituted human skin equivalent, EpiDerm™, made by MatTek, which, happily, promises to survive longer than its competitors. We are using our experience with Skin² and EPISKIN to speed up the acceptance of EpiDerm, not because we have any particular interest in MatTek or its products, but because we do not want much valuable experience to be wasted or the undoubted promise of this kind of test system to be lost.
Figure 2: A schematic representation of the European Centre for the Validation of Alternative Methods (ECVAM) validation process

New test method

Prevalidation

Formal validation

“Similar” test method

Catch-up validation

Define structural and performance characteristics

Validated test method
The ECVAM Prevalidation Study on the Use of EpiDerm for Skin Corrosivity Testing

Manfred Liebsch,1 Dieter Traue,1 Christa Barrabas,1 Horst Spielmann,1 Patricia Uphill,2 Susan Wilkins,2 Janet P. McPherson,2 Christiane Wiemann,3 Tanja Kaufmann,3 Martina Remmele3 and Hermann-Georg Holzhütter4

1ZEBET, BgVV, Diedersdorfer Weg 1, 12277 Berlin, Germany; 2Huntingdon Life Sciences, Huntingdon, Cambridgeshire PE18 6ES, UK; 3BASF AG, Department of Product Safety, 67056 Ludwigshafen, Germany; 4Institut für Biochemie, Humboldt-Universität zu Berlin, Monbijoustrasse 2a, 10117 Berlin, Germany
ECVAM’s Modular Approach to Validation 2003

Test definition

Within-lab. variability

Transferability

Between-lab. variability

Predictive capacity

Applicability domain

Minimum performance standards

Reproducibility

Relevance

“Validated”
(ESAC)

“Standardised Test”
(INVITTOX protocol)
The Principles of Weight of Evidence Validation of Test Methods and Testing Strategies

The Report and Recommendations of ECVAM Workshop 58

Michael Balls,1 Patric Amcoff,2 Susanne Bremer,3 Silvia Casati,3 Sandra Coecke,3 Richard Clothier,4 Robert Combes,1 Raffaella Corvi,3 Rodger Curren,5 Chantra Eskes,3 Julia Fentem,6 Laura Gribaldo,3 Marlies Halder,3 Thomas Hartung,3 Sebastian Hoffmann,3 Leonard Schechtman,7 Laurie Scott,3,6 Horst Spielmann,8 William Stokes,9 Raymond Tice,9 Drew Wagner2,8 and Valérie Zuan3

1FRAME, Nottingham, UK; 2Environment Directorate, OECD, Paris, France; 3ECVAM, Institute for Health & Consumer Protection, EC Joint Research Centre, Ispra, Italy; 4School of Biomedical Sciences, University of Nottingham, Nottingham, UK; 5Institute for In Vitro Sciences, Gaithersburg, MD, USA; 6SEAC, Unilever, Sharnbrook, Beds., UK; 7National Center for Toxicological Research, Food and Drug Administration, Rockville, MD, USA; 8ZEBET, Federal Institute for Risk Assessment (BfR), Berlin, Germany; 9National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
The ECVAM International Validation Study on *In Vitro* Tests for Acute Skin Irritation: Report on the Validity of the EPISKIN and EpiDerm Assays and on the Skin Integrity Function Testa

Horst Spielmann,1 Sebastian Hoffmann,2 Manfred Liebsch,1 Phil Botham,3 Julia H. Fentem,4 Chantra Eskes,2 Roland Roguet,5 José Cotovio,5 Thomas Cole,6 Andrew Worth,6 Jon Heylings,3 Penny Jones,4 Catherine Robles,7 Helena Kandárová,¹ Armin Gamer,8 Marina Remmele,8 Rodger Curren,9 Hans Raabe,9 Amanda Cockshott,10 Ingrid Gerner11 and Valérie Zuan2

1National Centre for Alternative Methods (ZEBET), Berlin, Germany; 2ECVAM, Institute for Health & Consumer Protection, European Commission Joint Research Centre, Ispra, Italy; 3Syngenta, Macclesfield, UK; 4Unilever, Sharnbrook, UK; 5L’Oréal, Clichy, France; 6ECB, Institute for Health & Consumer Protection, European Commission Joint Research Centre, Ispra, Italy; 7Sanofi Aventis, Montpellier, France; 8BASF, Ludwigshafen, German; 9Institute for In Vitro Sciences, Gaithersburg, MD, USA; 10Health and Safety Executive (HSE), Bootle, UK; 11Federal Institute for Risk Assessment (BfR), Berlin, Germany
INTRODUCTION

1. Skin irritation refers to the production of reversible damage to the skin following the application of a test substance for up to 4 hours [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)](1). This Test Guideline provides an in vitro procedure that, depending on country requirements, may allow determining the skin irritancy of chemicals as a stand-alone replacement test, as a screen, or within a testing strategy in combination with, if appropriate, a weight of evidence approach.
III. APPROACHES TO VALIDATION

Prospective Validation Studies ................................................................. 20
Retrospective Assessment of Validation Status ........................................ 21
“Modular” Approaches to Assessing Validation Status .............................. 22
Performance Standards for Test Methods ................................................ 24
Use of Performance Standards for Catch-up Validation ......................... 24
Use of Performance Standards for Modified Test Methods .................... 25
Validation of Patented Methods ............................................................. 25
Validation of Test Batteries/Testing Strategies ........................................ 25
Validation of (Quantitative) Structure-Activity Relationships - (Q)SARs .... 26