



Update on ECVAM Activities, Recent Workshops & Validation Studies

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on behalf of the ECVAM team

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<http://ecvam.jrc.it>





Key responsibilities in ECVAM



- **Unit management**
Enrico Sabbioni (infrastructure & safety)
Laura Gribaldo (staff issues, ESAC)
- **Action 1321 (Q)SAR**
Andrew Worth
- **Action 1322 Validation for chemicals & cosmetics**
Valerie Zuang / Silvia Casati
- **Action 1323 Validation for emerging areas**
Marlies Halder / Sandra Coecke
- **Action 1324 Database & Scientific information system**
Annett Roi
- **Integrated Projects**
Susanne Bremer (ReProTect)
Pilar Prieto (A-Cute-Tox)
- **Marie-Curie Training**
Raffaella Corvi



Key Areas covered by Actions

- QSAR(s)
- Topical toxicity
- Systemic toxicity
- Sensitisation
- Carcinogenicity
- Reproductive toxicity
- Toxicokinetics
- Ecotoxicology
- Biologicals
- Strategic developments
- Databases & SIS

Action 1321 - QSAR
Computational Toxicology
(QSAR(s))

Action 1322 – Alternatives
Validation of alternative tests
for the chemicals & cosmetics
legislations

Action 1323 – Emerging
Validation for emerging areas
(pharmaceuticals, biologicals,
biomaterials & other products) and
enabling technologies

Action 1324 – ECVAM dbAlm
ECVAM Database Service on
Alternative Methods



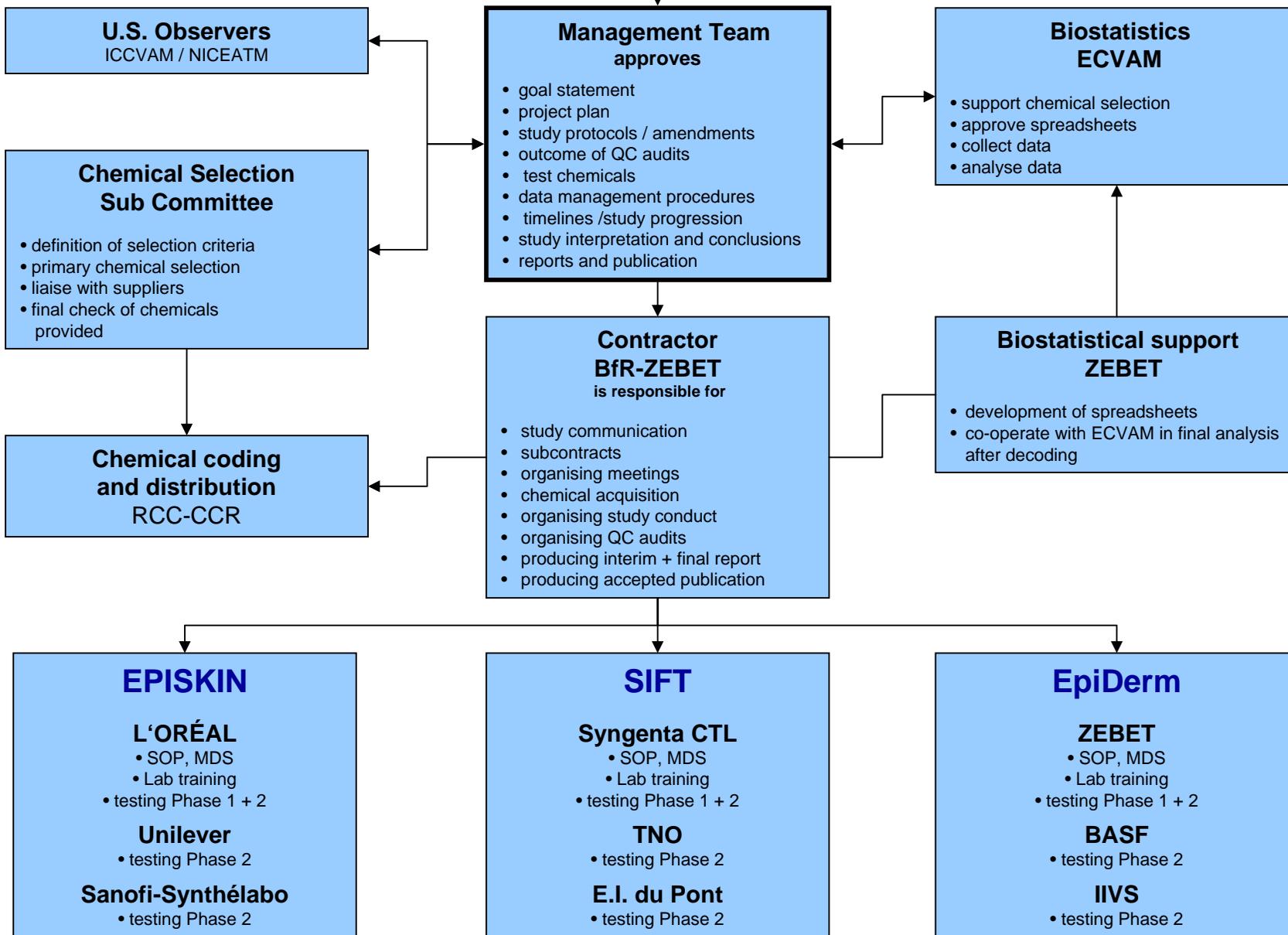
**Update on
ECVAM validation study on
in vitro methods for acute skin irritation**

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Sponsor ECVAM

Joint Research Centre





STUDY DESIGN

The experimental part consists of two phases:

PHASE 1: Preliminary phase

- Optimisation and confirmation of standard test protocols and prediction models (20 coded chemicals tested in the lead laboratories)
- Training of participating laboratories



PHASE 2: Definitive phase

- evaluation of the interlaboratory reproducibility and predictive ability of the tests (3 labs/test; ~ 60 coded chemicals)



Timeframe

2003		2004												2005			
11	12	01	02	03	04	05	06	07	08	09	10	11	12	01	02	03	04
<ul style="list-style-type: none"> Contract Sub-contracts (lead labs and RCC-CCR) Chemical acquisition for Phase 1 Chemical distribution 		<i>Lead-labs:</i> <ul style="list-style-type: none"> conduct phase 1 			<i>ECVAM:</i> <ul style="list-style-type: none"> Data Analysis Phase 1 Chemical acquisition for Phase 2 <i>MT:</i> <ul style="list-style-type: none"> Decide about phase II <i>BfR:</i> <ul style="list-style-type: none"> Subcontract labs 2 & 3 <i>Lead-labs:</i> <ul style="list-style-type: none"> train other labs 2 & 3 			<i>All labs:</i> <ul style="list-style-type: none"> Conduct phase 2 						<ul style="list-style-type: none"> Data Analysis Phase 2 Publication Submission to ESAC 			



3rd Management Team Meeting (June 2004)

Objectives:

- **To review the results from phase 1**
- **To plan phase 2 of the validation study in the light of the outcome of phase 1**



Summary of Phase 1

TEST	REPRODUCIBILITY	PREDICTIVE ABILITY		
		Overall (n=20)	I (n=9)	NI (n=11)
Epiderm	VERY GOOD – identical predictions from each run with the same chemical	15/20 (75%)	5/9 (56%)	10/11 (91%)
EPISKIN	VERY GOOD – identical predictions from each run with the same chemical	16/20 (80%)	6/9 (67%)	10/11 (91%)
SIFT	GOOD – variability in the TEWL and ER endpoints has impact on the I/NI predictions for some chemicals	9/20 (45%)	2/9 (22%)	7/11 (64%)



Update on ECVAM Activities on Biologicals and Ecotoxicology

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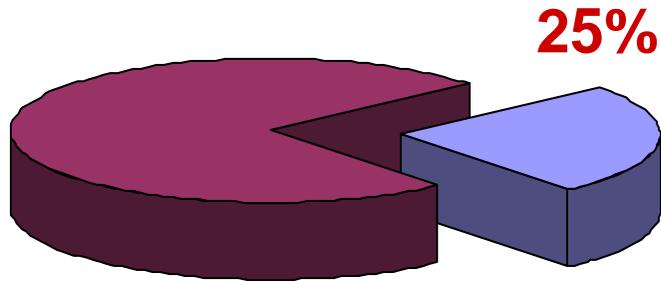
Biologicals are ...

**... products which are produced by or
derived from a living organism**

- **Vaccines**
- **Antitoxins**
- **Immunoglobulins**
- **Hormones**
- **Blood products**
- **Poly- and monoclonal antibodies**



Rationale



Tetanus infected mouse

- Large numbers of animals!
- High distress levels!
- Alternatives are available!



ECVAM Workshop 4: Alternatives to Animal Testing in the Quality Control of Immunobiologicals: Current Status and Future Prospects. Hendriksen *et al.*, *ATLA* 22, 420-434, 1994



Main activities & achievements

- **Organisation of workshops & meetings**
 - Nine reports are published in ATLA & on ECVAM website
 - Workshops/Meetings planned for 2005
 - WS on physico-chemical methods
 - WS consistency of production approach
 - Follow-up of WS 48: replacement of the NIH test for rabies vaccines (joint activity with Ph.Eur., FDA, NIBSC, WHO)
 - Alternatives in quality control of pertussis vaccines (WHO & Ph.Eur.)
 - Botulinum toxin (for therapeutical use)
- **Funding / management of (pre)validation & feasibility studies**
 - 11 are finalised
 - four methods are accepted by the European Pharmacopoeia
 - One validation study planned for 2005
 - serological methods for potency testing of whole-cellular pertussis vaccines



Main activities & achievements

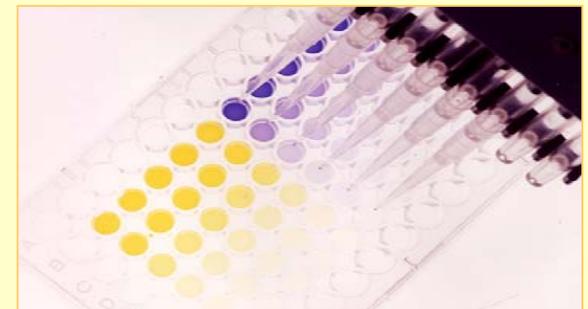
- **Task Force on Biologicals**

- Established in 2003
- Members: regulators, manufacturers, animal welfare officers
- Comments on monographs, guidelines, other regulatory issues
 - resulted in the deletion and reduction of several animal tests in Ph.Eur.
- Identification of promising Three Rs methods
- Requests for revision of monographs
- Contact to national competent authorities and manufacturers



Pyrogenicity

- **Validation study of 6 in vitro methods finalised in 2003**
- **Compilation of the dossiers for the ESAC & ICCVAM Peer Review Process are in progress**
- **Catch-up validation of Whole Blood Test and PBMC test using cryopreserved blood**
 - blinding
 - data evaluation





Shellfish toxin testing

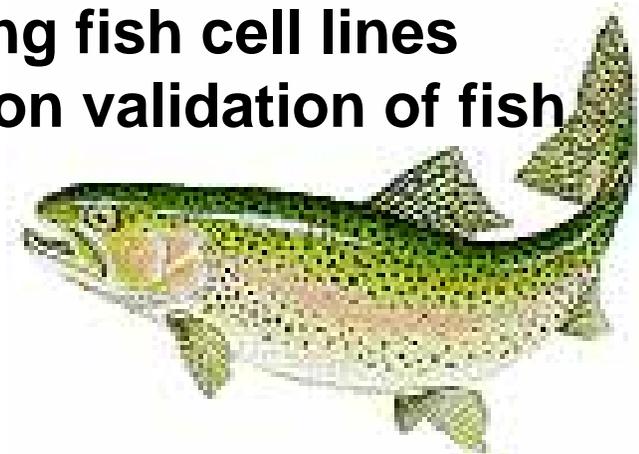
- **ECVAM/DG SANCO Workshop planned for January 2005**
 - Identify alternative methods & select for validation
 - Reference material preparation
 - Develop regional testing strategy





Ecotoxicology

- ECVAM workshop in 2001, report published in 2003
- Task Force established in 2003
- Workshop with ECETOC on Three Rs approaches
- Projects ongoing & planned
 - Evaluation of *Threshold approach* (= reduction of acute fish tests)
 - Optimisation of cytotoxicity tests using fish cell lines
 - Collaboration with German authority on validation of fish embryo test





Preliminary Conclusions & Recommendations of Recent ECVAM Workshops

- **Metabolism: A Bottle-neck in *In Vitro* Toxicological Test Development**
- **Dendritic Cells as a Tool for a Predictive Identification of Skin Sensitisation Hazard**
- **Weight-of-Evidence Validation**
- **Chemical Effects on Mammalian Fertility**
- **Chronic Toxicity**
- **QSARs and Applicability Domain**



Workshop on “Metabolism: A Bottle-neck in *In Vitro* Toxicological Test Development”

January 2004

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Goals

- 1) to identify toxicokinetic & metabolism issues in the different key areas for regulatory toxicity testing
- 2) to integrate the obtained knowledge into strategies towards the replacement of in vivo testing

Topics

- 1) kinetics / integration
- 2) metabolism / methods
- 3) target organs

Participants

Hans Ahr(Bayer AG)

Bas J. Blaauboer (IRAS)

Josè Castell (Hospital Universitari La Fe)

Robert Combes (FRAME)

Charles L. Crespi (Gentest)

Michael L. Cunningham (NIEHS)

Greetje Elaut (VUB)Free

Andreas Freidig (TNO)

Jean-François Gherzi-Egea (INSERM U 433)

Andre Guillouzo (INSERM U456)

Peter Hoet (KUL)

Magnus Ingelman-Sundberg (Karolinska Ins.)

Walter Janssens (SIPH)

Bernhard Ladstetter (Merck KGaA)

David Leahy (Cyprotex PLC)

Anthony Long (LHASA Ltd)

Annarita Meneguz (Istituto Superiore di Sanità)

Mario Monshouwer (Pharmacia, Gruppo Pfizer Inc.)

Siegfried Morath (University of Konstanz)

Fred Nagelkerke (Leiden University)

Olavi Pelkonen (University of Oulu)

Lysianne Richert (University of Besancon)

Beatrice Schaack (CEA Grenoble)

Emanuela Testai (Istituto Superiore di Sanità)

Winfried Steiling (Henkel KgaA)

Joan-Albert Vericat (NeuroPharma, S.A.)

ECVAM staff from relevant key aereas



Preliminary conclusions

- **Inherent metabolising ability of any in vitro system affects chemical toxicity**
- **Validation requires careful selection of chemicals with clear information on the role of metabolism in their toxicities**
- **Human-based metabolising systems should be used (e.g. human hepatocytes). Their suitability for regulatory testing needs to be demonstrated (*e.g. by comparing the predictivity of rodent- and human-based metabolising systems in the same in vitro test for a set of chemicals with adequate human data*).**
- **Biokinetic modelling is in principle ready for validation**
However, a sufficient number of chemicals with good quality data need to be found for use as a test set.



Preliminary recommendations

- **Establish a database of chemicals that are toxic to humans, either with or without metabolic activation, and which are detoxified as a consequence of metabolism**
- **Organisation of an ECVAM workshop on in vitro biokinetics**
- **Creation of a depository of reference chemicals for validation of metabolising systems**
- **Validation of physiologically-based pharmacokinetic modelling**
- **Inherent basal biotransformation competence of any in vitro test should routinely be characterised during development & before recommendation for toxicity testing**



**Workshop on
“Dendritic Cells as a Tool for a Predictive
Identification of Skin Sensitisation Hazard”**

April 2004

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Goals

- 1) to identify promising models
- 2) to identify necessary research activities

Topics

- 1) State of the art of DC-based in vitro tests for skin sensitisation
- 2) Monocytic cell lines (U937- THP-1)
- 3) Reconstructed epidermis with Langerhans cells

Participants

Pierre Aeby	COSMITAL
David Basketter	UNILEVER
Andrea Cavani	IDI
Frank Gerberick	P&G
Peter Griem	CLARIANT
Ian Kimber	SYNGENTA
J.P. Lepoittevin	Strasbourg University
Jean Meade	NIOSH
Marc Pallardy	INSERM
Nathalie Rougier	Biopredict International
Françoise Rousset	L'OREAL
Federica Sallusto	IRB
Geert Verheyen	Vito

ECVAM staff from relevant key areas





Preliminary conclusions

- DC play a key role in skin sensitisation. Therefore, DC-based tests should have a prominent role in any testing strategy but need further standardisation
- Models based on human monocytic cell lines (U937, THP-1) have shown to be useful in detecting skin sensitisers. These models are the most advanced in terms of optimisation and evidence of inter-laboratory reproducibility is available for the THP-1 cell line
- Reconstituted human skin models with Langerhans cells are still at R&D level and need further optimisation



Workshop on “Weight of Evidence Validation”

May 2004

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Goal

Develop principles & guidelines for validation that incorporate WoE approaches to permit the use of all information via a structured, systematic and transparent review process

Participants

Michael Balls	FRAME
Len Schechtman	FDA
Patric Amcoff	OECD
Horst Spielmann	BfR
Richard Clothier	FRAME
William Stokes	NTP NIEHS
Rodger Curren	IIVS
Ray Tice	NICEATM/ILS-Inc
Bob Combes	FRAME
Drew Wagner	OECD
Julia Fentem	Unilever
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Preliminary recommendations

- Different kinds of weighting approaches should be considered and defined to guide validation bodies on when, where and how to use them
- The principles and guidelines developed should provide flexibility, both in terms of process and decision making, to ensure that the valued element of scientific expertise and sound judgment are not superseded
- Additional effort should be extended to identify, assess and define systematic approaches and processes appropriate for WoE validation
- A statistical workshop should be deployed to identify acceptable approaches to integrate and analyze data from independent sources
- Historic and ongoing validation efforts should be leveraged to extract key learnings and develop practical WoE guidelines



Workshop on “Chemical Effects on Mammalian Fertility”

June 2004

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Goal

- 1) Identification of target tissue and mechanism
- 2) Evaluation of in vitro models
- 3) What is missing?

Participants

Donatella Balduzzi	Istituto Sperimentale Italiano Lazzaro, Italy
Rita Cordvrint	EggCentris, Belgium
George Daston	Procter & Gamble, USA
Andrea Galli	Istituto Sperimentale Italiano Lazzaro, Italy
Susan Laws	EPA, USA
Giovanna Lazzari	Laboratorio di Tecnologie, Italy
Ulla Liminga	Medical Product Agency, Sweden
Johan Smith	VUB, Belgium
Marcello Spano	ENEA, Italy
Ine Waalkens	TNO, Netherlands
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Preliminary conclusions

- **Identification of 26 cells, tissues, organs and biological mechanisms involved in fertility that are targeted by reproductive toxicants**
- **Presentation of in vitro models being evaluated by ReProTect or EPA in order to define the toxicological information they can provide**
- **Identification and prioritisation of in vitro models (11) that need to be further explored for the integration into a testing strategy**
- **Identification of “black boxes” (7) for which currently the participants could not name any in vitro model**
- **Information exchange on 5 in vitro tests between ECVAM (2 tests) and the EPA (3 tests) that are currently in the test optimisation phase/ validation phase**
- **Agreement on a questionnaire with 4 questions concerning fertility that should be sent to the regulators.**



Preliminary recommendations

- **ECVAM should carry out literature search on alternative methods regarding “black boxes”**
- **Cell lines developed in ReProTect should be available from a public cell bank (attention: patents!)**
- **Further development of in vitro models identified to be relevant in a testing strategy**
- **Organisation of ECVAM workshop on pharmacokinetics and integration into a testing strategy for reproductive toxicants**



Workshop on “Chronic Toxicity In Vitro: A new 3Rs Challenge”

September 2004

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Goal To discuss, evaluate and agree on alternative approaches for in vivo chronic toxicity testing

Topics In vitro versus in vivo; in vitro models; how to model long-term/short-term exposures; “omics” approaches; toxicokinetic modeling, relevance of integrated systems to predict NOEL/LOEL

Participants

- | | |
|--------------------------|--|
| Alan W. Baird | University College, Dublin, Ireland |
| Jose Castell | Hospital La Fe, Valencia, Spain |
| Wolfgang Dekant | University Würzburg, Germany |
| Peter Hoet | K.U.Leuven, Belgium |
| Carl Westmoreland | Unilever, UK |
| Armin Wolf | Novartis Pharma AG, Switzerland |
| Jens Noraberg | NeuroScreen ApS, Denmark |
| Jules Griffin | University of Cambridge, UK |
| Paul Dietl | University of Innsbruck, Austria |
| Bas Blauboer | IRAS, The Netherlands |
| Jayne Wright | Syngenta CTL, UK |
| Jerry J. Heindel | NIEHS, USA |
| Luciana Marocchio | GSK Safety Assessment, UK |

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Preliminary Conclusions

- The identification of potential hazards resulting from long-term, repeated exposure is an important aspect of toxicity testing for the protection of human and the environment and should integrate biokinetic modeling, biomarkers, and omics
- The development and validation of procedures for long-term testing with in vitro systems represents a very demanding challenge. Based on new developments, basic bioscience and instrumental analysis is now possible to attempt to meet these challenges
- Omics should be used to identify early markers of toxicity that occur in the process of long-term responses and that are mechanistically linked to the pathology.



Preliminary recommendations

- **Need of a worldwide approach combining expertise of the many areas of in vivo toxicology, basic biomedical research, cell tissue culture, omics and bio-informatics, database developments**
- **New in vitro models and approaches need to be developed and further validated.**
- **The validation of the approach will need high quality data from databases or storage samples.**
- **The work should be co-ordinated taking into consideration the current and future needs of regulators to speed up the acceptance process.**
- **Methods to predict NOELs based on in vitro models by using appropriated kinetic modelling should be developed and evaluated for risk assessment.**



Workshop on “QSAR Applicability Domain (AD)”

September 2004

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Goal

- 1) To describe state-of-the art in the identification of AD
- 2) To provide guidance to model builder on how to define AD
- 3) To identify areas for further research

Participants

Tom Aldenberg (RIVM, NL)
Mark Cronin (LJMU, UK)
Paola Gramatica (Univ. of Insubria, I)
Joanna Jaworska (P&G, B)
Scott Kahn (Accelrys, USA)
Gilles Klopman (Multicase, USA)
Carol Marchant (Lhasa, UK)
Glenn Myatt (Leadscope, USA)
Nina Nikolova (BAN, BG)

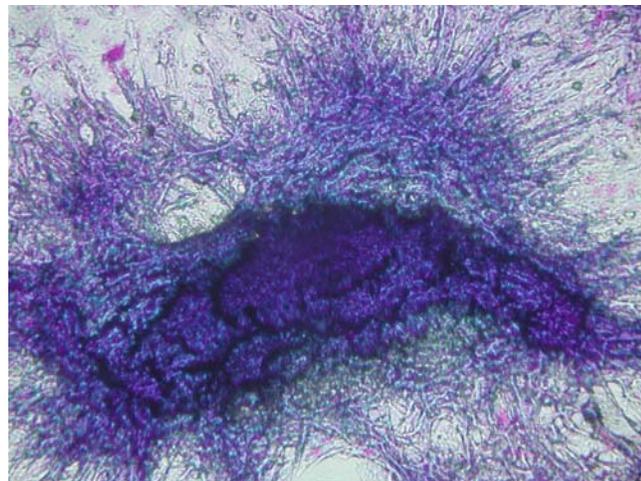
Grace Patlewicz (Unilever, UK)
Roger Perkins (FDA, USA)
David Roberts (Independent)
J. van de Sandt (NTO, NL)
Terry Schultz (Univ. of TN, USA)
David Stanton (P&G, USA)
Gilman Veith (OECD, F)
Chihae Yang (Leadscope, USA)

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Outcome of Recent Consultation Meetings

- **In vitro micronucleus test**
- **Cell transformation assay**
- **Biokinetics**
- **Validation of QSARs for Estrogen and Androgen Receptor Binding**



Consultation Meeting on “In Vitro micronucleus test (MNT)”

April 2004

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Goals

- 1) Establish validation status of in vitro MNT
- 2) Review of six ring trials

Participants

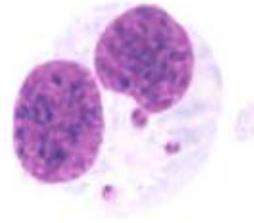
Robin Fielder	Health, UK
Peggy Guzzie	Pfizer, Fr
Micheline Kirch-Volders	Vrije Universiteit Brussel, B
David Kirkland	Covance, UK
Elisabeth Lorge	Biologie Servier, F
Lucia Migliore	University of Pisa, I
Hannu Norppa	Finnish Institute of Occupational Health, FI
Philippe Vanparys	Johnson& Johnson, B

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Conclusions & follow-up

- Experts felt that *in vitro* MNT is sufficiently validated
- Retrospective formal validation based on compilation & analysis of available data to be submitted to ESAC for review.
(1st draft June 2004)



Consultation Meeting on “Cell Transformation Assay (CTA)”

April 2004

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Goals

Agree on a proposal for the study design of a prevalidation study on CTA

Participants

Silvio Albertini	Hoffman-La Roche
Marilyn Aardema	P&G
Peggy Guzzie	Pfizer
Jean Roch Meunier	L'Oreal
Albrecht Poth	RCC
Karl-Rainer Schwind	BASF
Leonard Schechtman	ICCVAM
Noriho Tanaka	Hatano Research Institute, Japan
Makoto Umeda	Hatano Research Institute, Japan
Paule Vasseur	University of Metz, F
Haizhou Zhang	Covance

ECVAM staff



Conclusions & follow-up

- **Start prevalidation of CTA on SHE and Balb/C 3T3 cells**
- **ECVAM started negotiation on contract study**



Consultation Meeting on “Biokinetics”

August 2004

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Goals

To discuss possible future ECVAM activities in the fields of in vitro kinetics and in the mathematical modelling of kinetic processes

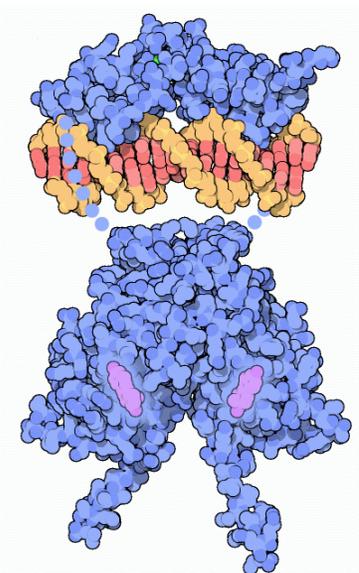
Participants

Sonja Beken	DG Public Health Protection, B
George Loizou	Health and Safety Laboratory, UK
Bas Blaauboer	IRAS, Utrecht University, NL
Jose Castell	Hospital Universitario La Fe, S
Charles Crespi	Gentest, USA
David Leahy	Cyprotex PLC, UK
Mario Monshouwer	Pharmacia, Gruppo Pfizer Inc., I
Olavi Pelkonen	University of Oulu, FIN
Darach Golden	Trinity College, IR
Martin Spendiff	Health and Safety Laboratory, UK
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Conclusions & follow-up

- **To perform weight of evidence validation study on methods for blood/tissue partitioning (standardised under GLP)**
- **To investigate validity of artificial membranes (as a model for passage through barriers) by screening the ECVAM/ICCVAM compounds and evaluated their benefit in comparison with more complex in vitro models.**
- **To organise Good Modelling Practice workshop in 2005.**
- **To perform feasibility study to evaluate validation principles and develop process for applying these principles.**



Consultation Meeting on “Validation of QSARS for Estrogen and Androgen Receptor Binding”

August 2004

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ihp



Goals

To prepare a roadmap for validation of (Q)SAR models to predict ER/AR activity

Participants

Patric Amcoff

OECD, F

Steven Bradbury

EPA, USA

Rajni Garg

Clarkson University, USA

Miriam Jacobs

University of Sussex, UK

Jun Kanno

NIHS, Japan

Ana Saliner

University of Girona, S

Pat Schmieder

EPA, USA

William Stokes

NICEATM, USA

Weida Tong

NCTR, FDA, USA

William J. Welsh

University of Medicine & Dentistry of NJ , USA

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Conclusions & follow-up

- Investigate regulatory applications of QSARs for ER/AR binding
- Develop guidance for validating QSARs for ER/AR binding
- Defining applicability domains
- Selection of QSAR models for validation
- Guidance on reference data and selection of test chemicals



Reports to be published soon

**ECVAM workshop report on
“Strategies to replace in vivo acute systemic
toxicity testing”**

**ECVAM-ICCVAM Workshop report on
“Validation principles for toxicogenomics-based
tests”**

