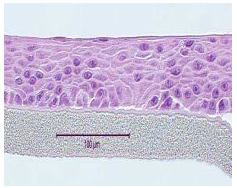
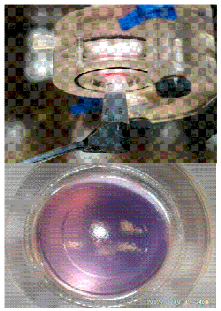
Recent Developments in the Colipa PT-SCAAT Eye Programme for Development of *In Vitro* Alternatives

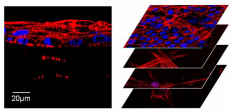
Pauline McNamee[1] (Chairperson), Sandrine Bessou-Touya[2], José Cotovio[3], Lieve Declercq[4], Ann De Smedt[5], Bart De Wever[6], Claudine Faller[7], John Harbell[8], Penny Jones[9], Beatrice Le Varlet[10], Monique Marrec-Fairley[11], Wolfgang Pape[12], Uwe Pfannenbecker[12], Klaus Schroeder[13], Magalie Tailhardat[14], Christine Van den Berghe[3], Freddy Van Goethem[5]

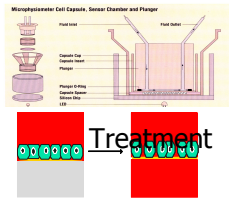
[1]The Procter & Gamble Company, Egham, Surrey, UK
[2]Laboratoire Pierre Fabre, Castres, France
[3]L'Oréal, Aulnay Sous Bois Cedex, France
[4]Estee-Lauder Companies, Oevel, Belgium
[5]Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium
[6]Phenion, Düsseldorf, Germany
[7]Cosmital Wella, Marly, Switzerland
[8]Mary Kay Inc, Dallas, Texas, USA
[9]Safety and Environmental Assurance Centre, Unilever, Sharnbrook, UK
[10]Links Ingénierie, Montpellier, France
[11]COLIPA, Brussels, Belgium
[12]Beiersdorf, Hamburg, Germany
[13]Henkel, Dusseldorf, Germany
[14]LVMH, St. Jean De Braye Cedex, France

Eye Irritation Programme Portfolio









Method development/optimisation of existing models

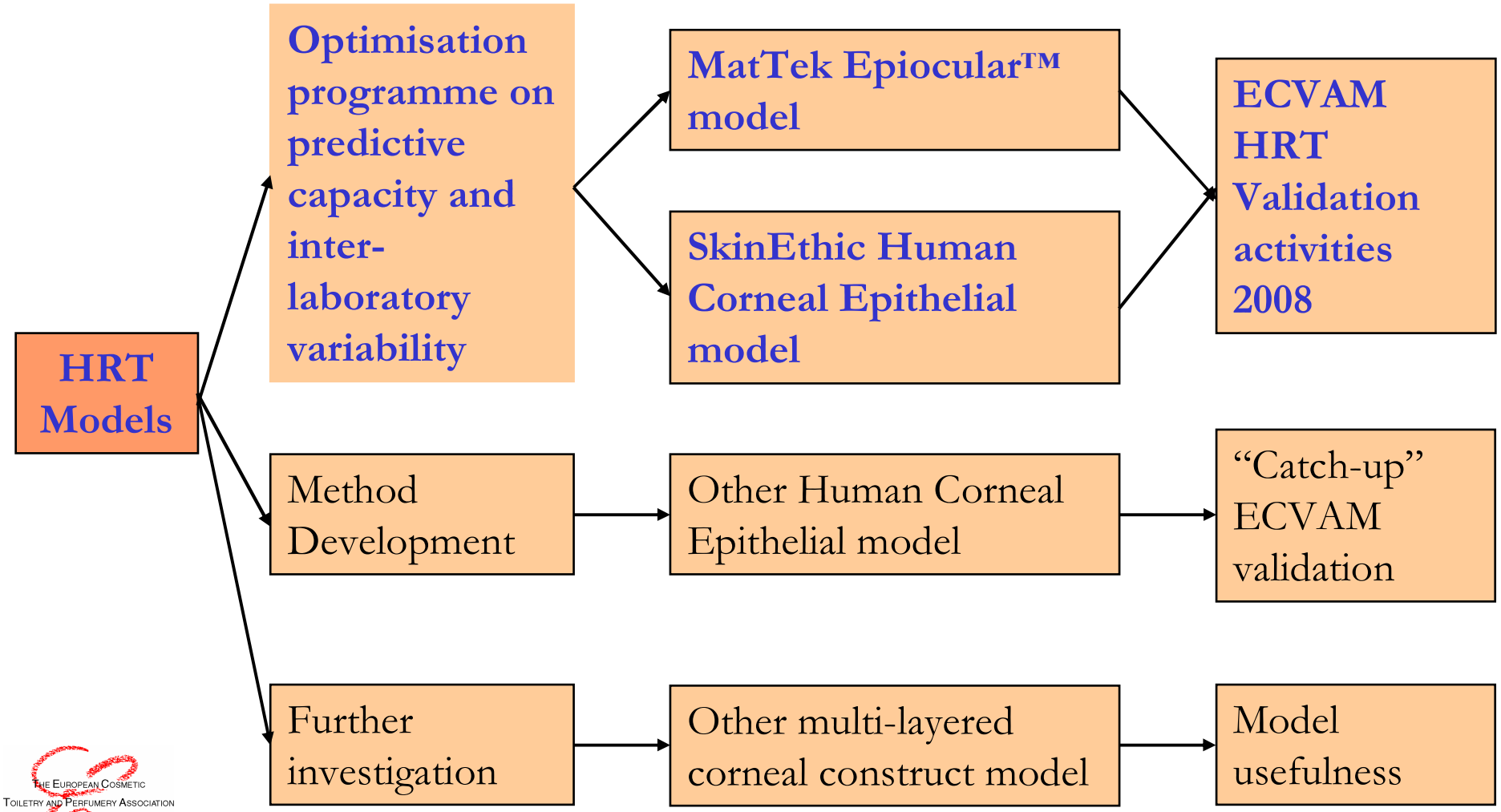
- Focus on Human Reconstructed Tissue (HRT) Models

Integrated research projects

- *In vitro* corneal culture eye irritation assay
- Cell culture models for ocular toxicity studies
- Genomics project

Collaborative activities with external partners e.g. ECVAM, Academia

Method Development/Optimisation of Current *In Vitro* Methods



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Optimisation Programme

MatTek Epiocular™

Tissue Construct
- Non-keratinised epithelium derived from normal human keratinocytes

Protocol
- MatTek corporation
- Liquids: 30 min + 12 min post-exposure soak + 120 min post-exposure incubation
- Solids: 90 min + 12 min post-exposure soak + 18h post-exposure incubation

Endpoints
- *In vitro*: viability (MTT)
- *In vivo* endpoint: EU risk phrases; GHS categories

Prediction Model
- 2 *in vitro* classes
- Irritant = viability ≤ 60%
- Non-irritant = viability > 60%

SkinEthic HCE Model

Tissue
- Immortalised human corneal epithelial cells

Protocol
- Van Goethem *et al.* (Toxicology *In Vitro* 20 (2006) 1-17)
- 10 min exposure

Protocol
- L'Oréal optimised protocol
- 1h exposure + 16h post-exposure incubation

Endpoints
- *In vitro*: viability (MTT)
- *In vivo* endpoint: EU risk phrases; GHS categories

Prediction Model
- 2 *in vitro* classes
- Irritant = viability < 60%
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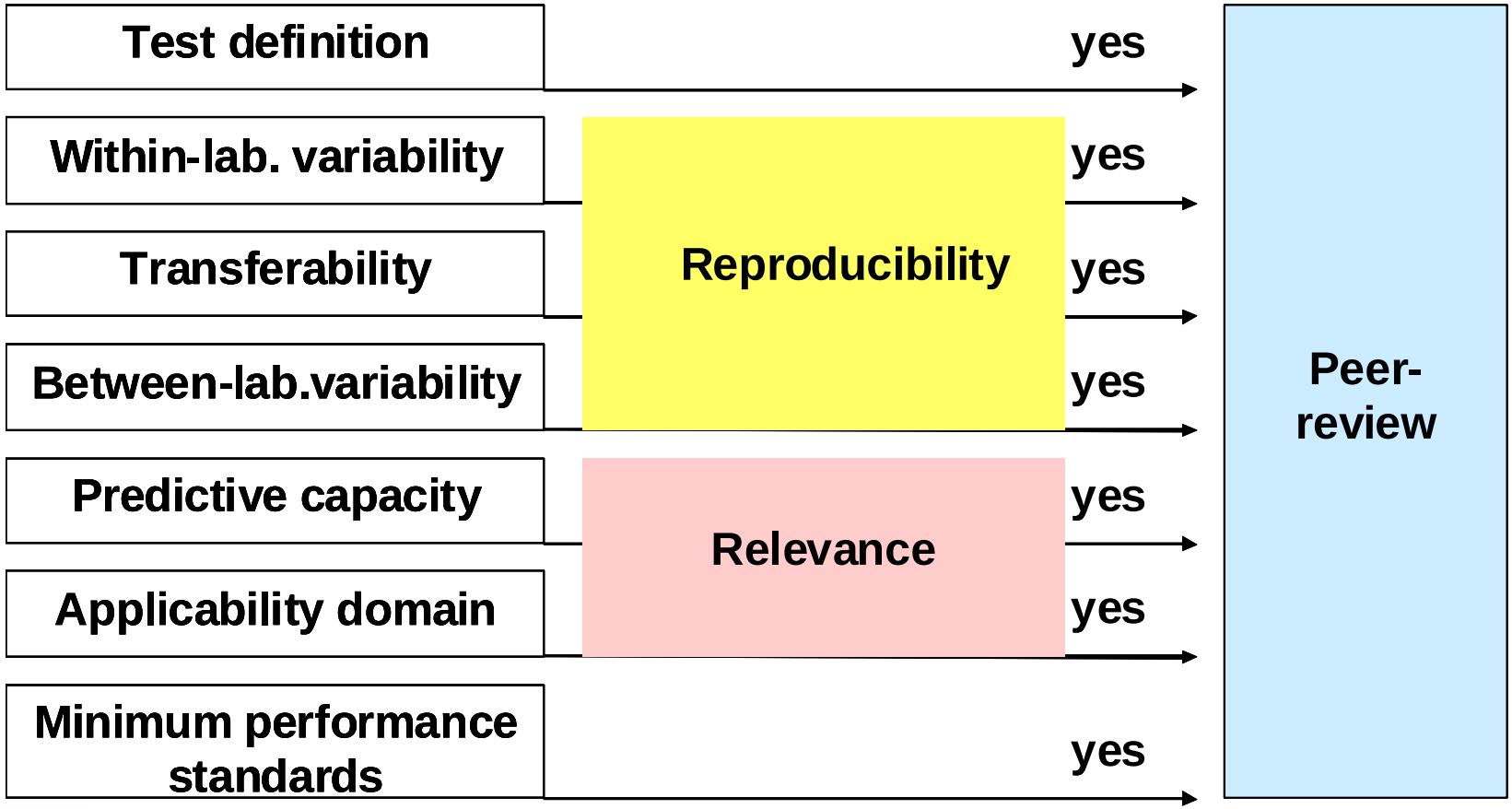
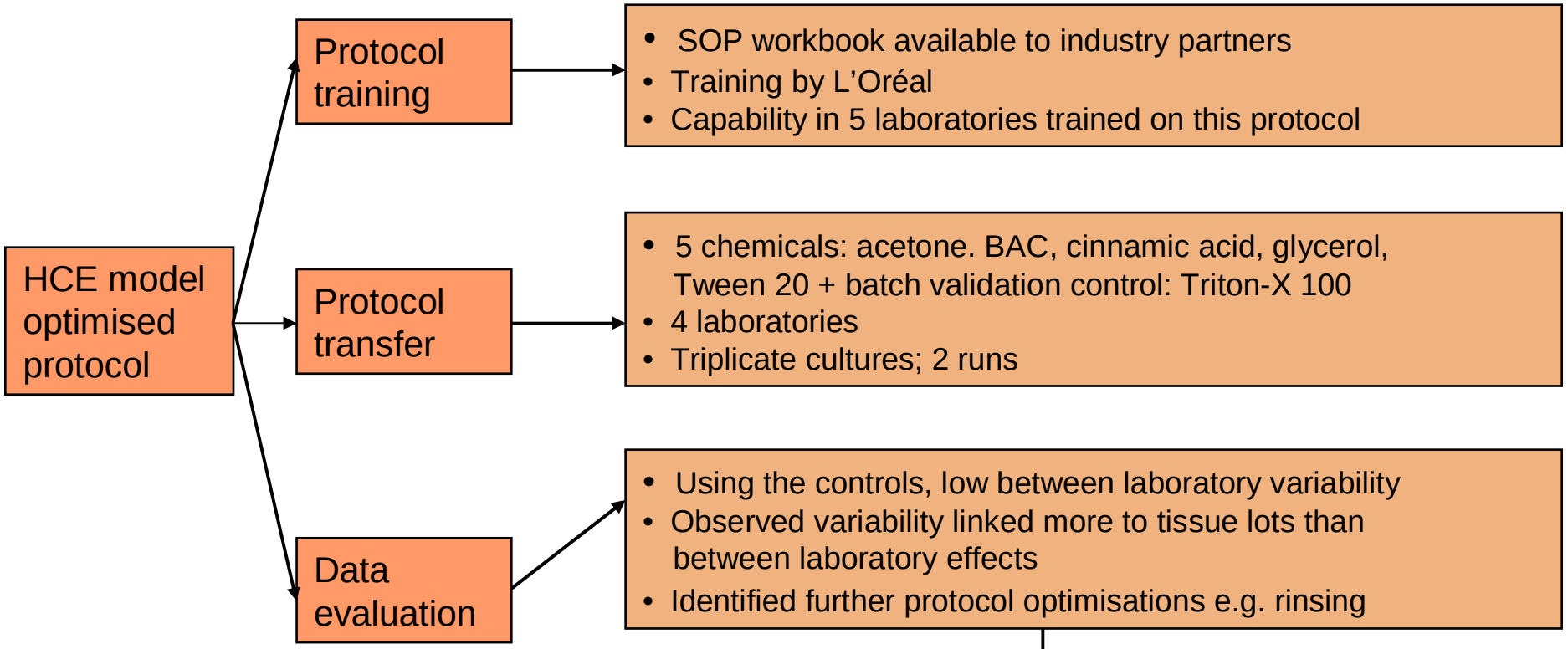
ECVAM Modular Approach



Hartung *et al*. 2004. ATLA **32**, 467-472

Colipa Optimisation Programme
Reproducibility: SkinEthic HCE



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Colipa Optimisation Programme
Reproducibility: MatTek EpiOcular™

HCE model optimised protocol

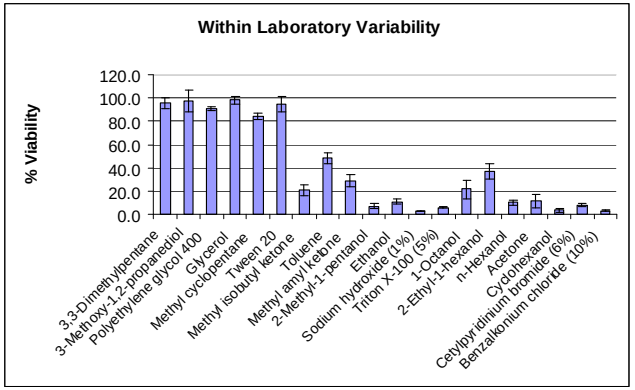
Protocol training

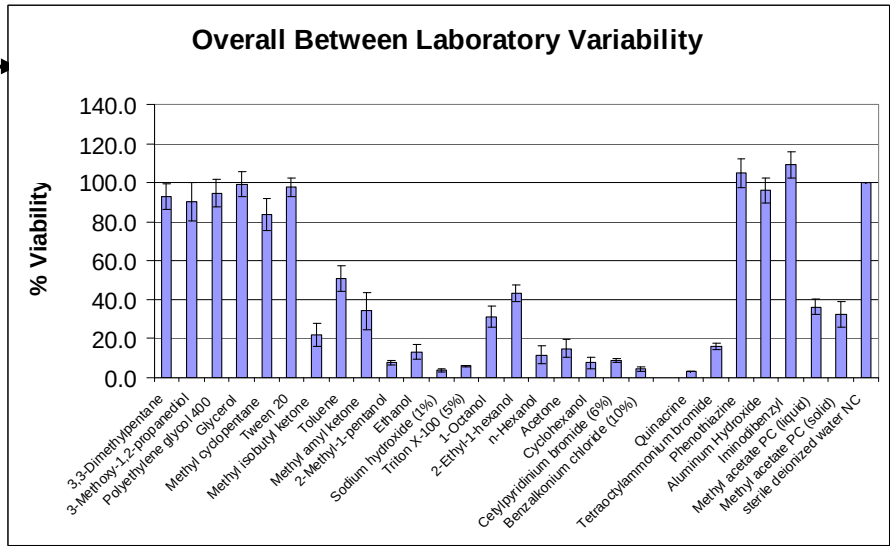
- SOP workbook available to industry partners
- Training by MatTek
- Capability in 7 laboratories

Protocol transfer

- 25 coded chemicals (20 liquids, 5 solids)
- 3 labs tested 20 liquids; 4 labs tested 15 liquids, 5 solids
- Triplicate cultures; 2 runs

Data generation





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Colipa Optimisation Programme
Predictive Capacity

- Majority of data derived from the model owners/producers
- Additional data generated
  - Chemicals of interest to industry
  - Gaps in chemical class
  - Under-represented chemical classes
  - Chemicals from a national validation organisation dataset
- Data demonstrate
  - Domain of Applicability is very important to effective understanding of predictive capacity
  - Availability of robust in vivo data is critical

Colipa reports of the industry optimisation programme for the HRT assays have been submitted to ECVAM in Feb/March 2008 for consideration to enter formal validation in 2008

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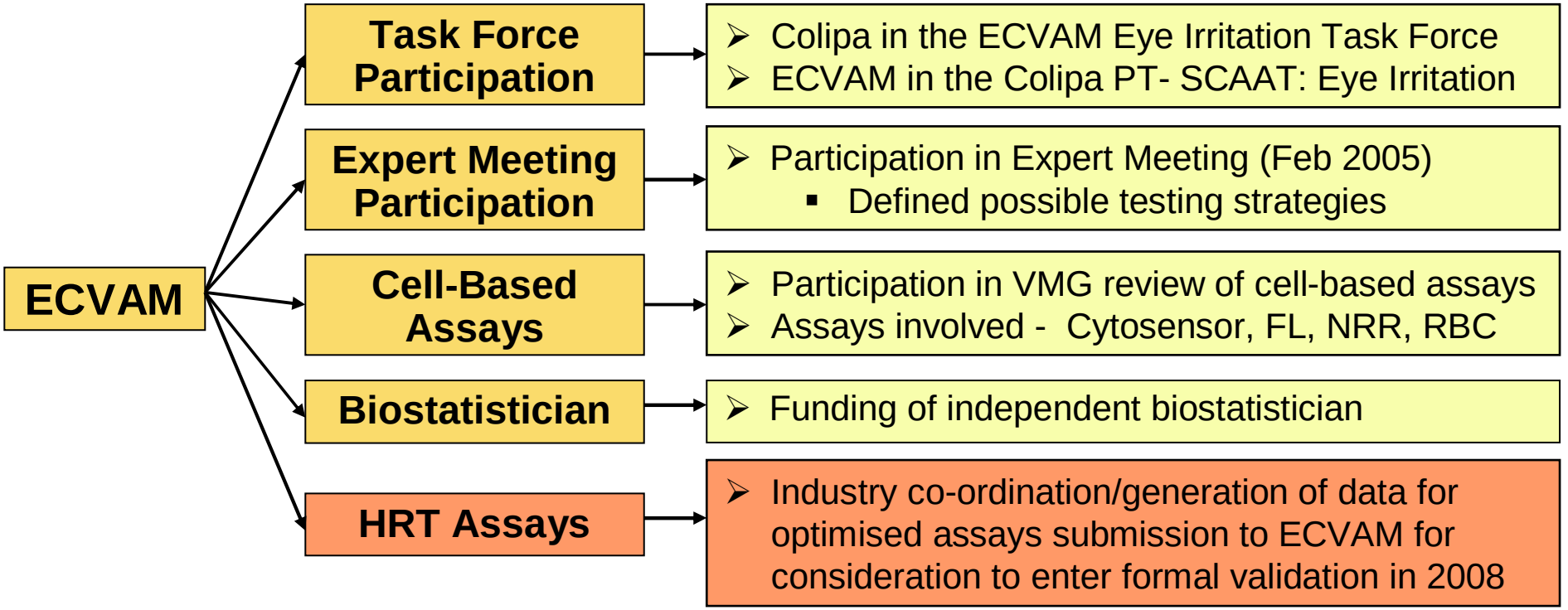
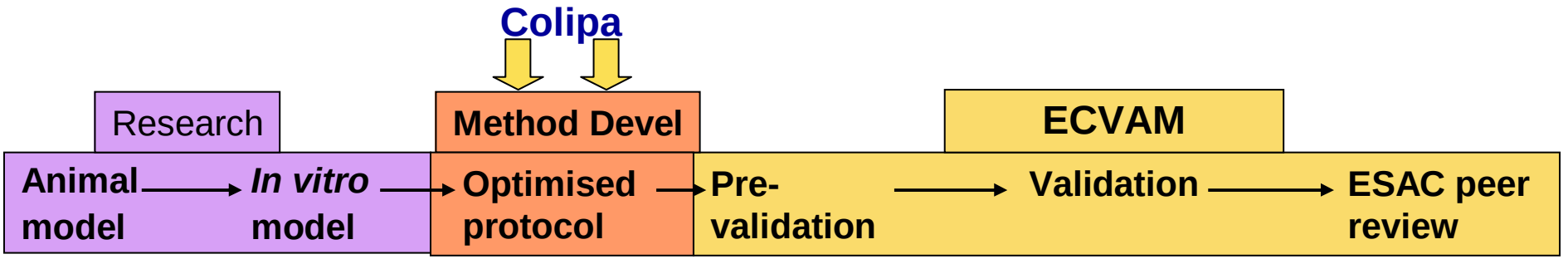
External Collaborative Activities





Research Programme Approach

Experience From Earlier Validation Studies

6 major validation studies conducted between 1991-1997:

- EC/HO, COLIPA
- BGA/BMBF
- MHW/JCIA
- CTFA, IRAG

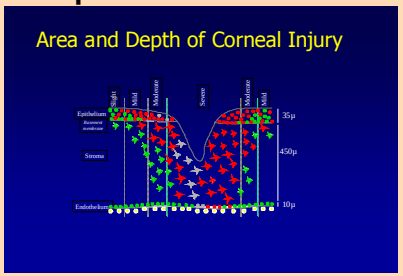
Mechanisms Workshops

Colipa Workshop on Mechanisms of Eye irritation - 1997

ECVAM workshop on Eye Irritation Testing: The Way Forward - 1998

Basic Research

Mechanistic work (Maurer *et al*): area/depth of corneal injury are principal factors in early responses and eventual repair after accidental eye exposure



Mechanistically based and focused on the cornea

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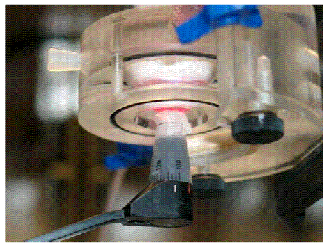
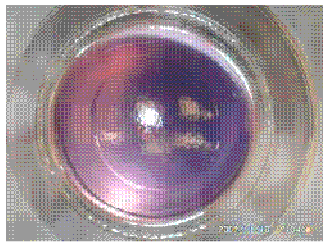
Research Programme Objectives

- Better understanding of cellular and molecular mechanisms of chemically induced eye irritation
- Identification of endpoints related to dynamics of injury and recovery
- Lead to new, appropriate *in vitro* endpoints, more predictive of *in vivo* response of human eye to irritants
- Development of prediction models for pre-validation of new and/or improved non-animal methods to proceed to formal validation

Research Programme: Project 1
In Vitro Corneal Culture Assay (Aachen)





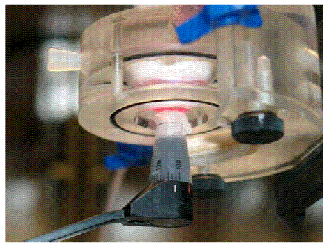
Purpose

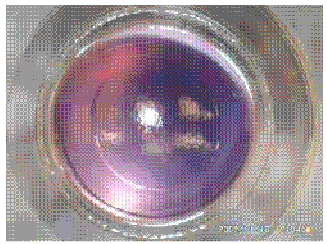
- Develop *in vitro* model of excised corneas maintained in culture to allow observation of injury/recovery after chemical exposure
- Investigate whether kinetics/patterns of change in physiological function and signals of injury released from the perfused cornea *in vitro* can predict a chemicals potential to damage the eye - focus on recovery

Evaluation methods

- biomicroscopy, morphology, pachymetry and glucose/lactate turnover for system viability/stability
- LDH, cytokines (IL-1α, IL-2, IL-6 IL-8, MIP1), growth factors (FGF, VEGF) and morphology

Research Programme: Project 1
In Vitro Corneal Culture Assay (Aachen)





Outcome

- Developed new isolated perfused corneal culture model maintained in steady state culture conditions for a period of time
- Determined viability/stability of the isolated perfused corneal culture system morphologically and metabolically
- Defined the parameters to be used routinely to confirm system viability and stability
- Determined model suitability to investigate wound healing by mechanical abrasion
- Exposed the defined isolated perfused corneal system to model toxicants
- Identified possible endpoints for further development in models evaluating chemically induced eye injury

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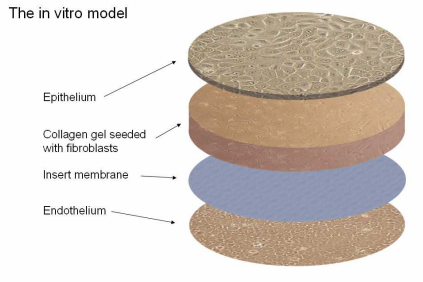
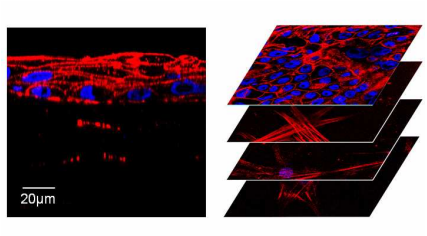
Research Programme: Project 2
Cell Culture Models for Ocular Toxicity (Bristol)





Purpose

- Sequentially build 3-D human corneal constructs consisting of epithelium, stroma and endothelium to better understand underlying mechanisms of action
- Identify new endpoints for magnitude of injury/quality of repair in human corneal models to enable prediction of nature/severity of toxicant effects

Evaluation methods

- Light and confocal microscopy
- Characterization of surface markers/differentiation
- Barrier formation assessment
- Membrane damage
- Metabolic activity
- Profiling of cytokine secretion

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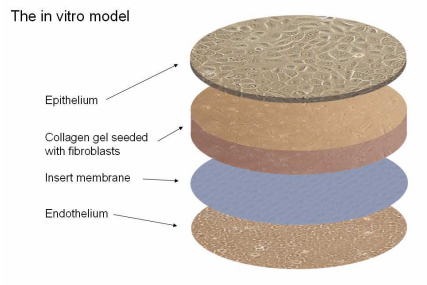
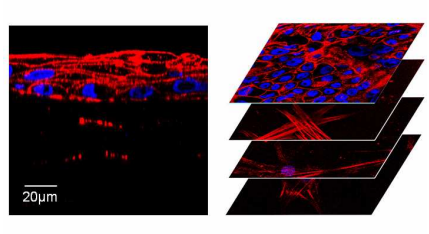
Research Programme: Project 2
Cell Culture Models for Ocular Toxicity (Bristol)





Outcome

- Investigation and development of increasingly complex constructs (monolayer, stratified epithelia, stromal equivalents, 2 & 3 layers) using human corneal and conjunctival cell lines (culture conditions, growth characteristics and suitability for use in 3D constructs)
- Investigated physiological responses to ocular injury by evaluating responses to model toxicants in monolayers and increasingly complex constructs using different human corneal and conjunctival cell lines
- Preliminary evaluation of construction of a 3-layer model by the addition of an endothelium cell layer
- Availability of a 3-D, 2-layer bioengineered human corneal construct for further development and evaluation

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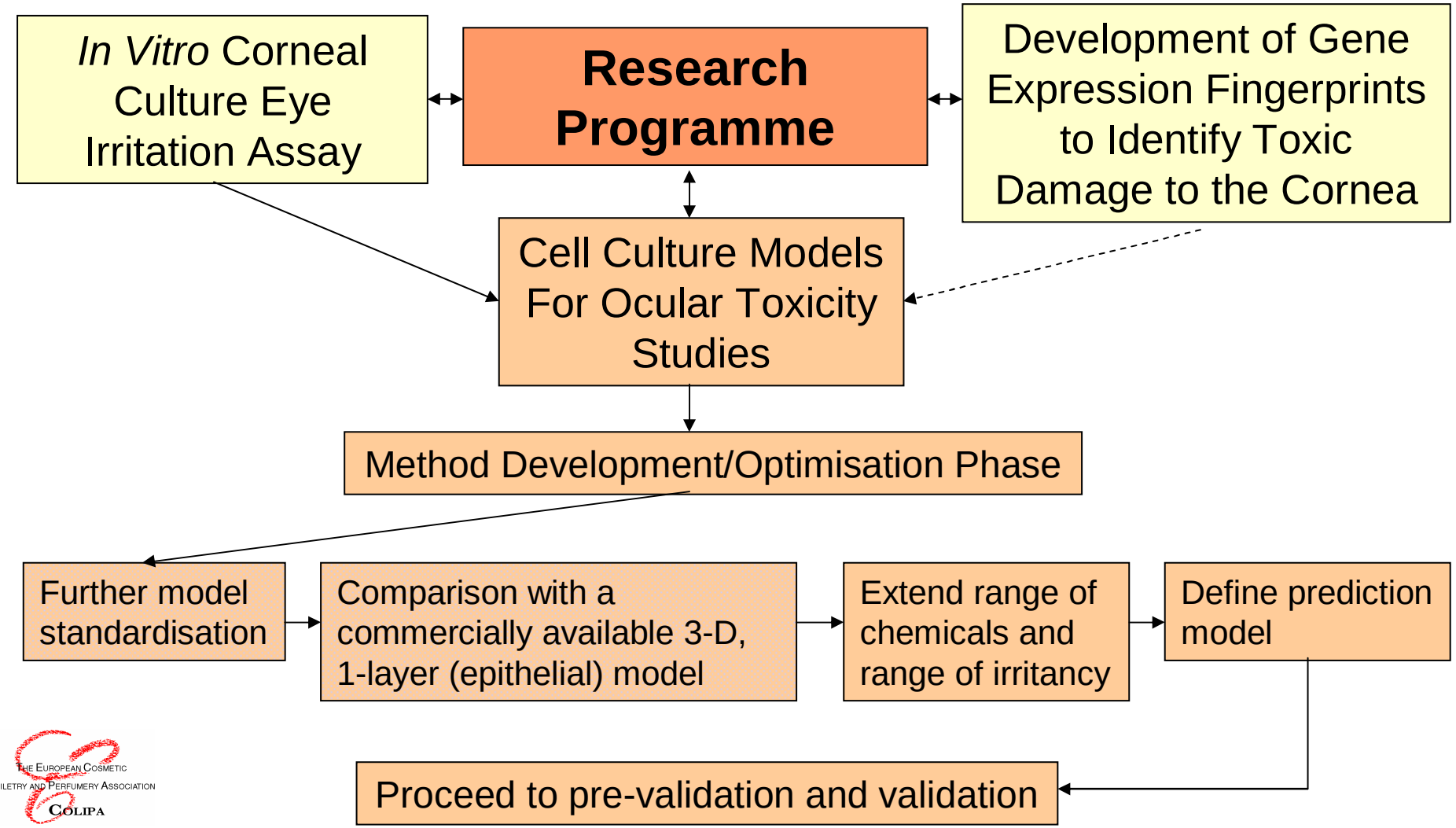
Research Programme: Project 3 Genomics (Cardiff)

- Genomics Project (started in 2006)
  - Development of a gene fingerprint directory to identify chemicals toxic to a bioengineered human cornea
  - Proof of concept that generic chemicals will cause differential gene expression in human bioengineered corneas and commercially available Human Corneal Epithelial (HCE) models
- Need to expand the project
  - Further understanding of proof of principle
  - Value of a genomics approach to identify new endpoints for reapplication into newly developed and/or existing optimised *in vitro* models
- New project proposal currently under review

COLIPA Eye Irritation Research Programme



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  - Ann De Smedt (Johnson & Johnson))
  - Lieve Declercq (Estee Lauder)
  - Bart De Wever (Phenion)
  - Claudine Faller (Cosmital Wella/P&G)
  - John Harbell (Mary Kay)
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