In Vitro Testing in Contract Research: A Valid Alternative ?

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RCC Harlan Harlan TEKLAD SafePharm Laboratories *Testing for a Safer Future* ILS

Presentation Outline

- **Contract Research Organisations – Setting the Scene**
- **Why *in vitro*? From the ‘old’ to the ‘new’**
- ***In vitro* testing services - SafePharm and RCC**
- **Concluding comments**

Contract Research Organisations – Setting the Scene

- CRO, CTO (testing) CSO (services)
- In existence last 70 years
- Many private, a few part Government owned
- Mission/Purpose – to provide testing or other services to industry (and generate revenue)
- Some non-profit making
- Recent acquisitions by multinational corporations
- Listing by Gad (2003)

Gad (2003). The Selection and use of Contract Research Organisations. A Guide for the Pharmaceutical and Medical Device Industries. Taylor and Francis.

What services?

Health Effects assessment
(hazard assessment & safety evaluation)

- Short term (acute) toxicology
- Sub chronic & chronic toxicology
- Developmental and Reproductive Toxicology
- Genetic Toxicology
- Carcinogenicity
- Safety Pharmacology

Other Services

- Drug synthesis
- Drug metabolism
- Formulation development
- Efficacy studies
- Pharmaco/toxicokinetics
- Ecotoxicology (terrestrial, avian and aquatic toxicology)
- Chemical & bioanalytical services
- Regulatory support

Which Industry Sectors?

- Pharmaceuticals/biopharmaceuticals
- Fine Chemicals
- Raw materials and Isolated Intermediates
- Consumer Products and ingredients
- Plant Protection Products
- Biocides
- Medical Devices

SafePharm Laboratories Limited and RCC

Both wholly owned by Harlan Sprague Dawley Inc.

Each in existence for over 30 years

SafePharm Laboratories

Testing for a Safer Future

RCC

Linking science to progress

SafePharm Laboratories Limited

- Founded in 1970
- Acquired by HSD July 2007
- Central UK
- 25 acre site
- GLP Accredited

SafePharm Laboratories

Testing for a Safer Future

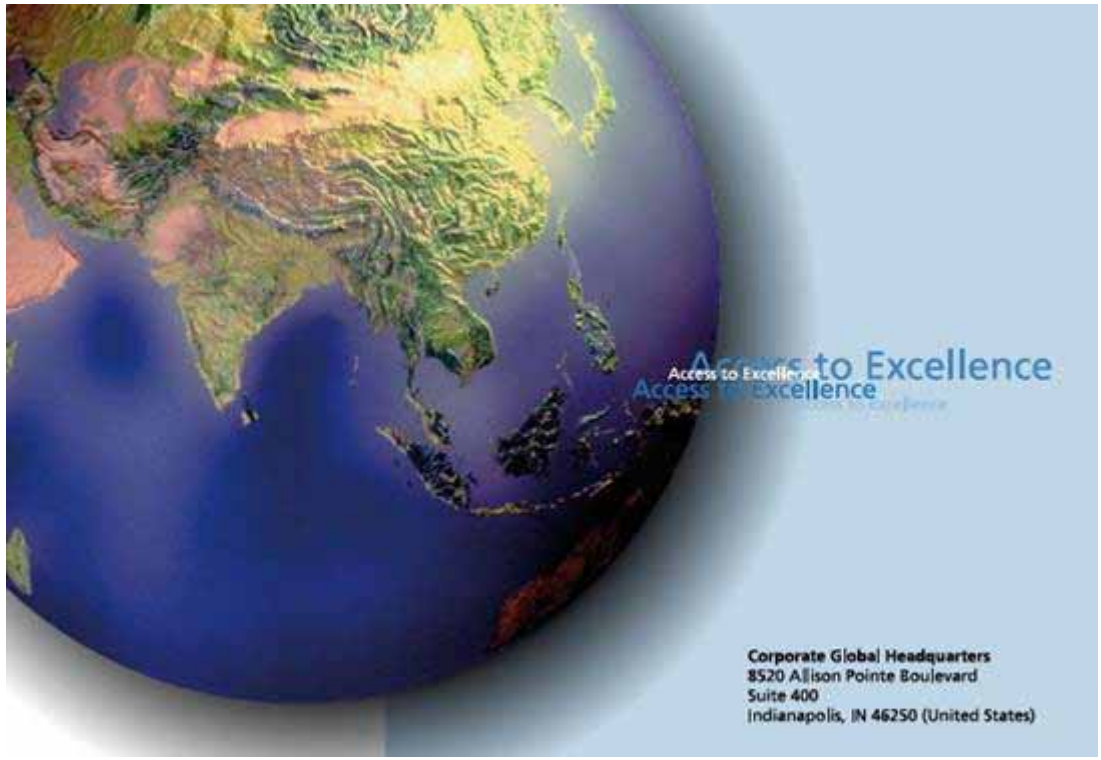
RCC

- **Founded in 1977**
- **Acquired by HSD 2005**
- **Sites in Switzerland, Germany, Spain, India, Canada, Japan**
- **GLP Accredited**

RCC
Linking science to progress

SafePharm Laboratories Limited and RCC

= A New Global Contract Service Organisation



With a single identity – watch this space!

Why Use a Contract Laboratory?

- Service orientated
  - provide specialist services not available in-house at the sponsoring Company
  - and/or routine testing services (allows the sponsoring Company to allocate its resources to greater advantage)
- Well established CROs have an extensive clientbase and wealth of experience
- Systems optimised for speed and quality and cost effectiveness
- GLP accredited
- Not unusual for a Company to identify primary & secondary CROs for provision of services
- Viewed as partnerships instead of customer-provider

WHY *IN VITRO* ?

FROM THE ‘OLD’ TO THE ‘NEW’

The ‘old’: *In Vivo*

Examples of mammalian studies

- Short term (acute) toxicology
- Sub chronic & chronic toxicology
- Developmental and Reproductive Toxicology
- Genetic Toxicology
- Carcinogenicity
- Safety Pharmacology

Variety of species

- Rodent (e.g. rat, mouse), non-rodent (e.g. rabbit, dog, pig, non-human primate)

The ‘new’: *In Vitro*

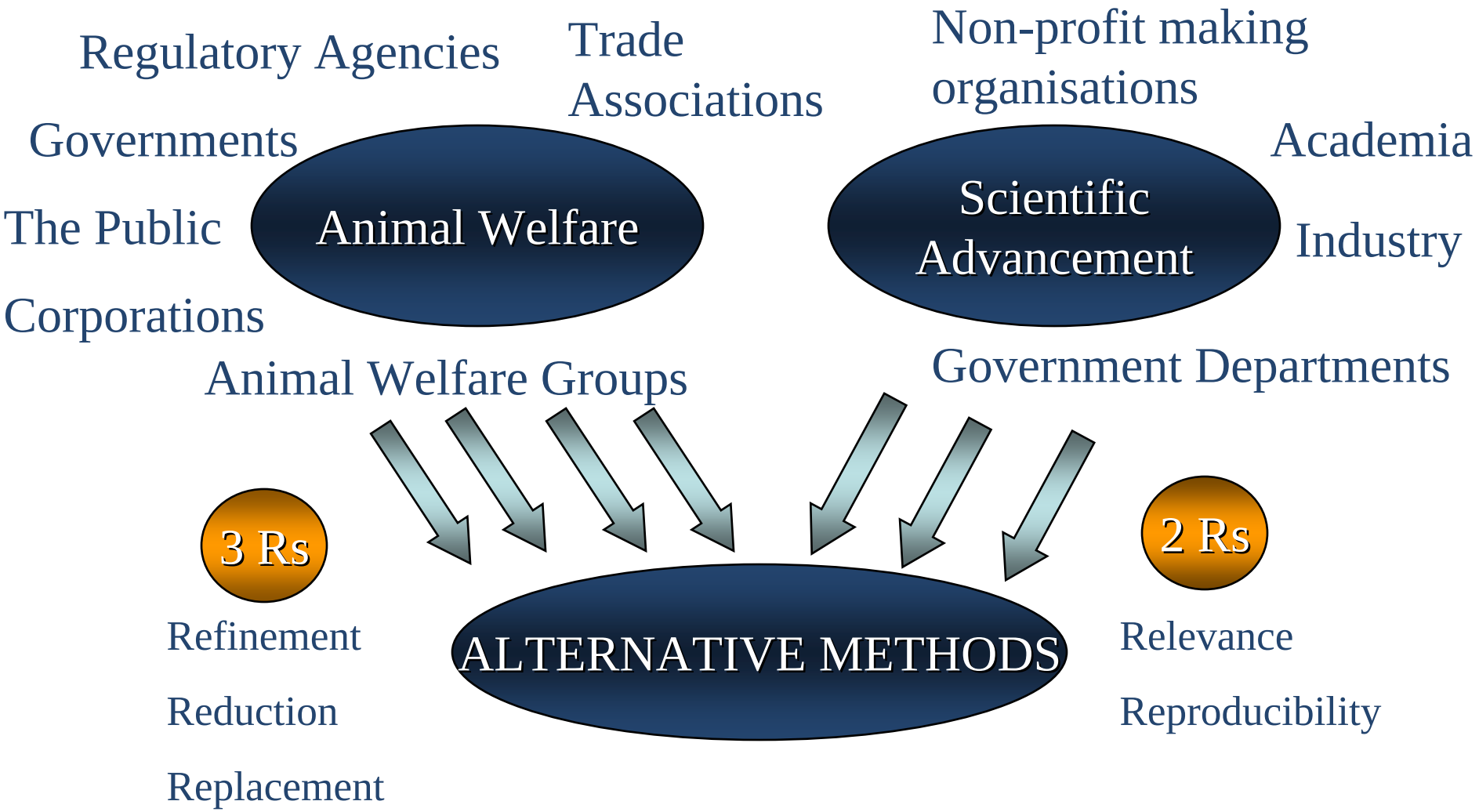
In Vitro Testing

- 20 years ago, little demand
- Not a realistic business opportunity
- Many assay systems available
- Very few validated

The situation is changing

Why are Alternative Tests Required?

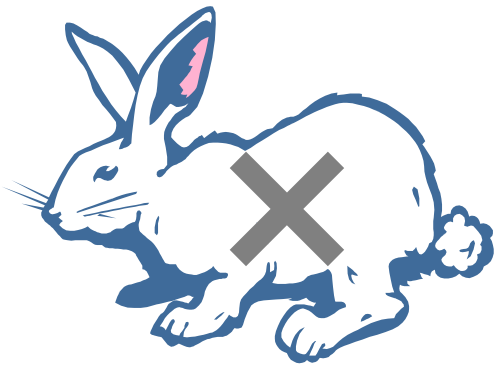
Scientific, Societal and Regulatory Pressures



Why are Alternative Tests Required?

Drawbacks of mammalian studies

- Species differences
- Health status
- Some subjective endpoints
- Responses are assessed but mechanisms are rarely evaluated
- Reproducibility
- Lack of performance standards
- Laboratory space
- ANIMAL WELFARE



Why are Alternative Tests Required?

Regulatory Framework: Europe

- Council Directive 86/609/EEC (the ‘animal welfare’ Directive)
- EU Cosmetics Directive 76/768/EEC
- New EU Chemicals Regulation (REACh) *

*Registration, Evaluation, Authorisation and restriction of Chemicals

National Legislation e.g.

- UK Animals (Scientific Procedures) Act 1986

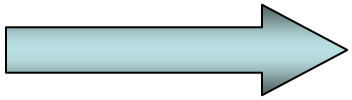
In vivo studies: Implementation of the 3Rs

Reduction (of severity) and refinement have been used with success

- Acute oral toxicity OECD 401 replaced: OECD 420, 423, 425
- Skin sensitisation OECD 406 replaced: OECD 429 LLNA
- Acute inhalation toxicity OECD 403: potential replacement by OECD 433 and 436
- Incorporating screening tests where applicable

How are *in vitro* methods being used by CROs?

- Used extensively as screening tools
- Integration into tiered testing strategies
- For product selection (benchmarking)
- As full replacement methods where validated and regulatory accepted methods are available
- To elucidate mechanisms of action



Reduction of severity of procedures

Reduction in animal numbers

How are *in vitro* methods being used by CROs?

- Drug discovery
- Efficacy
- Bioavailability - absorption
- Metabolism
- Safety Assessment
- Hazard Determination

Challenges to adoption of *in vitro* methods

- Rather limited availability of validated models/test guidelines (but expanding)
- Adoption as official test guidelines has been slow
- History of use of mammalian models. Fear of the unknown
- New skills required
- Relatively low demand
- Cost??

How are Alternatives Used at SafePharm?

- A proactive approach, but almost no pure R&D
- Involvement in prevalidation and validation studies
- Promising new methods, or newly validated methods are investigated and internally ‘validated’ using appropriate reference chemicals
- Screening methods incorporated into ‘ethical testing strategies’

In Vitro Testing at Safepharm: Notable milestones & achievements

- **1987:** *In vitro* Genetox testing batteries e.g. Ames, Chromosome Aberration, Mouse Lymphoma Assays

- **1999:**

  Decision to actively develop the use of non-genetox alternative methods for hazard identification:

  Initially *ex vivo* local tolerance - introduction of REET (IRE) & TER corrosivity

- **2000:** Formation of Alternative Toxicology Section

In Vitro Testing at Safepharm: Notable milestones & achievements

- **2000:** Internal validation of B.41 3T3 NRU phototoxicity assay completed

- **2004:** completion of ‘catch-up validation’ of SkinEthic RHE corrosivity model (OECD 431)

- **2005:**

  Submission of IRE protocol to ICCVAM

  Opening of new *in vitro* testing laboratory

In Vitro Testing at Safepharm: Notable milestones & achievements

- **2006:**

  Completion of ‘catch-up validation’ of CellSystems EST-1000 corrosivity model (OECD 431)

  Completion of pre-validation of the Slug Mucosal Irritation Assay (SMI) for eye irritation

  Introduction and in-house validation of BCOP and HETCAM
- **2007:** In-house validation of Episkin skin irritation test (ECVAM protocol)
- **2007/8:** Utilisation of SkinEthic Human Oral Epithelium model

Range of Services

Range of services offered influenced by

- Demand

- availability of appropriate methods

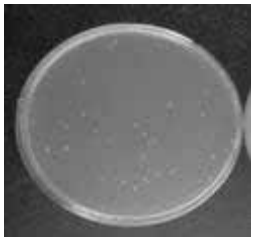
- benefits in terms of animal welfare and/or improved science

IN VITRO TESTING SERVICES

Genetic Toxicology

Bacterial mutagenicity test

e.g. ‘Ames’ test using *Salmonella typhimurium*



Chromosome aberration test

e.g. Human Lymphocytes, CHL Cells

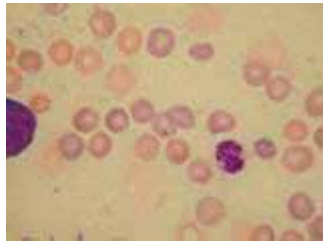
Genetic Toxicology

Mammalian cell mutation test

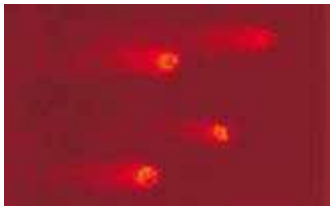
e.g. Mouse Lymphoma L5178Y TK +/- Assay

Mammalian Bone marrow test

e.g. in vitro mouse micronucleus test



Comet Assay



Local Tolerance

Skin corrosivity

Skin irritation

Eye irritation

Oral mucosal irritation

SKIN CORROSIVITY

1. *Ex Vivo TER*

2. Membrane Barrier

Transcutaneous Electrical Resistance Assay (OECD 430)

An *Ex Vivo* Test for Skin Corrosivity

- Utilises skin discs taken from the shaved dorsal region of humanely killed rats (aged 28-30 days)

Skin discs held in place over the end of a PTFE tube, epidermal side uppermost, using a rubber ‘O’ring

Transcutaneous Electrical Resistance Assay (OECD 430)

An *Ex Vivo* Test for Skin Corrosivity

TER Measurement
(24-hour exposure)

Corrositex™

An *In Vitro* Membrane Barrier Test (OECD 435)

Commercial Test Kit

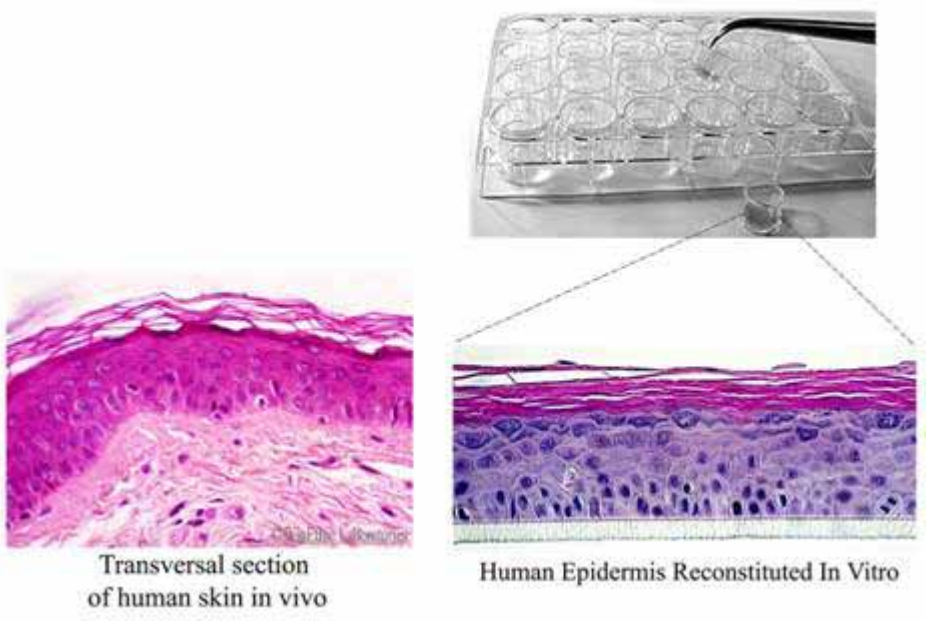
Chemical Detection System (CDS)

SKIN CORROSIVITY
HUMAN TISSUE MODELS

Skin Corrosivity: Human Tissue Models (OECD 431)

Human Tissue Equivalents e.g. Episkin, SkinEthic RHE, MatTek Epiderm, Cellsystems EST-1000

- Three dimensional tissue
- Cultured from normal, human epidermal keratinocytes
- Forms a highly differentiated tissue that closely resembles human epidermis



Photograph courtesy of SkinEthic

Skin Corrosivity: Human Tissue Models (OECD 431)

- Validated method (OECD 431 and Method B.40 of Annex V to 67/548/EEC)
- Endpoint is tissue viability (MTT reduction assay)

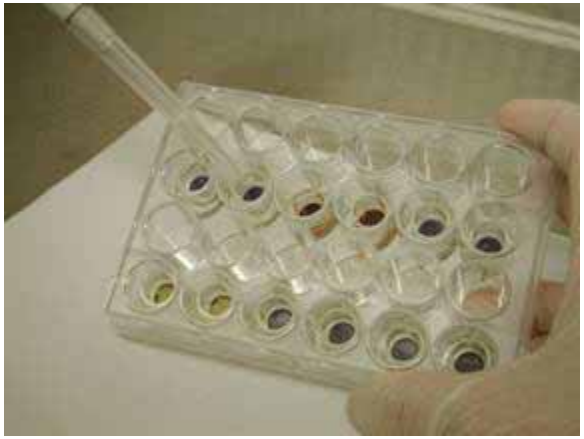
Skin Corrosivity: Human Tissue Models

Topical application

Incubation

Washing

MTT Assay

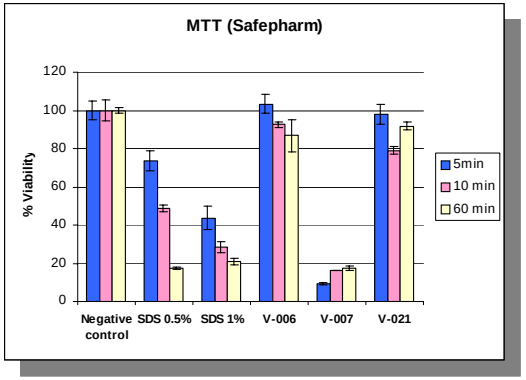


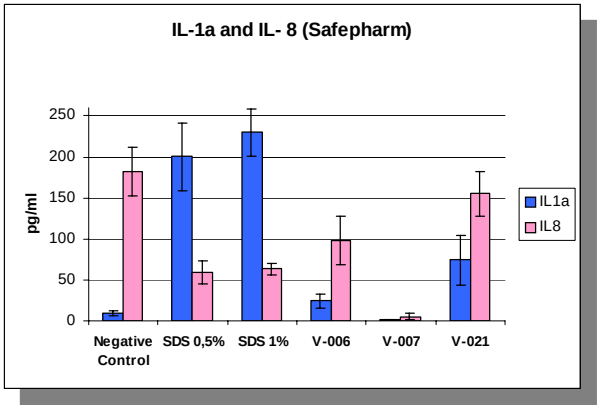
Alternative Tests at SafePharm

SKIN IRRITATION
HUMAN TISSUE MODELS

Multi-endpoint analysis (MEA)

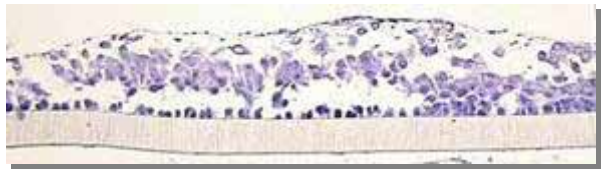
Tissue viability (MTT)





Release of inflammatory mediators

Negative control, 60 min exposure



SDS 0.5%, 60 min exposure

Histological examination

Skin Irritation: Human Tissue Models

Skin Irritation

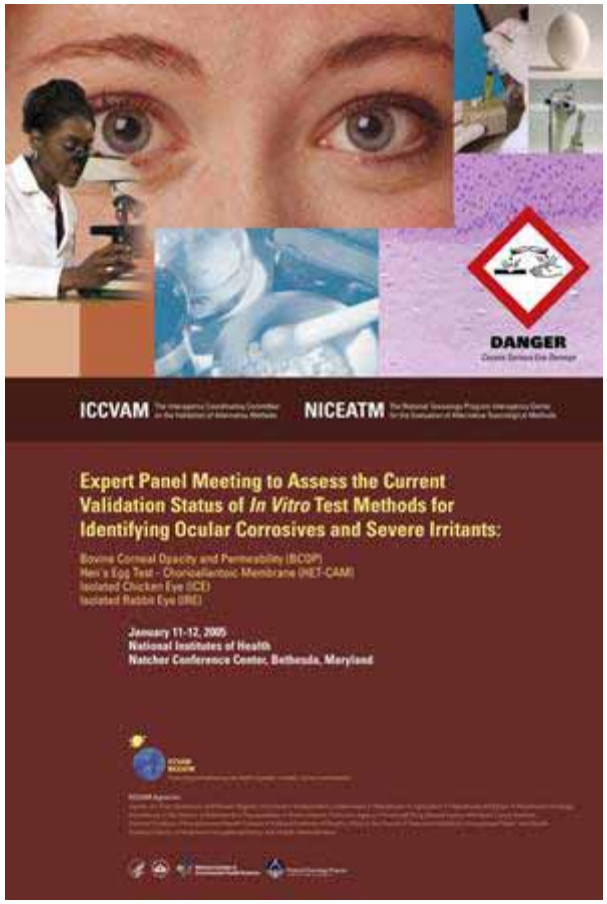
- Episkin: 15 minute + 42-hour post incubation
- Fully validated protocol. ESAC Statement issued

- Other commercial models: e.g. SkinEthic RHE, MatTek Epiderm, Cellsystems EST-1000
- Protocol variations

EYE IRRITATION

EX VIVO (ORGANOTYPIC) MODELS

Ex vivo (Organotypic) Models



- Isolated Rabbit Eye Test (IRE/REET)
- Bovine Corneal Opacity Test and Permeability Test (BCOP)
- Hen's Egg Test – Chorioallantoic Membrane (HET-CAM)
- Isolated Chicken Eye Test (ICE)
- BRDs and protocols available

http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox.htm

Ex vivo (Organotypic) Models

Within the EU, a positive results in one of these tests is acceptable for classification as an irritant (R41) without the need for further testing

EC (2004). Manual of Decisions for Implementation of the 6th and 7th Amendments to Directive 67/548/EEC on Dangerous Substances. Updated version 2004. 189 pp.

RABBIT ENUCLEATED EYE TEST (REET)

aka ISOLATED RABBIT EYE TEST (IRE)

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Rabbit Enucleated Eye Test (REET)

Full thickness corneal model

- ‘Validated’ in-house for screening of severe eye irritants
- Used at SPL since 1999
- Validated on behalf of GSK for worker safety assessment
- Has minimised exposure of animals to severe irritants
- ICCVAM BRD

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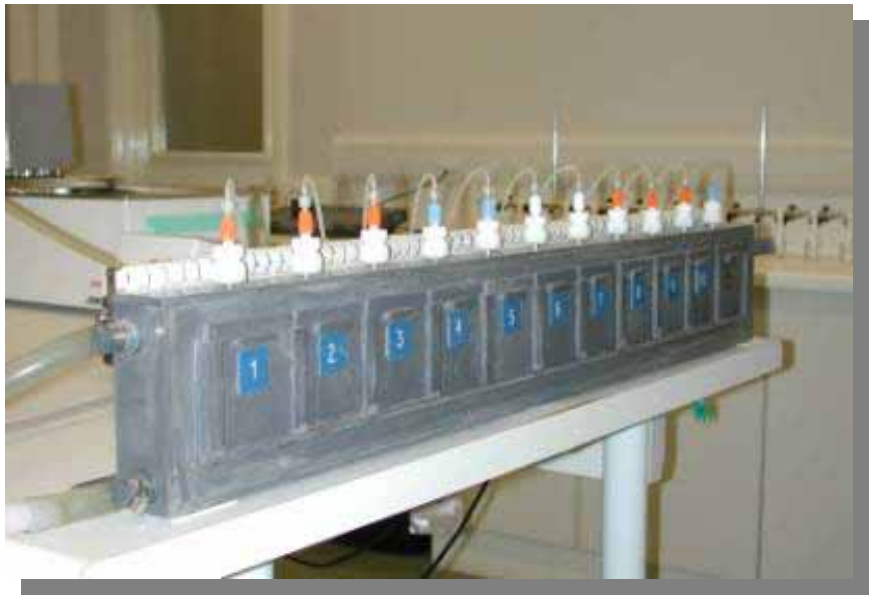
Rabbit Enucleated Eye Test (REET)

REET Apparatus



Superfusion Chambers

Rabbit Enucleated Eye Test (REET)

Observations

- Visual assessment of corneal opacity

- Uptake of sodium fluorescein dye by the cornea

Rabbit Enucleated Eye Test (REET)

Measurement of corneal thickness (optically or using ultrasonic pachymeter)

Slit-lamp biomicroscopic examination of the cornea

BOVINE CORNEAL OPACITY AND PERMEABILITY TEST (BCOP)

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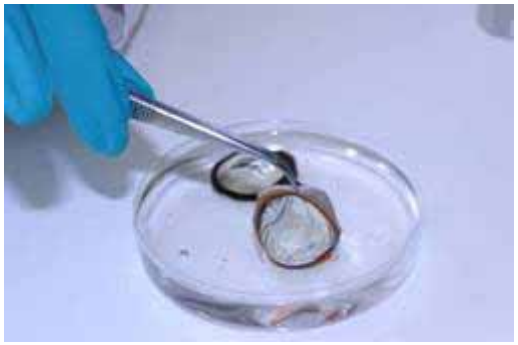
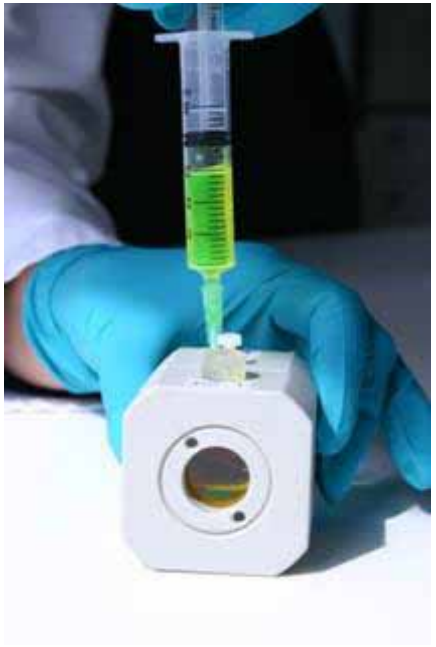
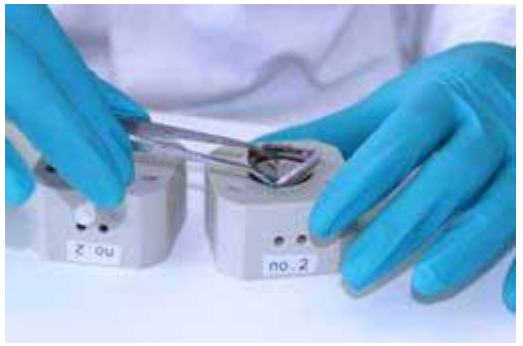
BCOP



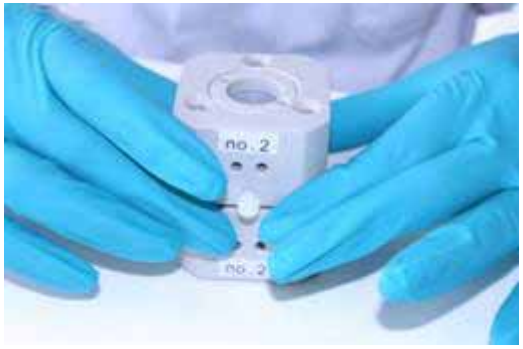
Full thickness corneal model

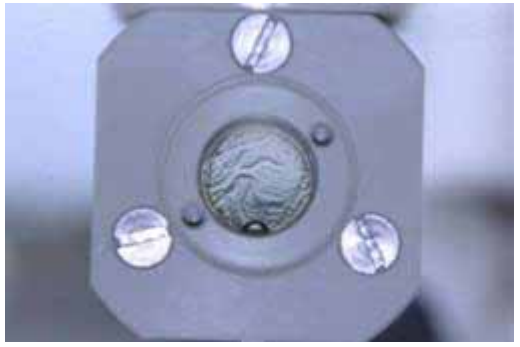
Bovine Cornea





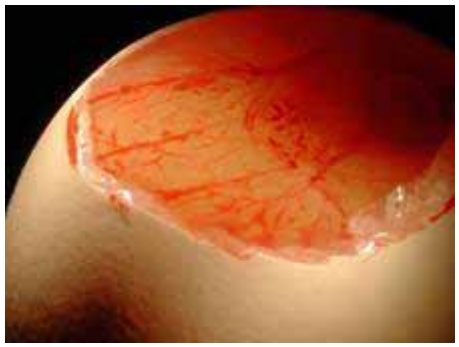
In vitro Score





Endpoints: Corneal Opacity & Permeability

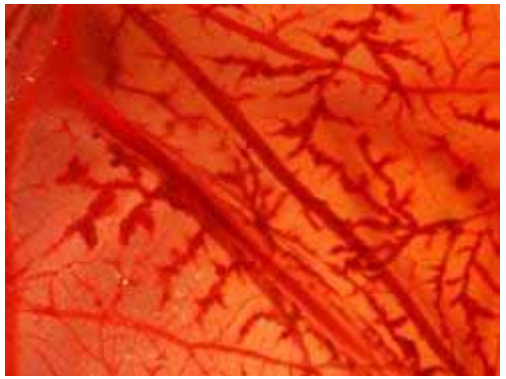
HET-CAM



Validated in-house for screening of severe eye irritants

Testing of formulations

ICCVAM BRD



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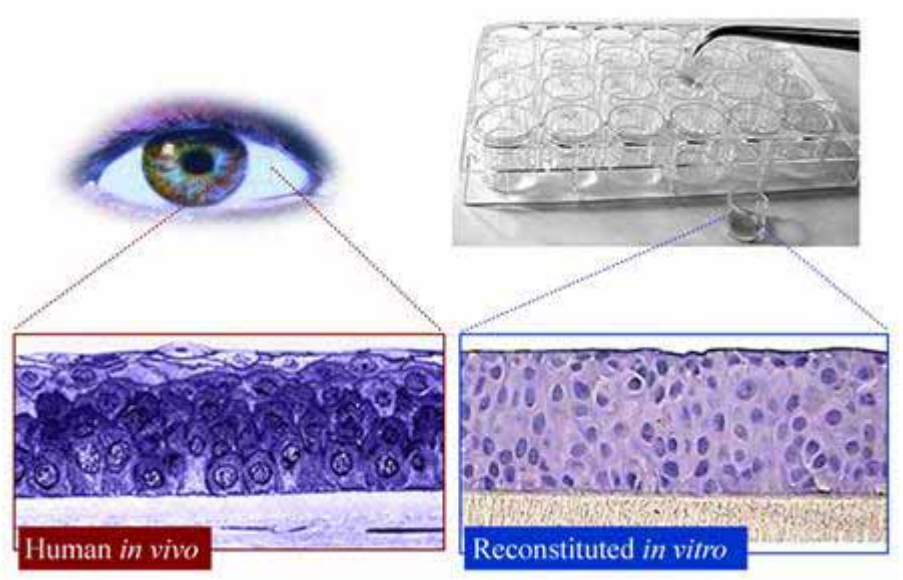
EYE IRRITATION

HUMAN TISSUE MODELS

Eye Irritation: Human Tissue Models

SkinEthic Human Reconstituted Corneal Epithelium (RHCE)

- Transformed human keratinocytes of the cell line HCE
- Forms a three dimensional corneal epithelium
- MEA approach



Photograph courtesy of SkinEthic

Other models are available e.g. MatTek Epiocular

Eye Irritation: Testing Strategy

Different models can be used within a battery of tests to evaluate eye irritation potential

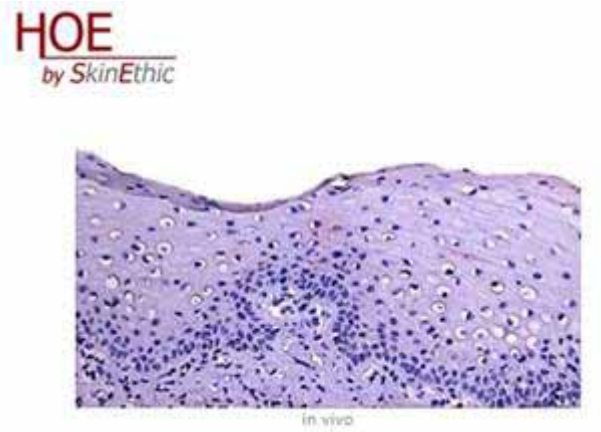
Example – in accordance with recommendations of ECVAM workshop 2005 (see EPAA website)

At SafePharm, full thickness (*ex vivo*) corneal models are used in conjunction with the human reconstituted corneal model to identify severe irritants and ‘non-irritants’

http://ec.europa.eu/enterprise/epaa/wg5_doc_oculaire.pdf

Oral Mucosal Irritation

Testing of oral care products e.g. dentifrice



Photograph courtesy of SkinEthic

MEA approach

BARRIER MODELS

IN VITRO DERMAL ABSORPTION

In Vitro Dermal Absorption

OECD Test Guideline 428

- Measures diffusion of chemicals into and across excised skin
- Use radiolabelled or non-radiolabelled test material
- Receptor fluid sampled at predetermined time-points

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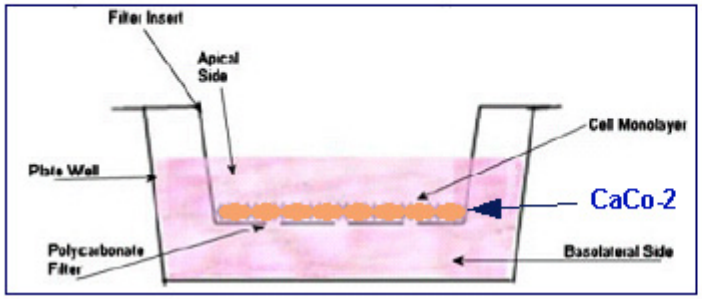
Other Barrier Models

Available Systems

- Intestinal uptake using CaCo-2 cells
- Blood brain barrier system using pig brain microvascular endothelial cells (BMEC)

CaCo-2



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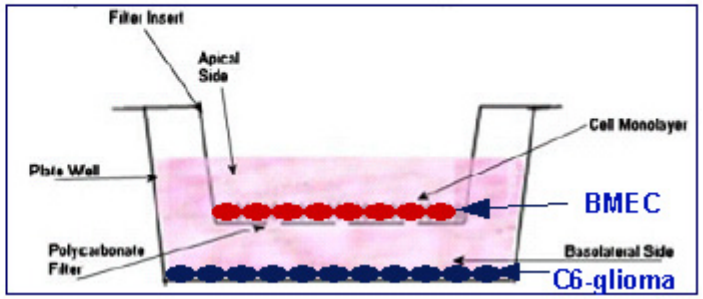

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PHOTOTOXICITY

In Vitro 3T3 NRU Phototoxicity Test

- OECD 432
- Method B.42 of Annex V to 67/548/EEC
- Mouse Fibroblast 3T3
- Determines the potential of chemicals to cause phototoxicity following exposure to UVA radiation (315 – 400 nm)

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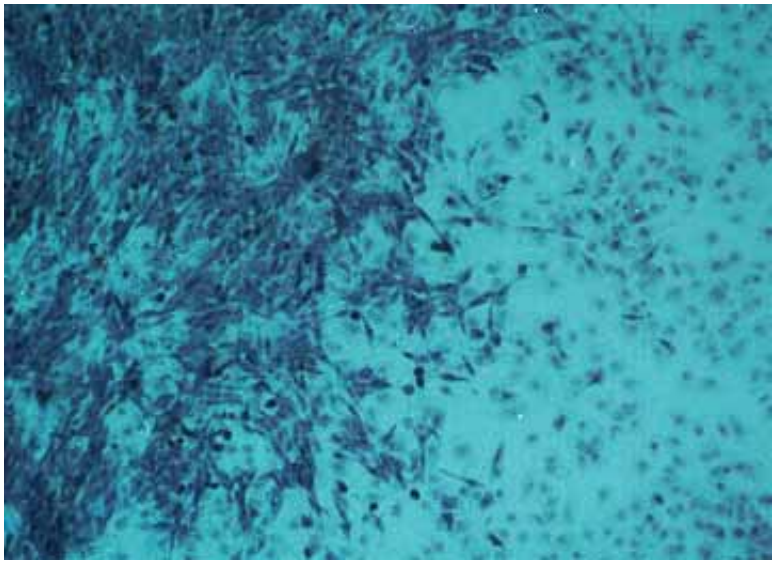
CARCINOGENICITY

Cell Transformation Test

Available Systems

- Balb/c 3T3 assay
- Syrian hamster embryo cell assay (SHE Assay)
- Evaluation of morphologically transformed colonies and foci



Applications

- Detection of non-genotoxic carcinogens

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METABOLISM

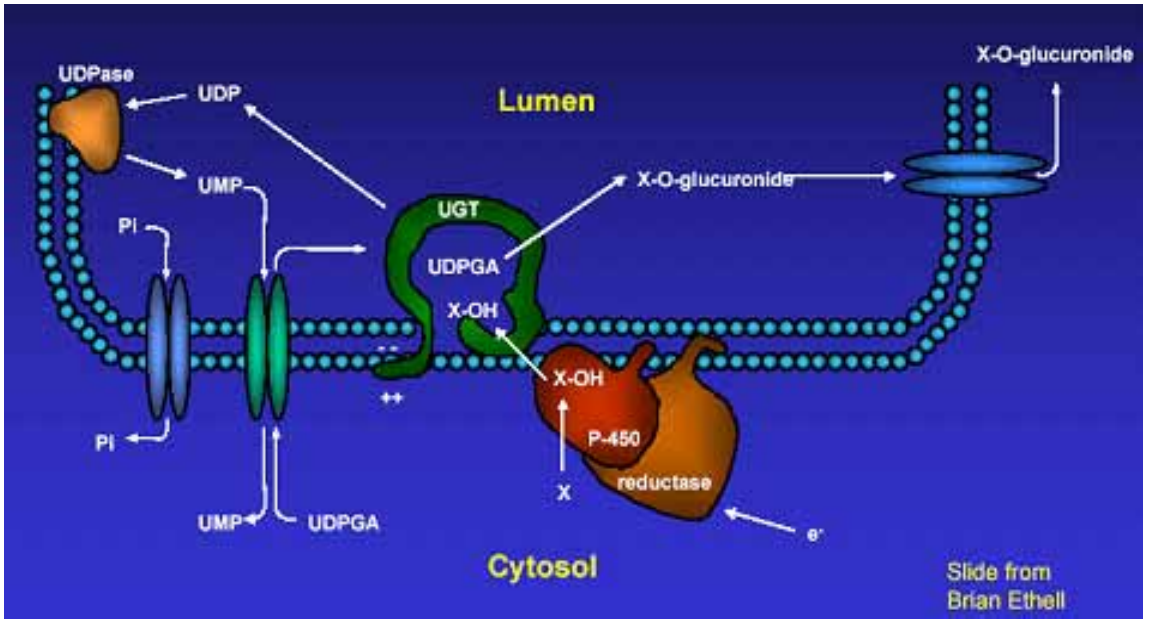
Metabolism *in vitro*

Fields of Work

- Metabolic stability with metabolic profiling
- Species specific kinetics (including rats, dogs, primates and humans)
- Drug-Drug interactions with phase I, II and III proteins (e.g. alterations of CYP and UGT activity, MDR1 interactions)

Available Systems

- Liver homogenates
- Hepatic microsomes
- Hepatocytes different species



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Other *In vitro* Systems

Cytotoxicity – various cells/cell lines

HERG Assay – QT prolongation

Concluding comments

- There are scientific, regulatory and animal welfare reasons for *in vitro* testing

- *In vitro* models are being routinely used for assessment of genotoxicity and are becoming firmly established in other areas of evaluation e.g. local tolerance

Concluding comments

•Outsourcing of testing to CROs is predicted to increase

•Will CROs be able to meet the demands of industry for *in vitro* testing?

Based on previous experience, the answer should be yes

- business opportunity
- resources will be provided

Concluding comments

•Will CRO's be positioned to undertake pure research?

Possibly, if sufficient funding is provided, but the priority is always likely to be with high throughput testing

• CRO's are widening their portfolio of *in vitro* assay systems

…. and where appropriate will 'scale up' their ability to test routinely on a large scale to meet regulatory, scientific and animal welfare demands

Acknowledgements

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Andrew Whittingham

Neil Warren

Helen Bytheway

Dr Wolfgang Voelkner

Dr Albrecht Poth

Thank you for your attention

Questions ?