New Models in the Validation Pipeline for Ocular Safety Testing

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ICCVAM Best Practices Workshop

William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD

January 19, 2011

Outline

- ECVAM Eye Irritation Validation Study (EIVS)
  - EpiOcular™ test method
  - SkinEthic™ test method
- Other non-animal ocular safety test methods and strategies
  - Fluorescein leakage test method
  - Antimicrobial Cleaning Product testing strategy pilot program
  - Isolated rabbit eye test method
- JaCVAM 2nd Validation Study
  - Short time exposure test method
  - *To be presented by Dr. Hitoshi Sakaguchi*

ECVAM Eye Irritation Validation Study (EIVS)

- Two *in vitro* test methods employing reconstructed human tissue (RhT) models
  - EpiOcular™ eye irritation test (EIT)
    - 3D construct prepared from non-transformed, human-derived epidermal keratinocytes
  - SkinEthic™ human corneal epithelium (HCE)
    - 3D construct uses immortalized human corneal epithelial cells
- Both test methods involve topical exposure of a test substance to the epithelial surface of the tissue construct, followed by cell viability measurement

ECVAM EIVS – Validation Management Team (VMT) Composition

- Validation Management Group
  - Stuart Freeman (Consultant) – Chair
  - Valérie Zuang (ECVAM) – Co-chair
  - Pauline McNamee (COLIPA) – Sponsor representative
  - João Barroso (ECVAM) Sponsor representative
  - Jan Lammers (TNO) – Coordinating organization representative
  - Carina de Jon-Rubingh (TNO) – Biostatistician
  - André Kleensang (ECVAM) – Biostatistician
  - Chantra Eskes (A.I.S.E.) – External scientist
  - Thomas Cole (ECVAM) – Chair of Chemicals Selection Group
- Lead laboratory representatives
  - Nathalie Alépée (L'Oréal) – SkinEthic
  - Uwe Pfannenbecker (Beiersdorf) – EpiOcular
- Liaisons
  - NICEATM – William Stokes
  - ICCVAM – Jill Merrill
  - JaCVAM – Hajime Kojima
  - Health Canada – Alison McLaughlin

ECVAM EIVS – Objective and Goal

- Objective:
  - *Validate the EpiOcular™ EIT and SkinEthic™ HCE in vitro eye irritation test methods in a formal inter-laboratory study, in order to incorporate these test methods in a Bottom-Up/Top-Down tiered testing strategy (as defined in an ECVAM workshop held in 2005, Scott L. et al., 2009), as e.g. the initial step in a Bottom-Up approach. The ultimate purpose of the test strategy will be to replace the regulatory Draize eye irritation test according to Test Method B.5 of EC Regulation 440/2008 (EC, 2008a) or OECD TG 405 (OECD, 2002)*
- Goal:
  - *Assess the relevance (predictive capacity) and reliability (reproducibility within and between laboratories) of the EpiOcular™ EIT and SkinEthic™ HCE test methods with a challenging set of coded test chemicals (substances and mixtures) for which high quality in vivo data are available*
    - *More specifically, the EIVS will assess the usefulness and validity of the EpiOcular™ EIT and SkinEthic™ HCE as stand-alone test methods to identify chemicals not classified as eye irritant ("non-irritant" chemicals) and their reliable discrimination from all classes of eye irritant chemicals*

ECVAM EIVS – Study Design

- 104 reference substances tested in at least 3 independent tests by each of 3 independent laboratories
- Chemical reactivity determined for all substances based on the Cysteine/Lysine Direct Peptide Reactivity Assay (DPRA)
  - As data from the DPRA analysis becomes available, subsets of 30-50 test substances will be distributed to the participating laboratories for viability assessment
- Two or more consecutive testing phases to allow for periodic opportunities to evaluate the frequency of technical errors and any other problems that might occur

Overview of the EpiOcular™ Test Method[1] (1)

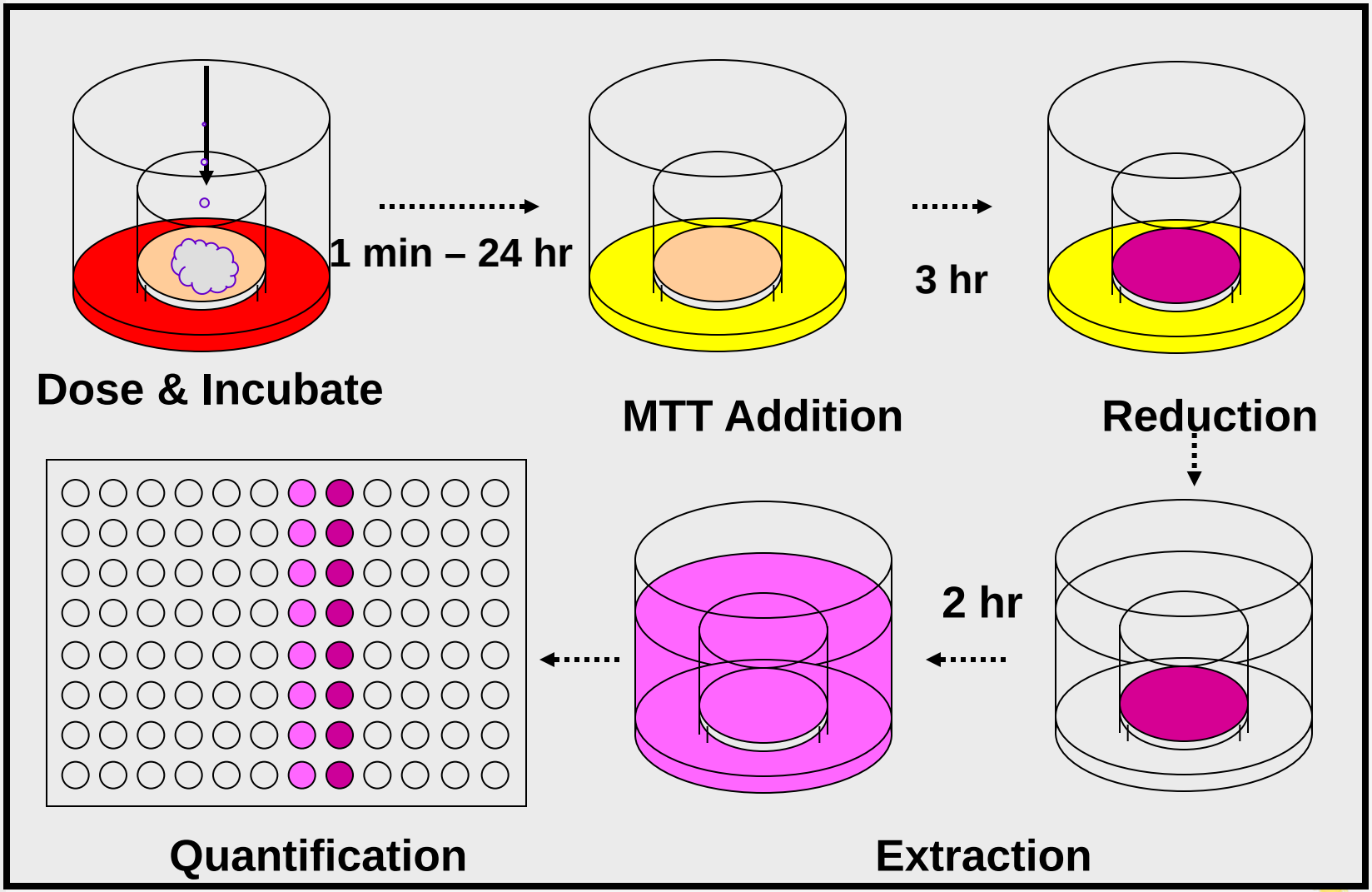
- 3-D tissue construct of normal human epidermal keratinocytes (NHEK)
  - Nonkeratinized, but stratified epithelium (5-8 cell layers) with an upper and central layer of squamous cells and a lower layer of rounded cells grown on a membrane in a specialized tissue culture insert with an air (apical) and liquid (basal) interface
  - Keratinocytes are normal, nontransformed, and nontransfected cells
  - Models the epithelial layer of the cornea, not the stroma or endothelium
  - Assumes *in vitro* cell viability correlates with a test substance's *in vivo* ocular irritation potential after corneal exposure
- Cell viability is measured by MTT reduction after topical exposure to the test substance

[1] Tissue construct produced by MatTek Corporation, Ashland, MA

Overview of the EpiOcular™ Test Method (2)

- Proposed decision criteria based on the viability of the treated tissues relative to the negative control-treated tissues
  - Nonirritant: If the test article-treated tissue viability is >60% relative to the negative control-treated tissue viability

EpiOcular™ Test Method Schematic[1]



[1]Provided by Rodger Curren IIVS, Inc.

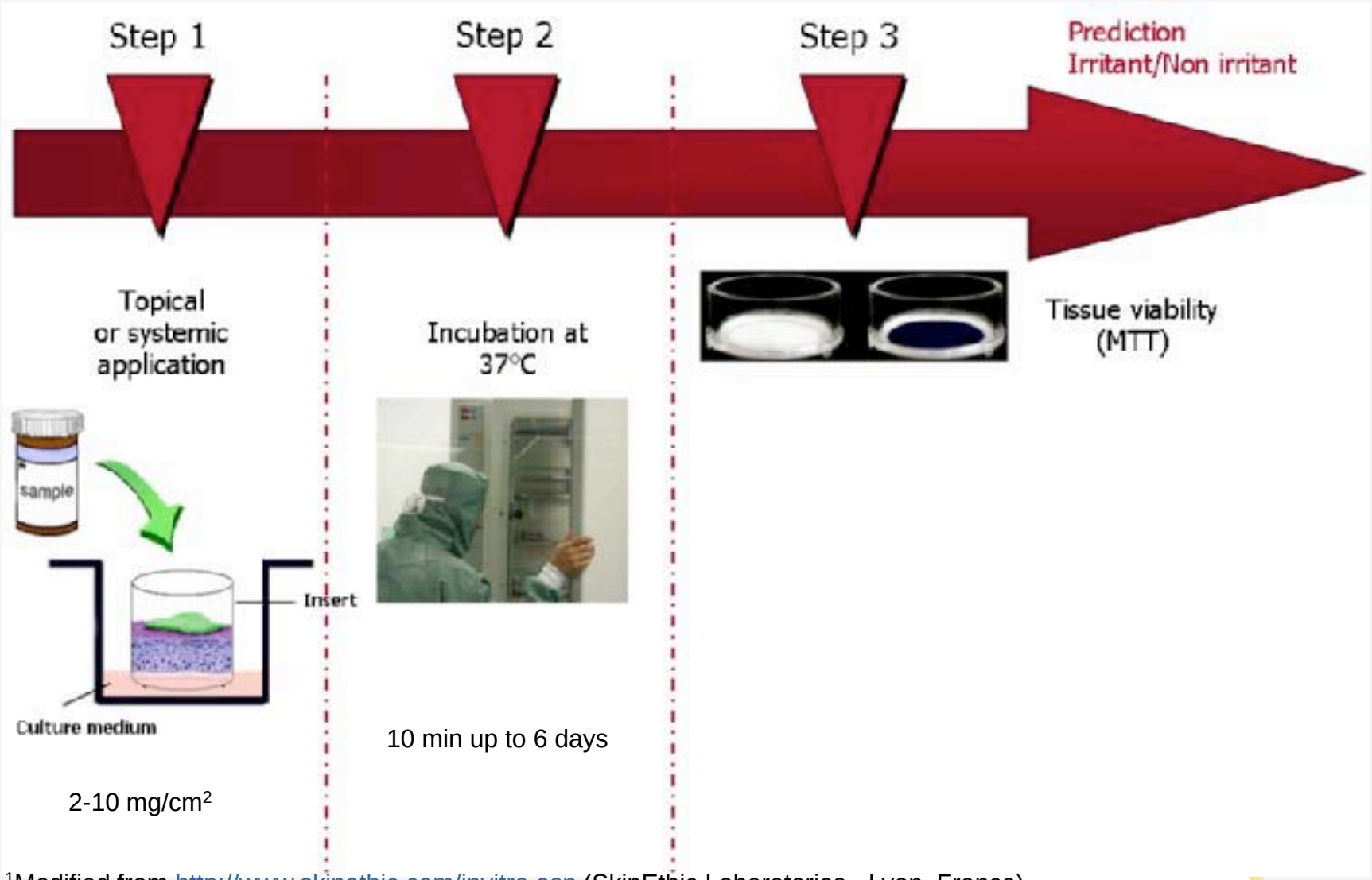
Overview of the SkinEthic™ Test Method (1)

- 3-D tissue construct of immortalized human corneal epithelial (HCE) cells
  - Cultured in a chemically defined medium and seeded on a polycarbonate membrane at the air–liquid interface
  - Multilayered epithelium resembling the *in vivo* corneal epithelium with a thickness close to 65 µm
- Substances are tested using 2 exposure times
  - Short exposure: 10 min exposure without post-treatment incubation
  - Long exposure: 60 min exposure followed by 16 h post-treatment incubation
- Cell viability is measured by MTT reduction after topical exposure to the test substance

Overview of the SkinEthic™ Test Method (2)

- Proposed decision criteria based on the viability of the treated tissues relative to the negative control-treated tissues
  - Estimated time to reduce cell viability to 50% of the negative control (i.e., phosphate-buffered saline)
  - Nonirritant: Mean tissue viability >50%

SkinEthic™ Test Method Schematic[1]



[1]Modified from http://www.skinethic.com/invitro.asp (SkinEthic Laboratories - Lyon, France)

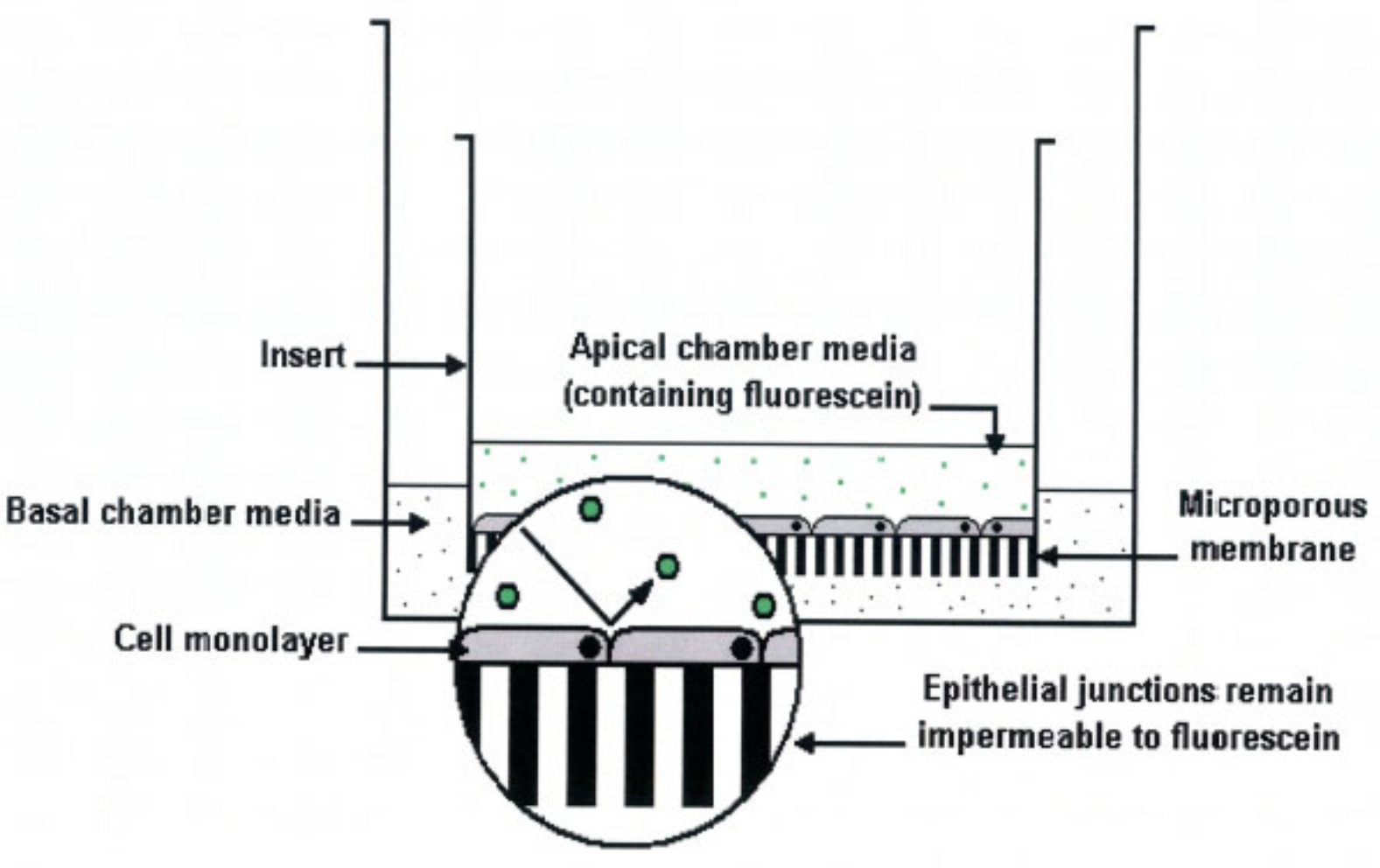
Draft OECD Test Guidelines Currently Under Consideration

- Cytosensor Microphysiometer (CM) Test Method
  - For identifying limited types of ocular corrosives and severe irritants and substances not labeled as irritants
  - Consistent with ICCVAM-recommended CM protocol
- Fluorescein Leakage (FL) Test Method
  - For identifying ocular corrosives and severe irritants
    - False-positive rate: 7% (7/103) to 9% (9/99)
    - False-negative rate: 54% (15/28) to 56% (27/48)
  - Specifically for water-soluble substances and mixtures
    - Limitations include strong acids and bases, fixatives, and highly volatile chemicals because their mechanisms of action are not measured by FL
    - Other limitations: solids; colored and viscous substances

Overview of the Fluorescein Leakage Test Method

- Uses Madin-Darby Canine Kidney (MDCK) CB997 tubular epithelial cells that are grown on permeable inserts and model the non-proliferating state of the *in vivo* corneal epithelium
- Amount of sodium-fluorescein dye that leaks through the cell layer is measured spectrofluorometrically following a short (1 min) exposure to the test substance
- Endpoint - concentration causing 20% fluorescein leakage relative to the value recorded for the untreated monolayer (0% leakage) and inserts without cells (100% leakage)
  - Expressed as FL_{20} (mg/mL)
- Proposed decision criteria based on the FL_{20} value
  - Irritant: $FL_{20} \leq 100$ mg/mL

Fluorescein Leakage Test Method Schematic[1]

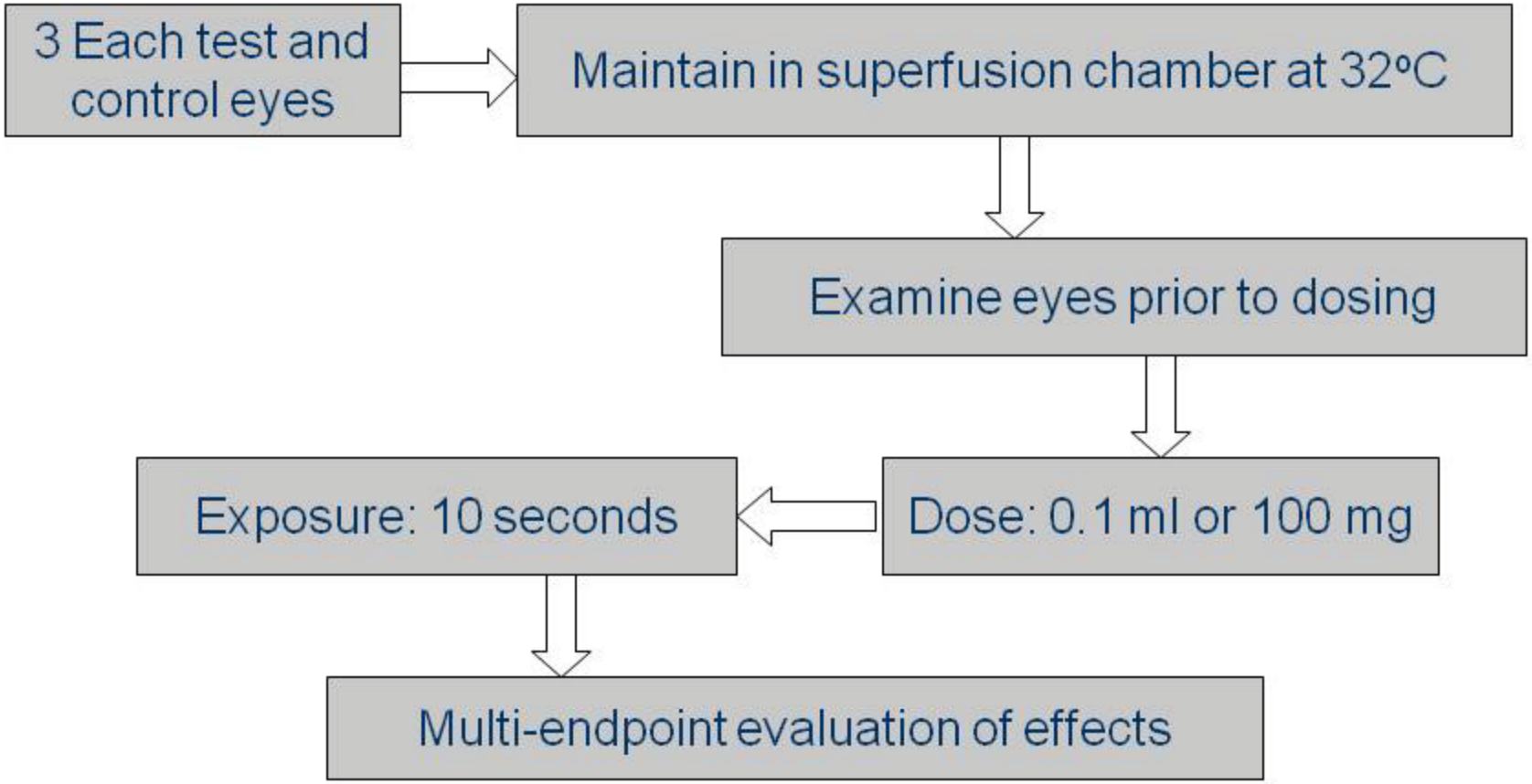


[1]Taken from: Wilkinson, PJ (2006)

The Isolated Rabbit Eye (IRE) Test Method

- Endpoints measured
  - Corneal opacity
  - Corneal swelling
  - Fluorescein penetration
  - Morphological effects on corneal epithelium
- Evaluated by ICCVAM/NICEATM in 2005 for identifying ocular corrosives and severe irritants
  - Recommended additional studies to expand the IRE database and optimize the IRE decision criteria
- Now undergoing further development and protocol optimization at Harlan Laboratories and GlaxoSmithKline
  - Use of IRE in combination with SkinEthic™ to develop “intelligent test strategy” for ocular irritation (SOT 2009; abstract 376)
  - *Work using a set of 30 diverse substances from the ICCVAM validation chemical database is underway* (SOT 2010; abstract 102)

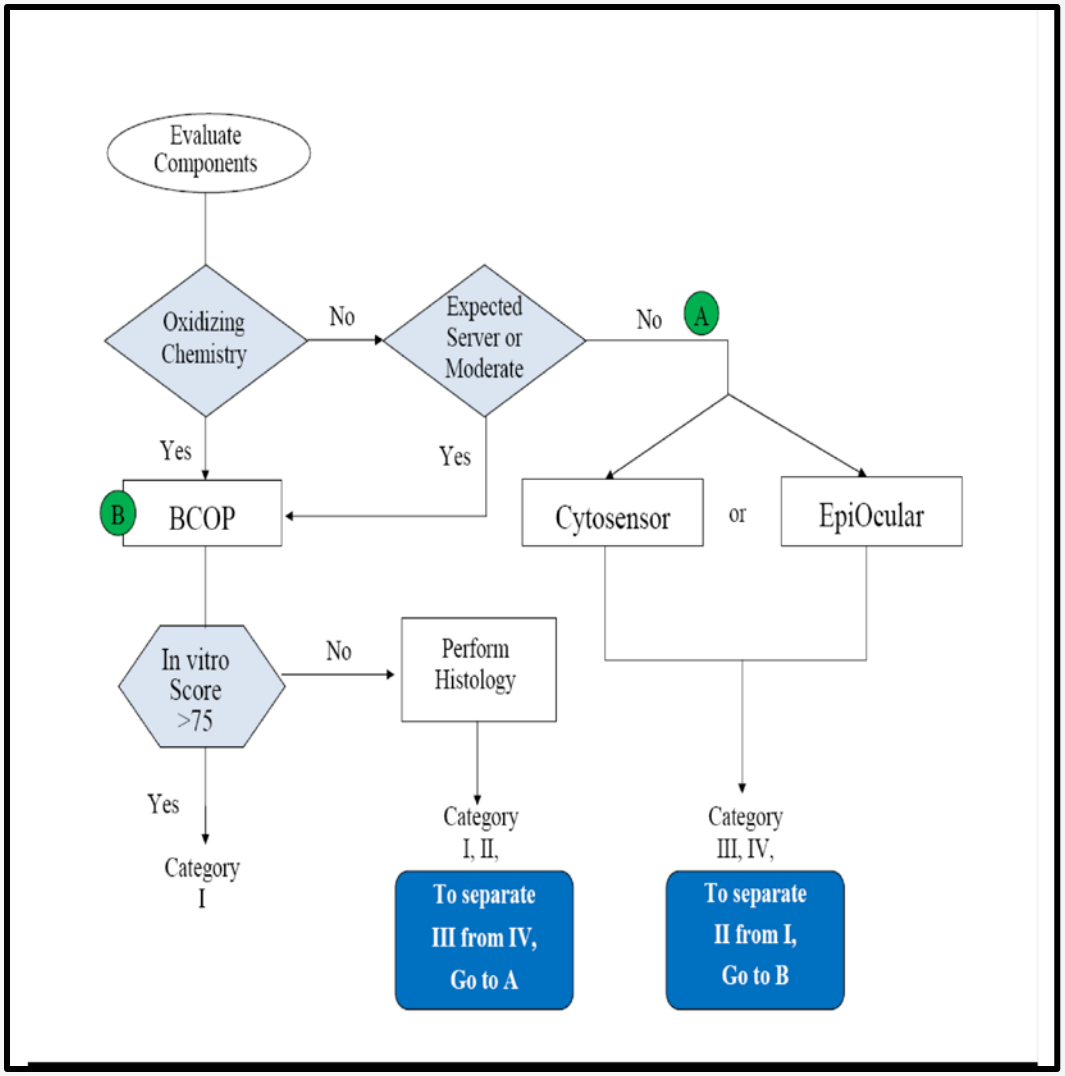
Overview of the Isolated Rabbit Eye Test Method



EPA Office of Pesticide Programs (OPP) Voluntary Pilot Program (initiated May 2009)

- Antimicrobial Cleaning Product Testing Strategy
  - Designed to evaluate the effectiveness of a specific alternative testing strategy, as a potential replacement for the rabbit eye test, for labeling antimicrobial products with cleaning claims
  - The proposed testing strategy uses three assays:
    - BCOP
    - CM
    - EpiOcular™
  - Intended to allow OPP to differentiate among the four eye irritation hazard categories used by the EPA
  - Along with the three alternative assays, OPP is asking participating registrants to submit available consumer incident data and any existing rabbit eye test results on similar or structurally-related chemicals or products as further support for the testing approach
  - To date, three submissions

AMCP Testing Strategy Proposal[1]



[1]Taken from the EPA Voluntary Pilot Program

Summary

- EpiOcular™ and SkinEthic™ test methods currently undergoing prospective validation
  - Coordinated by ECVAM
- Fluorescein Leakage and Cytosensor Microphysiometer test methods currently under consideration as Draft OECD Test Guidelines
- Voluntary pilot program at EPA: Antimicrobial Cleaning Products testing strategy
- Isolated rabbit eye test method undergoing further development and optimization