Section E

The Three Rs of Alternatives
Russell and Burch

- The Principles of Humane Experimental Technique, 1959
  - Replacement
    - Use cells in culture (in vitro) or computer models (in silico) instead of animals
  - Reduction
    - Use the right number of the right species of animal for the right experiment
  - Refinement
    - Reduce pain and distress, and use animals lower on the phylogenetic scale
Replacement

- Concept of replacement in ...
  - Basic science (academic activities)
  - Product development/discovery
  - Regulatory (scientifically valid vs. validation)
Replacement Alternative

- Pregnancy testing: monoclonal antibodies
- Pyrogen testing: LAL, cytokine release (RBC)
- Production of monoclonal antibodies
- Irritation, hypersensitivity: reconstituted tissue equivalents
Tissue Culture Insert Principle

Barrier Properties of Reconstituted Human Epidermis

- Reconstituted human epidermis lifted from the plastic insert after dispase II treatment for three minutes at room temperature

In Vitro Testing Approach

Topical application of test product

Multiple end-point analysis

Incubation at 37°C

Tissue viability (MTT)

Gene expression

Reconstituted Human Epidermis

culture medium

Insert

Reconstituted Human Epidermis (Day 12 & day 17)

Histology

Cytokine release in medium (IL-1α, IL-6, IL-8, TNFα, ...)

Refinement Alternatives

- Any modification of a procedure that decreases potential pain or distress, or uses animals lower in a phylogenetic category
  - Non-invasive monitoring: MRI
  - Implantable monitoring devices: B/P
  - Humane endpoints
Basic Method

Detection of Tumors in Vivo

- PC-3M-luc