

# **Safety of Topical Microbicides A Critical Review**

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# Safety of Topical Microbicides A Critical Review

- ❖ **Focus on vaginal safety**
- ❖ **Nonclinical**
  - ❖ *In vitro*
  - ❖ *In vivo*
- ❖ **Clinical**
- ❖ **An overview – discovery through Phase III**
- ❖ **Challenges and opportunities**





# NONCLINICAL SAFETY: From concept through clinical trial

**Discovery/early  
development**

**Pre- phase I  
safety**

**Pre-/Concurrent  
Phase II/III**





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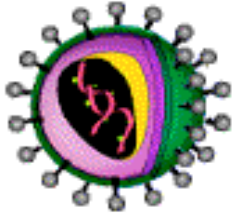
## *IN VITRO:*

- ◆ Cytotoxicity

## *IN VIVO:*

- ◆ Rabbit vaginal  
irritation  
(unformulated)





# NONCLINICAL SAFETY

## Discovery/Early Development

- ❖ ***In vitro* cytopathicity**
  - ❖ **Single cell type**
    - ❖ **PBMCs**
    - ❖ **Macrophages**
    - ❖ **Cells used to assess activity**
  - ❖ **Tissue-based/*ex vivo* systems**
    - ❖ **Origin of tissue**
    - ❖ **Hormonal state**
    - ❖ **Substitute for *in vivo* animal studies?**
  - ❖ **Therapeutic index**





## N-9 *In vitro* Results: Therapeutic Index

IC<sub>100</sub>: 0.05%    CC<sub>50</sub>: 1%    **TI: >20** (Hicks et al 1985).

IC<sub>100</sub>: 0.05%    CC<sub>50</sub>: 0.5%    **TI: >10** (Malkovsky et al 1988).

IC<sub>100</sub>: 0.01%    CC<sub>50</sub>: 0.01%    **TI: >1** (Bourinbaiar 1995).

Are epithelial cells are more resistant?

IC<sub>50</sub>: 0.005%    CC<sub>50</sub>: 0.0006%    **TI:0.12** (O'Connor et al 1995).  
(Hela cells)

Or cervical explants?

IC<sub>50</sub>: 0.005%    CC<sub>50</sub>: 0.0005%    **TI: 0.1** (*Shattock et al unpublished*)

**From R. Shattock**



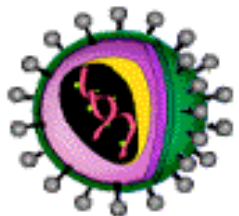


# NONCLINICAL SAFETY

## Discovery/Early Development

- ❖ **Additional safety assessments**
  - ❖ **Lactobacillus toxicity**
  - ❖ **Proinflammatory cytokines**
  - ❖ **Cellular activation**





# NONCLINICAL SAFETY: From concept through clinical trial

## Discovery/early development

### *IN VITRO:*

- ◆ Cytotoxicity

### *IN VIVO:*

- ◆ Rabbit vaginal irritation (unformulated)

## Pre- phase I safety

### *IN VITRO:*

- ◆ Genotoxicity

### *IN VIVO:*

- ◆ General toxicity
  - ◆ Acute, subacute & chronic
- ◆ Rabbit vaginal irritation (formulated)
- ◆ Genotoxicity
- ◆ Reproductive toxicity (Segment I)
- ◆ Pharmacokinetics
  - ◆ Absorption
- ◆ Hypersensitivity

## Pre-/Concurrent Phase II/III





# NONCLINICAL SAFETY

## Pre-Phase I

- ❖ **Genotoxicity**
  - ❖ **Bacterial gene mutation assay (e.g., Ames Salmonella test)**
  - ❖ **Mammalian gene mutation assay (e.g., Mouse Lymphoma test)**
  - ❖ ***In vivo* mammalian chromosomal aberration evaluation (e.g., micronucleus formation)**



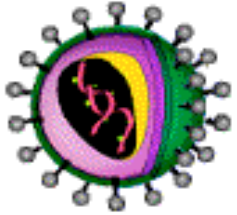


# NONCLINICAL SAFETY

## Pre-Phase I

- ❖ **Rabbit vaginal irritation model - Daily dosing for 10 days**
  - ❖ **RVI protocols vary substantially**
    - ❖ **Number of animals**
    - ❖ **Dose volumes**
    - ❖ **Depth of application**
    - ❖ **Scoring issues**
  - ❖ **Results show substantial variability**





## N9 in RVI toxicity tests:

- **Histological criteria:**
  - **Epithelial ulceration** (0-4+)
  - **Leucocyte infiltration** (0-4+)
  - **Vascular injection** (0-4+)
  - **Edema** (0-4+)
- **Composite score range:** 0-16
- **Traditional scoring:**
  - **Acceptable** 0-8
  - **Borderline** 9-10
  - **Unacceptable** 11-16
- **N9 generally scores 8-12**
- **Same agent can score 3-12**

From T. Moench





## Primate Model

- **Similar anatomy and physiology to humans**
- **Not confounded by concurrent STIs or compliance issues**
- **Standardized protocol –**
  - **Atraumatic application once daily for 4 days**
- **Assess safety throughout menstrual cycle**
- **Endpoints:**
  - **Colposcopic changes**
  - **Histology on biopsies**
  - **Microflora**
  - **pH**

**Adapted from T. Moench**





## Primate Model

- **N9 toxicity detected** (Patton et al., Am J Obs Gyn'99)
  - **Single dose yields observable changes**
  - **Toxicity progressive with repeated doses**
  
- **Columnar epithelium exposure limited**
- **Relatively small number of animals**
- **No simulation of sexual activity**





## Vaginal irritation caused by N-9

- **Studies to identify a N-9 exposure level capable of producing moderate vaginal inflammation in rhesus monkeys were undertaken.**
  - **Colposcopy and cervicovaginal biopsies were used to assess pathology.**
- **1 ml Gynol-II gel was used for all studies (37.5 mg N-9).**
- **Four animals exposed to 1 ml of N-9 gel twice a week for 2 weeks did not develop lesions.**
- **Six mature female macaques exposed to N-9 gel twice a day for 28 days developed vaginal irritation.**
- **The changes consisted of multifocal erythema and edema.**

From C. Miller





# NONCLINICAL SAFETY: From concept through clinical trial

## Discovery/early development

### *IN VITRO:*

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## Pre- phase I safety

### *IN VITRO:*

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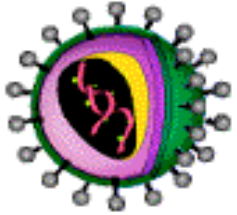
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  - ◆ Absorption
- ◆ Hypersensitivity

## Pre-/Concurrent Phase II/III

### *IN VIVO:*

- ◆ General toxicity
- ◆ Reproductive toxicity (segments II & III)
- ◆ Carcinogenicity





# NONCLINICAL SAFETY

## Pre-/Concurrent with Phase II/III

- ❖ **Reproductive toxicity**
  - ❖ **Segment I: to evaluate potential toxicity to fertility and early embryonic development**
  - ❖ **Segment II: to assess embryo-fetal development**
  - ❖ **Segment III: to evaluate the effects of the drug on peri- and postnatal development**

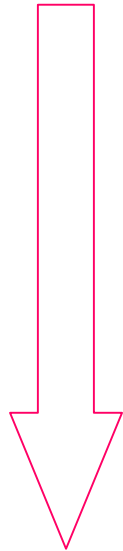




# Assessing Microbicide Safety in Clinical Studies: Range of Measures

Low-Tech  
Low-Cost

Findings  
Symptoms



Neutrophils in vaginal or cervical specimens

Vaginal flora by Gram stain

Visual inspection

Naked eye or hand lens

Colposcopy

Assessment of vaginal flora by quantitative culture

Hi-Tech  
Hi-Cost

Detection of immune mediators

**From S. Hillier**





## Colposcopy: Current state of knowledge

- Most studies of N-9 show colposcopic findings
- Women not using products have colposcopic findings
- Colposcopy can detect findings not seen with the naked eye
- Colposcopic findings do not correlate with symptoms and biopsy findings
- We do not know whether colposcopically detected findings predispose to HIV/STDs

**From C. Mauck**





## CLINICAL Challenges

- **Should safety be assessed:**
  - **minutes after application?**
  - **immediately following intercourse?**
  - **after 24 hours?**
- **Should safety be assessed:**
  - **with condoms?**
  - **throughout menstrual cycle?**
  - **in the context of (other) contraceptive use, including devices?**
- **Should safety be assessed in women representing all age groups engaging in sexual activity?**





## CLINICAL Challenges

- **Should safety be assessed in the upper reproductive tract?**
  - **Uptake of vaginal fluid into the peritoneum**
- **What is/are the most appropriate method(s) for assessing safety?**
  - **Symptoms**
  - **Naked eye exams**
  - **Colposcopy**
  - **Inflammatory markers**
  - **Other?**
- **“Ultimate safety issue is whether a microbicide protects against HIV.” – S. Hillier**





## Challenges & Opportunities

- **What is the relevance of these conventional & newly developed models/methods to the real world use of microbicide products?**
- **Once established by correlation with clinical outcome, will the predictive value of a particular model/method be dependent on mechanism of action or nature of microbicide e.g., chemical class?**
- **Standardization of some methods/models is needed:**
  - **To clarify interpretation of results**
  - **To permit more meaningful comparisons**
- **Iterative refinement of models/methods based on results from clinical trials and improved understanding of HIV transmission will help advance 2<sup>nd</sup> and 3<sup>rd</sup> generation microbicides.**

