Contribution of BRACVAM and Validations on going

## Octavio Presgrave

BraCVAM/FIOCRUZ

BACKGROUND

- **1998 – Law 9,605**

  - define penalties, including prision, to whom makes cruelty against animals

  - establishes the same penalties to whom perform painful experiments when there is an alternative method

- **2008 – Law 11,794**

  - regulates animal use in experimentation and education

  - creates CONCEA (National Council for the Control of Animal Experimentation)

  - every institution that uses animal must be registered

  - obliges all institutions that use animals to implement Ethics Committee on Animal Use (CEUA)

- **2009 – Decree 6,889**

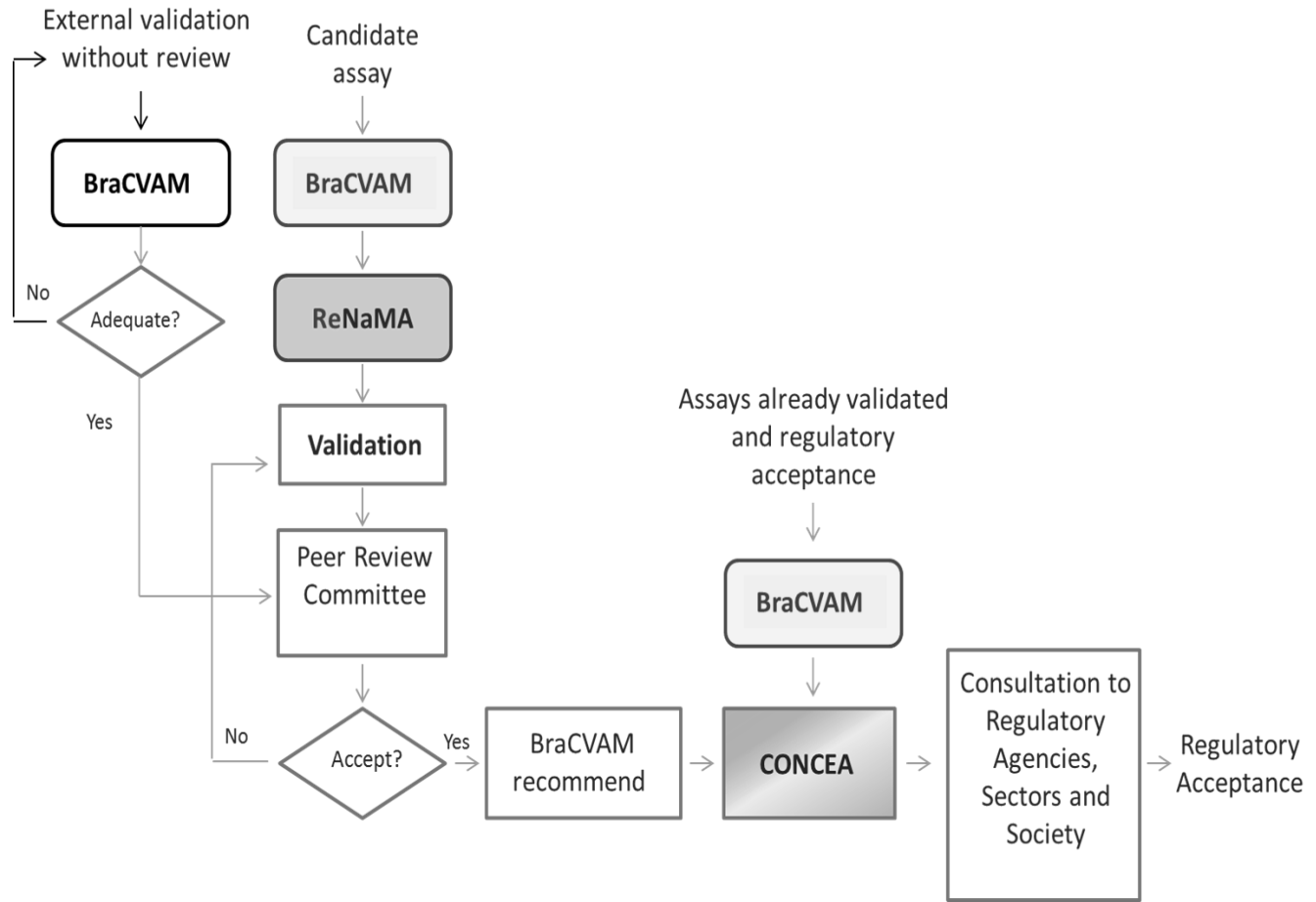 - states that an alternative method should be a validated and internationally accepted procedure

Validation process in Brazil…
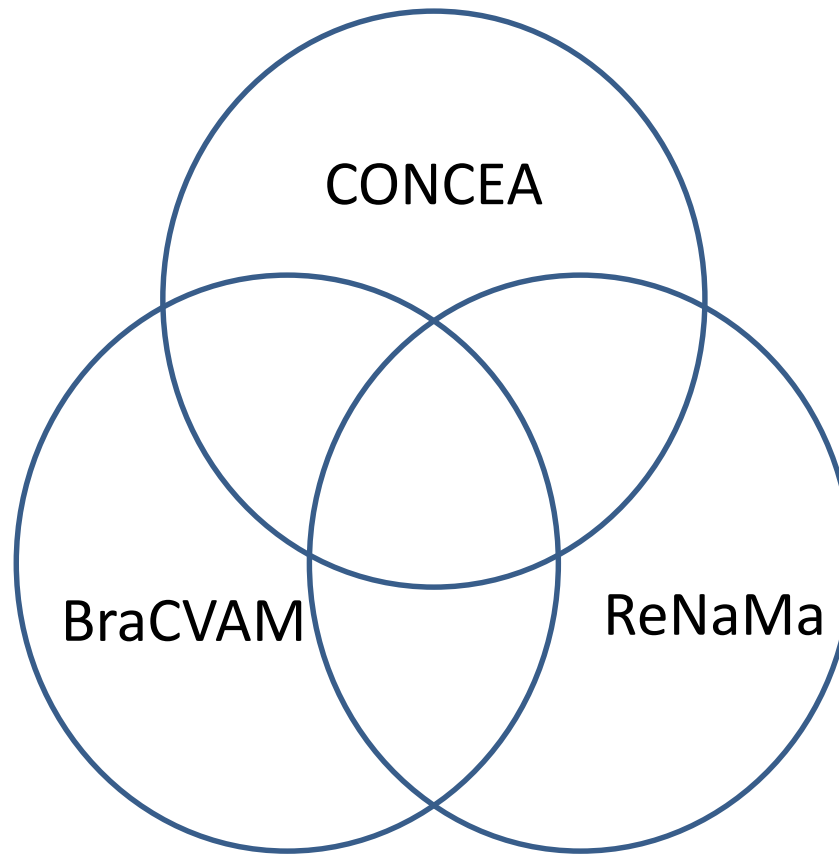
CONCEA – National Council for the Control of Animal Experimentaion
BraCVAM – Brazilian Centre for Validation of Alternative Methods
ReNaMa – National Network of Alternative Methods

External validation without review

Candidate assay

BraCVAM

Adequate?

No

Yes

BraCVAM

ReNaMA

Validation

Peer Review Committee

Accept?

No

Yes

BraCVAM recommend

Assays already validated and regulatory acceptance

BraCVAM

CONCEA

Consultation to Regulatory Agencies, Sectors and Society

Regulatory Acceptance

CONCEA

RN 18/2014
- 129
- 420
- 423
- 425
- 428
- 429
- 430
- 431
- 432
- 435
- 437
- 438
- 439
- 442A
- 442B
- 460
- 487

RN 31/2016
- 421
- 422
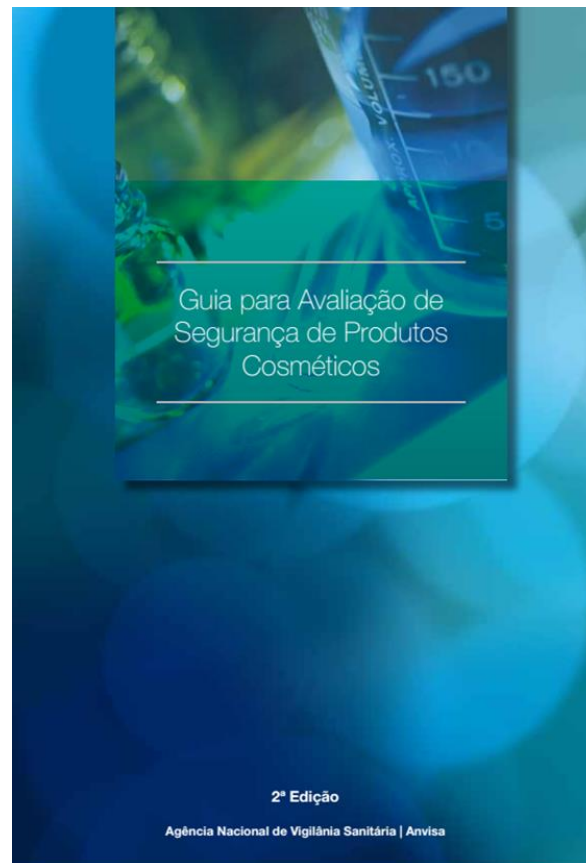- 442C
- 442D
- 491
- 492

+ BET

WHY HET-CAM?

ANVISA

- Guideline for the Safety Evaluation of Cosmetic Products

Andréa M. Nóbrega, Eloisa N. Alves, Rosaura F. Presgrave, Isabella F. Delgado

Avaliação da irritabilidade ocular induzida por ingredientes de cosméticos através do teste de Draize e dos Métodos HET-CAM e RBC

Andréa Martins da Nóbrega[1]
Eloisa Nunes Alves[2]
Rosaura de Farias Presgrave[3]
Isabella Fernandes Delgado[4]

Ensaios da membrana cório-alantoide (HET-CAM e CAM-TBS): alternativas para a avaliação toxicológica de produtos com baixo potencial de irritação ocular

Chorioallantoic membrane assays (HET-CAM and CAM-TBS): alternative tests for performing toxicological evaluation of products with low potential for ocular irritation

Amanda Gleyce Lima de OLIVEIRA[1], Ronald Santos SILVA[2], Eloisa Nunes ALVES[2], Rosaura de Farias PRESGRAVE[2], Octavio Augusto França PRESGRAVE[2], Isabella Fernandes DELGADO[3*]

POTENCIAL TÓXICO DE DENTIFRÍCIOS: AVALIAÇÃO COMPARATIVA ENTRE O TESTE DE IRRITAÇÃO DA MUCOSA ORAL EM HAMSTERS E O MÉTODO HET-CAM

TOXIC POTENTIAL OF DENTIFRICES: COMPARATIVE EVALUATION BETWEEN ORAL MUCOSAL HAMSTER IRRITATION TEST AND HET-CAM

Márcia da Conceição Corrado[1],
Eloísa Nunes Alves[1],
Octavio Augusto França Presgrave[1],
Rosaura de Farias Presgrave[1],
Rodrigo Netto Costa[2] e
Isabella Fernandes Delgado[3]*

RESUMO

Certos ensaios toxicológicos utilizados no controle de qualidade de produtos têm sido questionados por grupos defensores do bem estar animal por levarem ao sacrifício e ao sofrimento de um número significativo de animais. Além de implicações éticas, a busca por métodos alternativos é uma questão de grande relevância para os laboratórios oficiais de controle da qualidade. Neste contexto, o método HET-CAM (*Hens Egg Test-Chorion Allantoic Membra-*

Results from INCQS HET-CAM studies show that:

1) HET-CAM superestimates graduation; and

2) HET-CAM is better to identify non-irritants. If irritation is found, another assay need to be used.

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

6.   The BCOP test method can be used to identify chemicals inducing serious eye damage as defined by UN GHS, i.e. chemicals to be classified as UN GHS Category 1 (4). When used for this purpose, the BCOP test method has an overall accuracy of 79% (150/191), a false positive rate of 25% (32/126), and a false negative rate of 14% (9/65), when compared to in vivo rabbit eye test method data classified according to the UN GHS classification system (3) (see Annex 2, Table 1). When test chemicals within certain chemical (i.e., alcohols, ketones) or physical (i.e., solids) classes are excluded from the database, the BCOP test method has an overall accuracy of 85% (111/131), a false positive rate of 20% (16/81), and a false negative rate of 8% (4/50) for the UN GHS classification system (3). The potential shortcomings of the BCOP test method when used to identify chemicals inducing serious eye damage (UN GHS Category 1) are based on the high false positive rates for alcohols and ketones and the high false negative rate for solids observed in the validation database (1)(2)(3). However, since not all alcohols and ketones are over-predicted by the BCOP test method and some are correctly predicted as UN GHS Category 1, these two organic functional groups are not considered to be out of the applicability domain of the test method. It is up to the user of this Test Guideline to decide if a possible over-prediction of an alcohol or ketone can be accepted or if further testing should be performed in a weight-of-evidence approach. Regarding the false negative rates for solids, it should be noted that solids may lead to variable and extreme exposure

 Moreover, BCOP false negatives in this context are not critical since all test chemicals that produce an 3 < IVIS ≤ 55 would be subsequently tested with other adequately validated in vitro tests, or as a last option in rabbits, depending on regulatory requirements, using a sequential testing strategy in a weight-of-evidence approach.

applicability domain of the test method. Investigators could consider using this test method for all types of chemicals, whereby an IVIS > 55 should be accepted as indicative of a response inducing serious eye damage that should be classified as UN GHS Category 1 without further testing. However, as already mentioned, positive results obtained with alcohols or ketones should be interpreted cautiously due to potential over-prediction.

International Workshop on the HET-CAM Assay
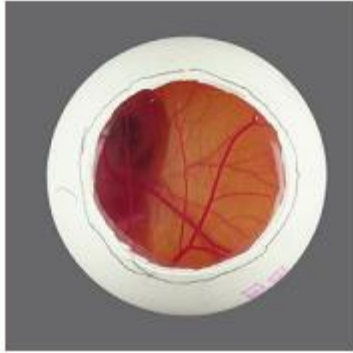
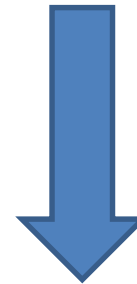29. and 30. October 2012

in cooperation with

epaa
The European Partnership
for Alternative Approaches to Animal Testing

SeCAM

The Federal Institute for Risk Assessment

HIGHLIGHTS

- HET-CAM is a test that should be studied

- Good Bottom-Up strategy

- Changes in graduation of phenomena

- Complementary informations

Histology of CAM alterations

Aims of the HET-CAM Validation

- Part of a battery

- Substances

$\Rightarrow$In the future: formulations

Advantages

✓ Economic: Low investment

✓ Scientific: High possibility of having good results

✓ Educational: Training BraCVAM and ReNaMA in the validation process

✓ Regulatory: A method already accepted by ANVISA

✓ International: Visibility of BraCVAM and ReNaMA

✓ Results: Active participation of Brazil in the alternative scenarium

HET-CAM Validation

- ## Validation Manager Group (VMG)

Chair: Dr. Thomas Hartung

Co-Chairs: Dr. Octavio Presgrave and Dr. Isabella Delgado

Sponsor: Dr. Luiz Henrique Mourão

INMETRO: Dr. Luciene Balottin

INCQS: Dr. Cristiane Caldeira

LNBio: Dr. Eduardo Pagani

Expert: Dr. Manfred Liebsch

- ## Observers

ECVAM – Dr. João Barroso

OECD – Dr. Anne Gourmelon

ANVISA – Dr. Joel Majerowicz

MAPA – Dr. Wanderson Silva

ICCVAM – Dr. Warren Casey

- ## Statistics

Dr. Sebatian Hoffmann

Dr. Wlamir Moura

HET-CAM Validation step-by-step

- Preliminary phase
  - VMG Meeting √
  - Trainning of laboratories √
  - Choosing of substances – 6 substances √
  - Testing/analysis √

- Second phase
  - Choosing of substances – 35 substances ←
  - Validation
  - Testing/Analysis
  - BraCVAM Advisor Committee
  - *Final report*

Challenges

- Integrate sectors, Ministries, NGOs

- Consolidate the validation process

- Consolidate BraCVAM activities

Thank you!!!