

# Safety assessment of cosmetics with no animal testing

## 2nd Pan-American

Conference for Alternative Methods, 23/08

Vanessa Rocha, PhD

Natura Innovation, Brazil



# Natura and Alternative Methods development in Brazil

## 2nd Pan-American

Conference for Alternative Methods, 23/08

Vanessa Rocha, PhD

Natura Innovation, Brazil



# NATURA & CO

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Natura Co-Founders  
London, 2017

# ANIMAL TESTING BAN AT NATURA

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Investments on innovation, infra structure and people



## **20 partnerships**

Universities and Research Institutes – skin models, allergy, genotoxicity, OMICS

**>67 in vitro models on safety and efficacy**

**20 patents on natural ingredients**

**>8 scientific papers, 21 posters on conferences**

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**2003**

**2018**

# Working Together

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Universities and  
Research Centers  
Validation Center

Trade Associations  
on  
Brazil and  
South America

RENAMA  
Networking on  
Alternative  
Methods  
MCTIC

# Working Together

Workshops, Symposiums, and Conferences

2007 – Workshop at Natura Cajamar

2010 – Bracvam publication



**COLAMA 2012**

**Alternativas Brasil**  
WORKSHOP INTERNACIONAL EM MÉTODOS ALTERNATIVOS AO USO DE ANIMAIS, COM ÊNFASE EM ALTERNATIVAS EMERGENTES

**UNIFESP**  
UNIVERSIDADE FEDERAL DE SÃO PAULO

**Fitocosmética Sustentable**  
*1º Simposio Internacional de Fitocosmética Sustentable*  
Buenos Aires, 26 y 29 de septiembre de 2017

**World Toxicologic Pathology Congress**  
April 21st to 26th 2018  
University of São Paulo, São Paulo/SP - Brazil

**2nd PAN-AMERICAN Conference for Alternative Methods**  
August 23-24, 2018  
Rio de Janeiro

**natura**

**AAT** **45 ANOS** **IN** **natura** **University of Windsor**

# Working Together

## Publications

Toxicology in Vitro xxx (2012) xxx-xxx



Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



Universities,  
Research Centers,  
Validation Center

Skin Irritation  
3D models  
2012

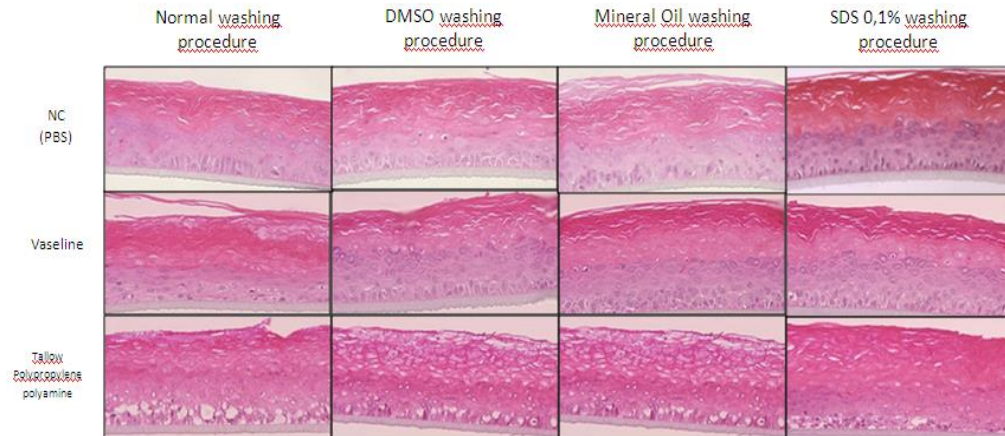
Improved procedures for *in vitro* skin irritation testing of sticky and greasy natural botanicals

J. Molinari<sup>a,\*</sup>, C. Eskes<sup>c</sup>, E. Andres<sup>a</sup>, N. Remoué<sup>a</sup>, V.M. Sá-Rocha<sup>b</sup>, S.P. Hurtado<sup>b</sup>, C. Barrichello<sup>a</sup>

<sup>a</sup>Natura Innovation et développement de produits, Paris, France

<sup>b</sup>Natura Inovação e Tecnologia de Produtos, Cajamar, São Paulo, Brazil

<sup>c</sup>SeCAM, Agno, Switzerland





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## Publications

Universities,  
Research Centers,  
Validation Center

BRACVAM  
Legislation



Regulatory Toxicology and Pharmacology

Volume 62, Issue 2, March 2012, Pages 393-403



Workshop Report

Regulatory assessment of *in vitro* skin corrosion and irritation data within the European framework: Workshop recommendations

Chantra Eskes<sup>a,\*,</sup> Véronique Detappe<sup>b,</sup> Herman Koeter<sup>c,</sup> Joachim Kreysa<sup>d,</sup> Manfred Liebsch<sup>e,</sup> Valérie Zuang<sup>d,</sup> Patric Amcoff<sup>f,</sup> João Barroso<sup>d,</sup> José Cotovio<sup>g,</sup> Robert Guest<sup>h,</sup> Martina Hermann<sup>i,</sup> Sebastian Hoffmann<sup>i,</sup> Philippe Masson<sup>k,</sup> Nathalie Alépée<sup>q,</sup> Luis Alfonso Arce<sup>l,</sup> Beat Brüscherweiler<sup>m,</sup> Tiziana Catone<sup>n,</sup> Rostislav Cihak<sup>o</sup> ... Olivier Depallens<sup>b</sup>

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SHORT COMMUNICATION

## Proposal for a Brazilian Centre on Alternative Test Methods

Chantra Eskes<sup>1,</sup> Vanessa de Moura Sá-Rocha<sup>2,</sup> Jadir Nunes<sup>3,</sup> Octavio Presgrave<sup>4,</sup> Dermeval de Carvalho<sup>5,</sup> Philippe Masson<sup>6,</sup> Ekaterina Rivera<sup>7,</sup> Sandra Coecke<sup>8,</sup> Joachim Kreysa<sup>8</sup> and Thomas Hartung<sup>9</sup>

<sup>1</sup>Independent Consultant, Ispra, Italy; <sup>2</sup>Natura, Cajamar, SP, Brazil; <sup>3</sup>Brazilian Association of Cosmetology, São Paulo, SP, Brazil; <sup>4</sup>National Institute of Quality Control in Health (INCQS), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil; <sup>5</sup>Biotox, Ribeirão Preto, SP, Brazil; <sup>6</sup>EVIC International, Paris, France; <sup>7</sup>Biological Science Institute, Federal University of Goiás, Brazil; <sup>8</sup>ECVAM, In Vitro Methods Unit, Institute for Health and Consumers Protection, European Commission Joint Research Center, Ispra, Italy; <sup>9</sup>Johns Hopkins University, Baltimore, USA and University of Konstanz, Germany

# Working Together

## Publications



Mutation Research/Genetic Toxicology and  
Environmental Mutagenesis

Volume 743, Issues 1–2, 18 March 2012, Pages 36–41



Universities,  
Research Centers,  
Validation Center

Successful micronucleus testing with the EPI/001 3D  
reconstructed epidermis model: Preliminary findings

E. Andres <sup>a</sup>  , J. Molinari <sup>a</sup>, N. Remoué <sup>a</sup>, V.M. Sá-Rocha <sup>b</sup>, C. Barrichello <sup>a</sup>, S.P. Hurtado <sup>b</sup>

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<https://doi.org/10.1016/j.mrgentox.2011.12.026>

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Micronucleus (Genotox)  
on 3D Skin model  
2012

# Working Together

## Publications



Toxicology in Vitro

Volume 27, Issue 4, June 2013, Pages 1220–1225



Givaudan<sup>®</sup>

The sensitivity of the KeratinoSens<sup>™</sup> assay to evaluate plant extracts: A pilot study

Eric Andres<sup>a</sup>, Vanessa M. Sá-Rocha<sup>b</sup>, Carla Barrichello<sup>a</sup>, Tina Haupt<sup>c</sup>, Graham Ellis<sup>d</sup>, Andreas Natsch<sup>a</sup>

Research Article

Journal of Applied Toxicology<sup>™</sup>

Received: 9 February 2015,

Revised: 6 April 2015,

Accepted: 13 April 2015

Published online in Wiley Online Libr

(wileyonlinelibrary.com) DOI 10.1002/jat.3172

**Probabilistic hazard assessment for skin sensitization potency by dose–response modeling using feature elimination instead of quantitative structure–activity relationships**

Thomas Luechtefeld<sup>a†</sup>, Alexandra Maertens<sup>a†</sup>, James M. McKim<sup>b</sup>, Thomas Hartung<sup>a,c,\*</sup>, Andre Kleensang<sup>a</sup> and Vanessa Sá-Rocha<sup>a,d</sup>



Toxicology in Vitro

Volume 30, Issue 1, Part B, 25 December 2015, Pages 318–324



Skin sensitizer identification by IL-8 secretion and CD86 expression on THP-1 cells

Carolina Bellini Parise<sup>a</sup>, Vanessa Moura Sá-Rocha<sup>b</sup>, Jane Zveiter Moraes<sup>a</sup>

Universities,  
Research Centers,  
Validation Center

Sensitization



# Working Together

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\_Inducing the development of alternative methods with suppliers;

\_Develop new technologies (OMICS, Bioprinting) with suppliers and Universities

\_Use new approaches on safety assessment of cosmetic ingredients

# Visit our posters - Friday


## #96



### Gene expression screening to identify activation profiles of sensitizing pathways in skin after exposition to cosmetic ingredients

Daniela Zimbari<sup>1</sup>, Cinzia Ferrari<sup>1</sup>, Caroline Stuker<sup>1</sup>, Juliana Regada<sup>1</sup>, Helen Andrade Arcuri<sup>1</sup>, Vanessa Rocha<sup>1</sup>  
<sup>1</sup>Natura Innovation and Product Technology, Cajamar, São Paulo, Brazil

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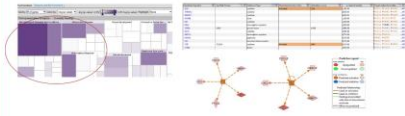
#### Abstract

Vegetable lipid-based ingredients have been exploited due to a great potential to promote new alternatives for skin. However, many ingredients have presented as a complex mixture of molecular interactions which when in combination with other ingredients in a topical formulation or when in contact with the skin, can be able to initiate some unexpected responses. In this study, a gene expression-based assay of 43 genes carefully selected due to their involvement in inflammatory and sensitizing pathways were performed after treatment of skin fragments with vegetable lipids, comparing to reference molecules as hexanoic acid and SDS. The gene expression profile of each sample allowed the identification of the major influence of two inflammatory pathways as IL-6 and interleukin extrinsic signaling with the combined up-regulation of HSPA1, MMP1, CXCL8, PTGS2, TNF, CXCL1 and CXCL2 genes to indicate these can be associated with adverse effects identified after the conduction of a clinical panel composed of 110 volunteers. Therefore, these data allowed to identify differential profiles of pathways' activation and inhibition by samples set and to orientate the search for a physiological response by a complementary metabolomic assay. In conclusion, this new *ex vivo* toxicological methodology proved to be useful in the correlation with some clinical features for a better understand of the complexity of the human skin exposed to cosmetics ingredients and, in this way, supporting to reduce the use of animal tests in cosmetic area.

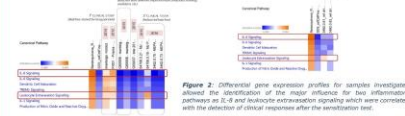
**Keywords:** vegetable ingredients, inflammatory pathways, gene expression.

#### Results and Discussion

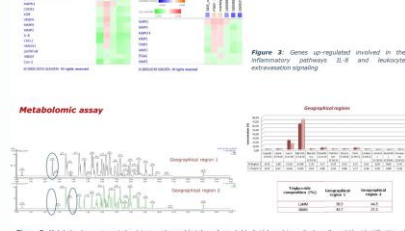
##### Gene expression screening



**Figure 1:** Fold of 43 genes involved in inflammatory pathways were defined based on previous large-scale studies that had shown differential profiles of activation for samples from different vegetable lipid-based ingredients and compared with hexanoic acid.



**Figure 2:** Differential gene expression profiles for samples investigated showed the identification of the major influence for two inflammatory pathways as IL-6 and interleukin extrinsic signaling which were combined with the detection of signal responses after the sensitization test.



**Figure 3:** Metabolomic assay carried out in samples and batches of vegetable lipid-based ingredients allowed the identification of different profiles of metabolites and fatty acids composition.

#### Methods

##### Ex vivo skin explant model:

Human skin explants from healthy donors were treated with "lipid" of vegetable lipid-based ingredients and reference molecules as hexanoic acid and SDS with a subsequent incubation for 72h at 37°C in 3% CO<sub>2</sub>, followed by RNA extraction and cDNA synthesis.

##### Gene expression screening:

A real time gene expression-based assay of 43 genes involved in inflammatory and sensitizing pathways were performed. Data analysis were carried out with Expression Suite Software v1.3.3 (Thermo Fisher Scientific, USA) and a threshold of 1.3 was considered. Significant modulation may further analyzed using Ingenuity Pathway Analysis (Qiagen Bioinformatics, USA) to determine the differential profile of activation of inflammatory pathways. Statistical evaluation was made using a t-test followed by a Benjamini-Hochberg (False Discovery rate). P-values below 0.05 were considered significant.

##### Metabolomic assay:

The lipidic extracts were analytically weighed and dissolved in THF to obtain a 15 mg mL<sup>-1</sup> concentration, which was used for the chromatography. A Waters HPLC OD Ultra Performance Liquid Chromatography coupled with Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometer (UPLC-ESI-QToF-MS) was utilized for analysis of the lipidic extracts. For UPLC-MS analysis, 2.5 µL of the extract was injected in HPLC on a reverse-phase analytical column Acuity (UPLC BEH C18 2.1 µm particles, 2.1 x 100 mm, 0.1 µm) at a flow rate of 0.60 mL min<sup>-1</sup> using a 0.1% formic acid (A) to acetonitrile (B) gradient (0.00-10.00 min, linear gradient from 20% to 80% A; 10.00-12.50 min, 0% A; 12.50-12.51 min, linear gradient 0% A to 95% A; 12.51-15.00 min, 95% A). Ionization parameters were as follows: capillary voltage of 3 kV; cone voltage of 15 V and a source temperature of 120 °C. Data acquisition in the reverse mode was performed by MS scanning a second analysis through the m/z range of 100-1000 Da and the data was analyzed using Masslynx software version 4.1 (Waters Corporation, Milford, USA).

##### Clinical panel:

110 volunteers were submitted to a sensitization protocol (MPT) after exposition to different samples and batches of vegetable lipid-based ingredients.

#### Conclusions

This alternative methodology based on *ex vivo* models and gene expression profile allowed a better understanding of the behavior of vegetable lipid ingredients applied on the skin in the modulation of potential skin sensitization signals. In addition, has come to light the involvement of specific chemical metabolites which would be responsible for those *ex vivo* and clinical responses, in order to improve the development process of extraction and characterization, as well as quality control methods for the storage of those vegetable ingredients.

## #98



### The influence of UVA protection on skin during sun exposure

Juliana Carvalhães Lago<sup>1</sup>, Sílvia Stuchi Maria-Engler<sup>2</sup>, Érica Aparecida de Oliveira<sup>2</sup>  
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#### Abstract

The skin is the largest organ of the human body and forms a physical barrier to the environment, protecting other organs against damage from ultraviolet radiation; determining the biological events that occur in skin after UV radiation exposure is essential to photoprotection studies. In this study, we evaluated the protection efficiency of sunscreens, considering their UVA protection against UV radiation, in specific biological markers of the skin, using a 3D skin model. We evaluated 5 groups: CN (non irradiated skin), CP (irradiated skin without treatment) e samples A1, A2, A3 (sunscreens containing UVB/UVA filters in the ratio 1:6; 1:2; 1:1 respectively). The samples were topically applied on the skin, followed by radiation exposure. All samples showed similar results, except CN. In treated groups, A1 presented higher number of markers cells, although A2 and A3 also presented them, but smaller number. Cyclosterin 10 and 14 indirectly demonstrated that there are no cytotoxic effects with no difference between the formulations. To evaluate keratinocytes differentiation, Flagelin expression was analyzed and both gene and protein level showed reduction of its expression on the CP with preserved result in treated samples. The CP presented higher level of p53 expression while treated samples showed a slight marking of nuclei, very similar to CN. The evaluation of 8-oxo-dG demonstrated higher protection profile for samples A2 and A3 against oxidative damage, but still with more damage than CN. Sun radiation significantly increased IL6 and IL8 release when compared with CN. The treated groups demonstrated expressive decreasing of IL6 and IL8 release, related to CP. The effect of treatments on MMP1 and MMP9 synthesis were evaluated and all irradiated groups presented increase of MMP9 synthesis than CN, although none had significant decrease with treatment. Regarding synthesis of MMP2, the A1 sample demonstrated higher concentration compared with CP and CN, while the others samples were only significantly different from CN.

**KEYWORDS:** Aging, Photobiology, UVR/UVB radiation

#### Results and Discussion

##### Histologic analysis



**Figure 1:** Keratinocytes and p53 expression after irradiation.



**Figure 2:** MMP9 and MMP2 expression after irradiation.



**Figure 3:** IL6 and IL8 release.



**Figure 4:** MMP9 and MMP2 synthesis.

#### Methods

##### Photoprotector samples

We evaluated 03 (three) photoprotectors products, with different concentration of UVB/UVA filter: A1 (1:6); A2 (1:2); A3 (1:1) besides Control irradiated with no photoprotection and no irradiation Control.

##### Gene expression assay

20 mg of photoprotector samples were applied on 30 equivalent skin, 30 minutes before exposition. The 3D skin was irradiated with 13 J/cm<sup>2</sup> using sun simulator. After 24 hours, the RNA was extracted and 96 gene expression real time PCR (RT-PCR) assay was performed.

##### Protein quantification assay

20 mg of photoprotector samples were applied on 30 equivalent skin, 30 minutes before exposition. The 3D skin was irradiated with 13 J/cm<sup>2</sup> using sun simulator. After 24 hours, the histologic analysis assay was performed with specific biomarkers.



**Figure 5:** Percentage of cells in different states.

#### Conclusion


According with the results, it was possible to identify radiation effect using 3D equivalent skin model. All three samples showed photoprotective characteristics, however, samples A2 and A3 presented more efficacy regarding the preservation of epidermis and dermis layer, decrease of DNA damage by raising 8-oxo-dG and p53, decrease of IL6 and IL8 synthesis. In addition, we observed reduced sun protection relative to sample A1, considering histologic biomarkers. It was not possible to determine efficacy of photoprotections products with higher UVA filters using gene expression profile assay. However, the use of gene expression assay allow us identify the biological mechanisms modulated during sun exposition and the gene expression behavior corresponding.

#### References

1. Kawanishi S, Inoue Y, Kawanishi S. A review of *in vitro* methods for identifying chemical sensitizers combining protein-ligand with ARE/ERK-mediated gene expression. *Toxicology and Applied Pharmacology*. 2012; 257(1):19-32.  
 2. Carvalhães Lago J, Stuchi Maria-Engler S, de Oliveira É. The influence and use of skin exposure: potential and treatment for dermatological diseases. *Journal of Dermatology Research and Clinical Practice*. 2011; 1(1):1-10.  
 3. Barone CC, Barone HR, Calhoun KR. Antioxidant vegetable oils of fat: Food Technology and Quality Control. *Antioxidants* (2021) Review from ON-Theme-Related-Label-Description/Description-Time-Title (Epub) 2021 September 17. <https://doi.org/10.3390/antiox10091634>, 2020.


# Visit our posters - Friday


## #136



**Non-animal testing strategy for the safety assessment of a new botanical extract developed for cosmetic application – A case study**

Marcelo Vieira<sup>1</sup>; Mayara Paludetti<sup>1</sup>; Cyro Zacarias<sup>1</sup>; Cintia Paes<sup>1</sup>; Vitor Fonseca<sup>1</sup>; Caroline Bianchi<sup>1</sup>; Bianca Rocha<sup>1</sup>; Vanessa Rocha<sup>1</sup>  
<sup>1</sup>Natura Innovation and Technology of Products  
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### Introduction

Since 2006 Natura does not use animal testing for the development of their products and ingredients. Although in vitro methods are increasingly replacing animal tests for this purpose, many parameters are still uncovered and some gaps need to be addressed by other alternative strategies. In this context, a combination of in vitro tests, in silico predictions and read-across approach were used to evaluate the safety use of this material.

**Objective:** The aim of the present study was to define the safety use of a new aromatic extract for cosmetic application based exclusively on a non-animal testing strategy.


### Methods



**Literature**

1. Toxicological data of the molecule


Using scientific bases, the toxicological data of the compound were collected in order to evaluate the safety for each endpoint. Data can also be found for read-across.



**In Silico**

2. Predictions in softwares


The in silico evaluation consists of computational simulations to predict the hazard of a molecule. It was used softwares that are based on QSAR models and structural alerts.



**In Vitro**

3. In vitro safety tests

Through the toxicological data collected from the literature and all the ingredients that form part of the raw material composition, C and D were defined. The in vitro tests to be performed.



**Safety Assessment**

4. Calculation of safe concentration

Through all the obtained results, the safe use concentration of raw material by category of product was calculated. It was used the Threshold of Toxicological Concern (TTC) and the Dermal Sensitization Threshold (DST).

### Results and Discussion

It was performed cytotoxicity (Figure 1) and phototoxicity (Figure 2) assays, both in 3T3 fibroblasts strain. It doesn't present cytotoxic activity and IC50 value at the concentrations tested. It was possible to observe a small drop in cell viability at the highest concentrations. In phototoxicity assays, although it showed a decrease in cell viability for both the irradiated sample and the non-irradiated sample, under the conditions tested,  $PIF = 1$  was considered non-phototoxic.

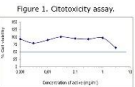


Figure 1. Cytotoxicity assay.

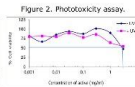


Figure 2. Phototoxicity assay.

For assessment of the genotoxic potential of the extract, it was performed the Bacterial Reverse Gene Mutation test (Ames test) and Mammalian Cell Micronucleus test. During the Ames test, under the test conditions, the sample did not induce frame-shift mutation or base-pair substitution in the genome of *Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537, at the concentrations tested in both the presence and in the absence of metabolic activation. With the preliminary results of Micronucleus assay, the sample showed no genotoxic effects.

### Conclusion

The present case-study demonstrated the challenges on estimate the hazard of botanical ingredients using in vitro assays, and offer ways to evaluate the safety use of the material based on exposure scenario, read across and in silico predictions, to overcome the lack of toxicological information.

### References

1. OECD. Guidelines for the Testing of Chemicals. Test No. 473. In vitro mammalian erythrocyte micronucleus test. Paris, 2001.

2. OECD. Guidelines for the Testing of Chemicals. Test No. 474. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.

3. OECD. Guidelines for the Testing of Chemicals. Test No. 475. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.

4. OECD. Guidelines for the Testing of Chemicals. Test No. 476. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.

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8. OECD. Guidelines for the Testing of Chemicals. Test No. 480. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.

9. OECD. Guidelines for the Testing of Chemicals. Test No. 481. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.

10. OECD. Guidelines for the Testing of Chemicals. Test No. 482. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.


11. OECD. Guidelines for the Testing of Chemicals. Test No. 483. In vitro mammalian lymphocyte micronucleus test. Paris, 2001.


## #137



**Rationale for safety assessment of an aromatic extract developed for cosmetic application based on non-animal testing strategy.**

Cintia Ferreira Paes, Alina Arselmini, Cyro Zacarias, Caroline Bianchi, Bianca Rocha, Vitor Bortolazzo, Marcelo Vieira, Mayara Paludetti, Vanessa Rocha  
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 Contact: cintiapaes@natura.net





### Introduction

The absence of toxic potential of a cosmetic ingredient can be evidenced based on a series of alternative methods and makes part of the hazard identification. With the ban on animal testing for evaluation of cosmetic products in Europe and other countries, in vitro methods, Dermal Sensitization Threshold (DST) rationale and a confirmatory clinical assays were used to analyze safety for cosmetic application of a new aromatic extract obtained from the flowers of a plant from the Asteraceae family.

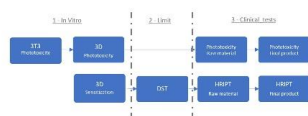
### Results and Discussion

In the chemical characterization, the extract was found to be composed of some sesquiterpenes (like bergamotene, nerolidol and spathulenol), monoterpene (such as verbenol and epi-linalool), and also hexadecanoic and linolenic acids. In lower levels, there were also some sesquiterpene lactones and polyacetylenes, substances of concern that are already described in the scientific literature for this family of plants. The extract did not show any mutagenic activity in the Ames Test. A phototoxic potential was verified in the 3T3 phototoxicity test, but in a tridimensional model, this potential was not confirmed. Regarding the sensitization potential, the extract was found to be a sensitizer in a tridimensional in vitro model. The DST applies the same principles as those used to develop the TTC to define a level of skin exposure where there is no appreciable risk of skin sensitization to an untested chemical. This rationale is applied and endorsed by the Research Institute of Fragrance Materials (RIFM).

### Methods

Considering the chemical characteristics of Asteraceae family, the strategy to guarantee the safety of cosmetics ingredients was:

- To conduct in vitro studies;
- To determine the limit for exposure of the allergens;
- After its approval, conduct the clinical studies.



The flowchart illustrates the safety assessment process. It starts with '1. In Vitro' (3T3 Cytotoxicity, 3T3 Phototoxicity, 3D Sensitization) leading to '2. Test' (Toxicology Test Product, Sensitization Test Product, DST, HRIPT Eye Product, HRIPT Test Product). This leads to '3. Clinical Study' (Toxicology Test Product, Sensitization Test Product).

### References

Schiff R, de Aji R, Roberts DW, Lallo J. Estimates of the Dermal Sensitization Threshold (DST) approach to inorganic chemicals classified as reactive. Regulatory Toxicology and Pharmacology 22 (2): 694-701 (2000).

SCCS (SAC). Review of the SCCS Review of Guidelines for the Review of Cosmetic Ingredients and their Safety Evaluation by October 2015. AQLAM, ed. Criteria for the Research Institute of Fragrance Materials, Inc. (RIFM) safety evaluation process for Fragrance Ingredients, Food and Chemical Toxicology, 2015.

# Working Together

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Trade Associations  
on  
Brazil and  
South America

ABIHPEC 2015 Creates a working group on Alternative to Animal Testing to:

- \_ Understand gaps and needs for cosmetic companies to attend Conceia normatives;
- \_ Contribute for the scientific progress with training, Workshops for companies.



L'ORÉAL



AVON



STIFEL



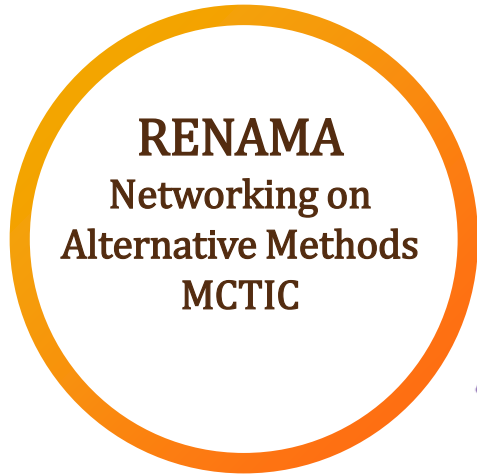
Givaudan<sup>o</sup>



Council of Latin American Cosmetic, Personal Care and Home Care Industries

# Working Together

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\_Associated Laboratory

\_LN BIO collaboration: eye irritation project (poster section) and Premasul training course



\_Inmetro collaboration: Medida Certa project (interlaboratory running of cytotoxicity and phototoxicity in partnership with Natura, Inmetro, In Vitro Cells (Alergisa), Kosmoscience and Chemyunion).



\_Natura and USP represents the associated laboratories



# Working Together

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## I MEETING OF RENAMA'S ASSOCIATED LABORATORIES Rio de Janeiro, 08/22/18

I ENCONTRO DOS LABORATORIOS  
ASSOCIADOS À REDE NACIONAL DE  
MÉTODOS ALTERNATIVOS



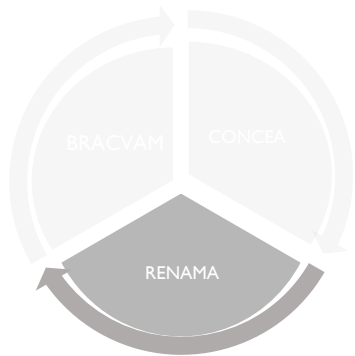
CONHECER,  
COLABORAR  
CONVERGIR

Rio de Janeiro  
22.08.2018



30 participants

# MAPPING WHAT WAS IMPLEMENTED



24 Methods  
(Normative  
18th and  
31st)

<b>Skin Irritation and Corrosion</b>	
OECD 430 - In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)	✓
OECD 431 - In vitro Skin Corrosion: Reconstructed human epidermis (RHE) test method	✓
OECD 435 - In Vitro Membrane Barrier Test Method for Skin Corrosion	X
OECD 439 - In Vitro Skin Irritation Reconstructed Human Epidermis Test Method	✓
<b>Ocular Irritation and Corrosion</b>	
OECD 437 - Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants	✓
OECD 438 - Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants	X
OECD 460 - Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants	✓
OECD 491 - Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Inducing Moderate Eye Damage	✓
OECD 492 - Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification	✓
<b>Phototoxicity</b>	
OECD 432 - In Vitro 3T3 NRU Phototoxicity Test	✓
<b>Skin Absorption</b>	
OECD 428 - Skin Absorption: In Vitro Method	✓
<b>Skin Sensitization</b>	
OECD 429 - Skin Sensitisation Local Lymph Node Assay	
OECD 442A - Skin Sensitization Local Lymph Node Assay: DA (non-radioactive)	X
OECD 442B - Skin Sensitization Local Lymph Node Assay: BrdU-ELISA (non-radioactive)	✓
OECD 442C - In Chemico Skin Sensitisation Direct Peptide Reactivity Assay (DPRA)	✓
OECD 442D - In Vitro Skin Sensitisation ARE-Nrf2 Luciferase Test Method	X
<b>Genotoxicity</b>	
OECD 487 - In Vitro Mammalian Cell Micronucleus Test	✓
<b>Acute Toxicity</b>	
OECD 420 - Acute Oral Toxicity - Fixed Dose Procedure	✓
OECD 423 - Acute Oral toxicity - Acute Toxic Class Method	✓
OECD 425 - Acute Oral Toxicity: Up-and-Down Procedure	X
OECD 129	✓
<b>Reproductive Toxicity</b>	
OECD 421 - Reproduction/Developmental Toxicity Screening Test	X
OECD 422 - Combined Repeated Dose Toxicity Study with the reproduction/Developmental Toxicity Screening Test	X
<b>Pyrogenic Contamination</b>	
Bacteria Endotoxin Test - Brazilian Pharmacopeia	X

# Working Together

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I MEETING OF RENAMA´S ASSOCIATED LABORATORIES  
Rio de Janeiro, 08/22/18

Outputs:

1. Impact that different laws can have on the implementation of Conceia´s Normatives and use of alternative methods
2. Training and qualification
3. Big projects including international partners

# JOINT EFFORTS TO GO FURTHER

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# 2016\_Video to celebrate 10 years with no animal testing (Natura 10 anos)

Portuguese

<https://www.youtube.com/watch?v=g79MMFUXrwQ>

Espanish

<https://www.youtube.com/watch?v=52DvKQRL0u8>

English

[https://www.youtube.com/watch?v=7\\_qCR-EEZE//](https://www.youtube.com/watch?v=7_qCR-EEZE//)





# ALTERNATIVE METHODS FOR SAFETY ASSESSMENT

## Integrated testing Strategies

