Safety assessment of cosmetics with no animal testing

2nd Pan-American

Conference for Alternative Methods, 23/08

Vanessa Rocha, PhD

Natura Innovation, Brazil

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Natura and Alternative Methods development in Brazil

2nd Pan-American

Conference for Alternative Methods, 23/08

Vanessa Rocha, PhD

Natura Innovation, Brazil

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Natura Co-Founders
London, 2017

ANIMAL TESTING BAN AT NATURA

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

2003 **2018**

Working Together

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Working Together

Universities,
Research Centers,
Validation Center

Workshops, Symposiums, and Conferences

2007 – Workshop at Natura Cajamar

2010 – Bracvam publication

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Skin Irritation
3D models
2012

Publications

Toxicology in Vitro xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

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

J. Molinari [a,*], C. Eskes [c], E. Andres [a], N. Remoué [a], V.M. Sá-Rocha [b], S.P. Hurtado [b], C. Barrichello [a]

[a] *Natura Innovation et développement de produits, Paris, France*
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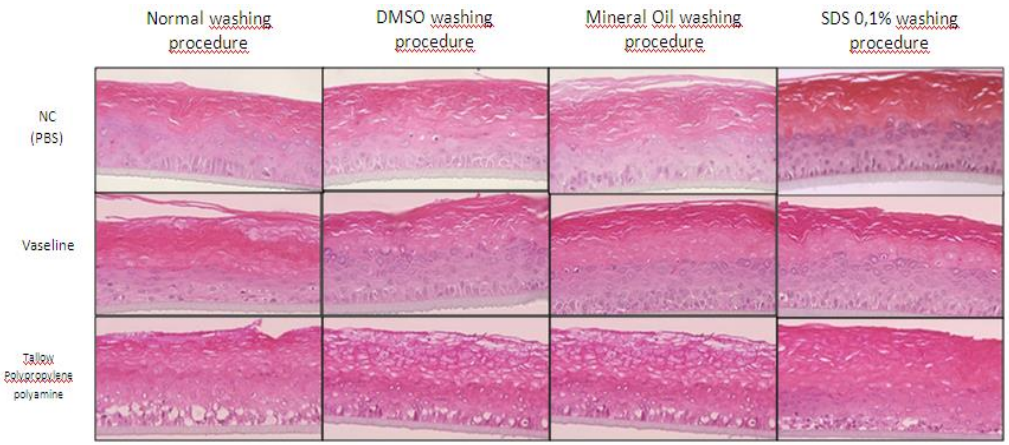
[c] *SeCAM, Agno, Switzerland*



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BRACVAM Legislation

Publications

ELSEVIER

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 [a], Véronique Detappe [b], Herman Koëter [c], Joachim Kreysa [d], Manfred Liebsch [e], Valérie Zuang [d], Patric Amcoff [f], João Barroso [d], José Cotovio [g], Robert Guest [h], Martina Hermann [i], Sebastian Hoffmann [j], Philippe Masson [k], Nathalie Alépée [g], Luis Alfonso Arce [l], Beat Brüschweiler [m], Tiziana Catone [n], Rostislav Cihak [o] ... Olivier Depallens [b]

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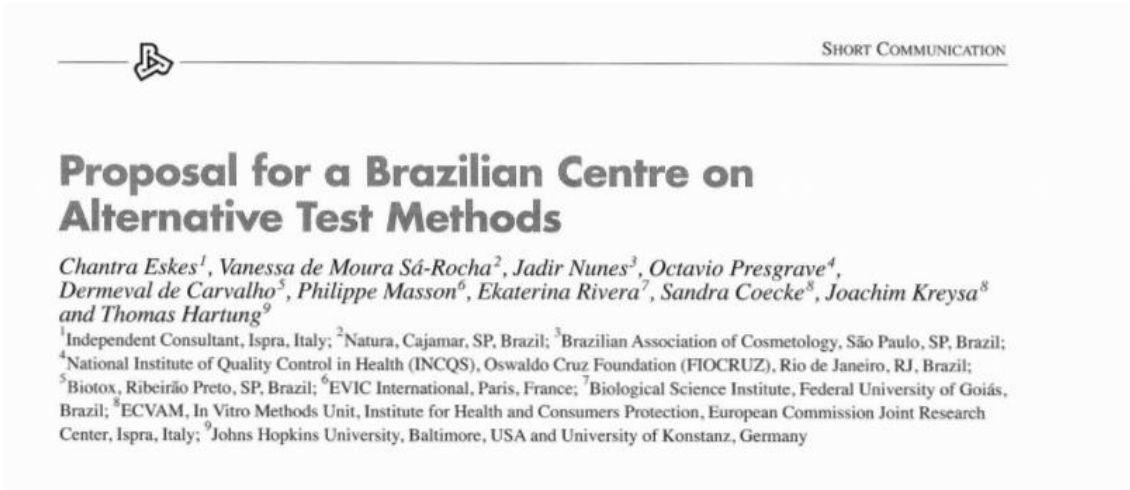

SHORT COMMUNICATION

Proposal for a Brazilian Centre on Alternative Test Methods

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

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

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Micronucleus (Genotox)
on 3D Skin model
2012

Publications

ELSEVIER

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

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

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

E. Andres [a], J. Molinari [a], N. Remoué [a], V.M. Sá-Rocha [b], C. Barrichello [a], S.P. Hurtado [b]

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

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Universities, Research Centers, Validation Center

Sensitization

Publications

Toxicology in Vitro
Volume 27, Issue 4, June 2013, Pages 1220–1225

The sensitivity of the KeratinoSens™ assay to evaluate plant extracts: A pilot study

Eric Andres[a], Vanessa M. Sá-Rocha[b], Carla Barrichello[a], Tina Haupt[c], Graham Ellis[d], Andreas Natsch[c]

Research Article
Journal of Applied Toxicology
Received: 9 February 2015, Revised: 6 April 2015, Accepted: 13 April 2015 Published online in Wiley Online Libra
(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[a†], Alexandra Maertens[a†], James M. McKim[b], Thomas Hartung[a,c*], Andre Kleensang[a] and Vanessa Sá-Rocha[a,d]

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[a], Vanessa Moura Sá-Rocha[b], Jane Zveiter Moraes[a]

Working Together

_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

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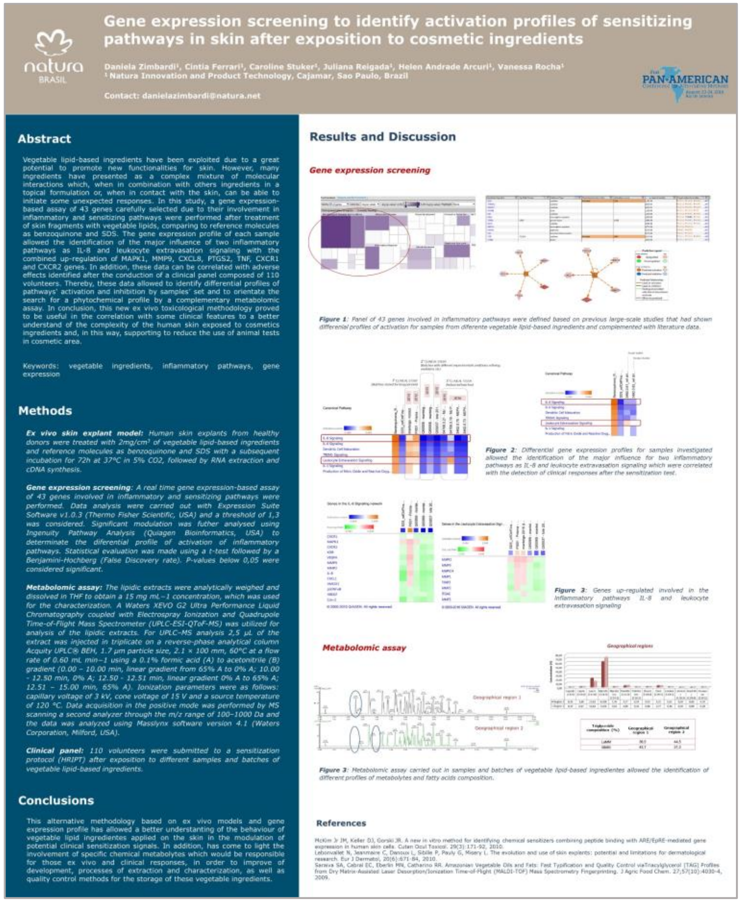

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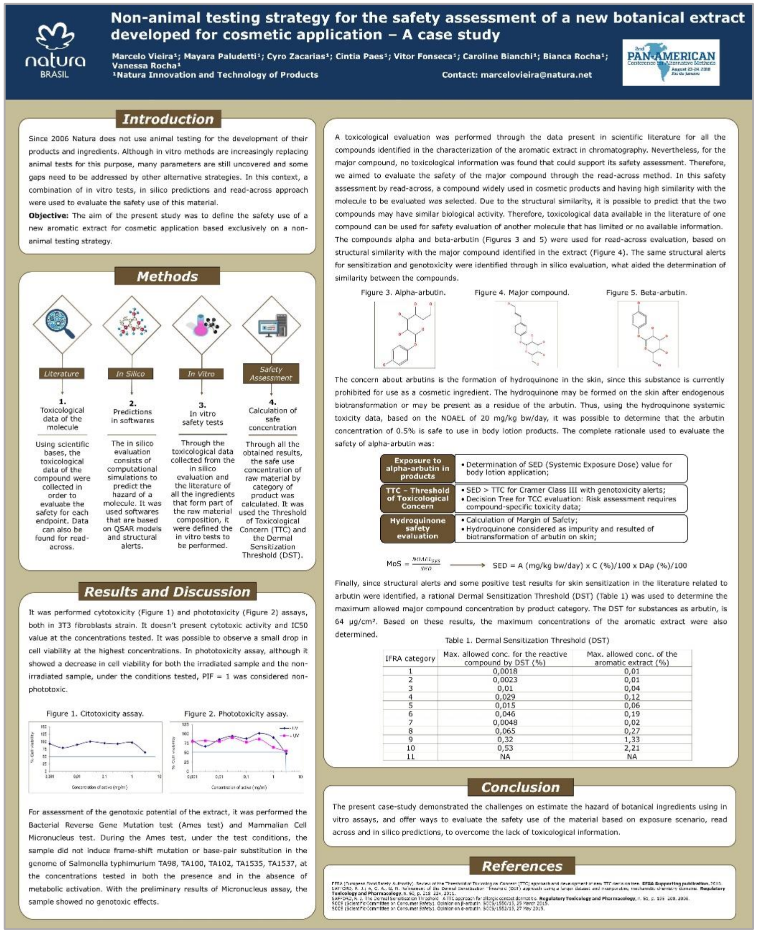

Non-animal testing strategy for the safety assessment of a new botanical extract developed for cosmetic application – A case study

Marcelo Vieira[1]; Mayara Paludetti[1]; Cyro Zacarias[1]; Cintia Paes[1]; Vitor Fonseca[1]; Caroline Bianchi[1]; Bianca Rocha[1]; Vanessa Rocha[1]
[1]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 in silico evaluation and the literature of all the ingredients that form part of the raw material composition, it 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 assay, 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. Citotoxicity 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.

A toxicological evaluation was performed through the data present in scientific literature for all the compounds identified in the characterization of the aromatic extract in chromatography. Nevertheless, for the major compound, no toxicological information was found that could support its safety assessment. Therefore, we aimed to evaluate the safety of the major compound through the read-across method. In this safety assessment by read-across, a compound widely used in cosmetic products and having high similarity with the molecule to be evaluated was selected. Due to the structural similarity, it is possible to predict that the two compounds may have similar biological activity. Therefore, toxicological data available in the literature of one compound can be used for safety evaluation of another molecule that has limited or no available information. The compounds alpha and beta-arbutin (Figures 3 and 5) were used for read-across evaluation, based on structural similarity with the major compound identified in the extract (Figure 4). The same structural alerts for sensitization and genotoxicity were identified through in silico evaluation, what aided the determination of similarity between the compounds.

Figure 3. Alpha-arbutin. Figure 4. Major compound. Figure 5. Beta-arbutin.

The concern about arbutins is the formation of hydroquinone in the skin, since this substance is currently prohibited for use as a cosmetic ingredient. The hydroquinone may be formed on the skin after endogenous biotransformation or may be present as a residue of the arbutin. Thus, using the hydroquinone systemic toxicity data, based on the NOAEL of 20 mg/kg bw/day, it was possible to determine that the arbutin concentration of 0.5% is safe to use in body lotion products. The complete rationale used to evaluate the safety of alpha-arbutin was:

Exposure to alpha-arbutin in products	• Determination of SED (Systemic Exposure Dose) value for body lotion application;
TTC – Threshold of Toxicological Concern	• SED > TTC for Cramer Class III with genotoxicity alerts; • Decision Tree for TCC evaluation: Risk assessment requires compound-specific toxicity data;
Hydroquinone safety evaluation	• Calculation of Margin of Safety; • Hydroquinone considered as impurity and resulted of biotransformation of arbutin on skin;

$$MoS = \frac{NOAEL_{sys}}{SED} \longrightarrow SED = A\ (mg/kg\ bw/day) \times C\ (\%)/100 \times DAp\ (\%)/100$$

Finally, since structural alerts and some positive test results for skin sensitization in the literature related to arbutin were identified, a rational Dermal Sensitization Threshold (DST) (Table 1) was used to determine the maximum allowed major compound concentration by product category. The DST for substances as arbutin, is 64 μg/cm². Based on these results, the maximum concentrations of the aromatic extract were also determined.

Table 1. Dermal Sensitization Threshold (DST)

IFRA category	Max. allowed conc. for the reactive compound by DST (%)	Max. allowed conc. of the aromatic extract (%)
1	0,0018	0,01
2	0,0023	0,01
3	0,01	0,04
4	0,029	0,12
5	0,015	0,06
6	0,046	0,19
7	0,0048	0,02
8	0,065	0,27
9	0,32	1,33
10	0,53	2,21
11	NA	NA

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.

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EFSA (European Food Safety Authority). Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA Supporting publication, 2016.
SAFFORD, R. J.; A, C. A.; G. N. Refinement of the Dermal Sensitisation Threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regulatory Toxicology and Pharmacology, n. 60, p. 218–224, 2011.
SAFFORD, R. J. The Dermal Sensitisation Threshold – A TTC approach for allergic contact dermatitis. Regulatory Toxicology and Pharmacology, n. 51, p. 195–200, 2008.
SCCS (Scientific Committee on Consumer Safety). Opinion on β-arbutin. SCCS/1550/15, 25 March 2015.
SCCS (Scientific Committee on Consumer Safety). Opinion on α-arbutin. SCCS/1552/15, 27 May 2015.

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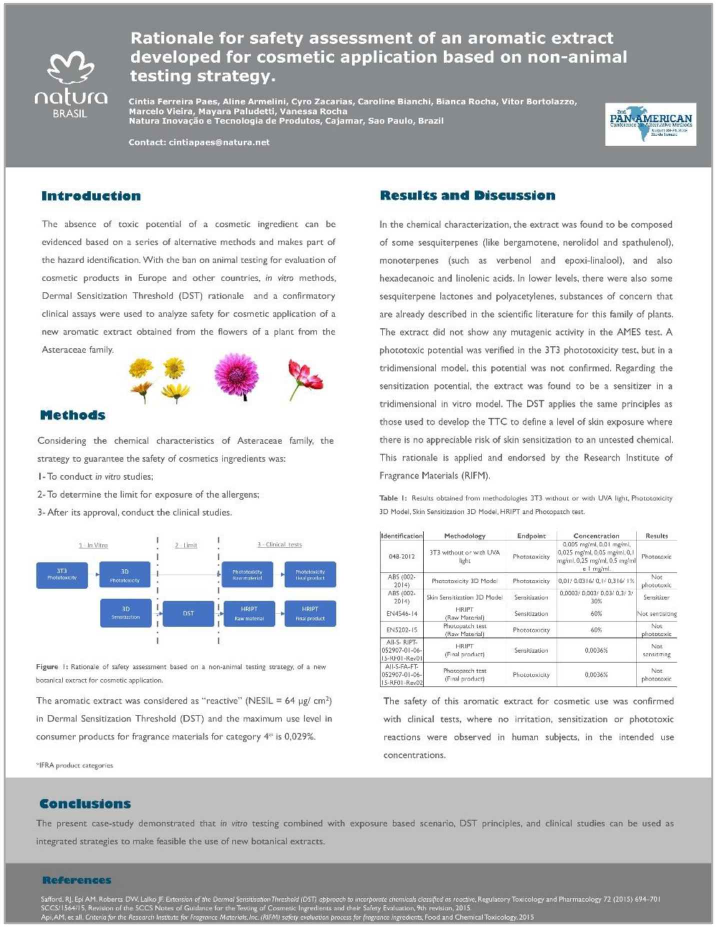

Rationale for safety assessment of an aromatic extract developed for cosmetic application based on non-animal testing strategy.

Cintia Ferreira Paes, Aline Armelini, Cyro Zacarias, Caroline Bianchi, Bianca Rocha, Vitor Bortolazzo, Marcelo Vieira, Mayara Paludetti, Vanessa Rocha
Natura Inovação e Tecnologia de Produtos, Cajamar, Sao Paulo, Brazil
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.

Methods

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

1- To conduct *in vitro* studies;

2- To determine the limit for exposure of the allergens;

3- After its approval, conduct the clinical studies.

1 - In Vitro: 3T3 Phototoxicity → 3D Phototoxicity; 3D Sensitization. 2 - Limit: DST. 3 - Clinical tests: Phototoxicity Raw material → Phototoxicity Final product; HRIPT Raw material → HRIPT Final product.

Figure 1: Rationale of safety assessment based on a non-animal testing strategy, of a new botanical extract for cosmetic application.

The aromatic extract was considered as "reactive" (NESIL = 64 μg/ cm²) in Dermal Sensitization Threshold (DST) and the maximum use level in consumer products for fragrance materials for category 4* is 0,029%.

*IFRA product categories

Results and Discussion

In the chemical characterization, the extract was found to be composed of some sesquiterpenes (like bergamotene, nerolidol and spathulenol), monoterpenes (such as verbenol and epoxi-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).

Table 1: Results obtained from methodologies 3T3 without or with UVA light, Phototoxicity 3D Model, Skin Sensitization 3D Model, HRIPT and Photopatch test.

Identification	Methodology	Endpoint	Concentration	Results
048-2012	3T3 without or with UVA light	Phototoxicity	0,005 mg/ml, 0,01 mg/ml, 0,025 mg/ml, 0,05 mg/ml, 0,1 mg/ml, 0,25 mg/ml, 0,5 mg/ml e 1 mg/ml.	Phototoxic
ABS (002-2014)	Phototoxicity 3D Model	Phototoxicity	0,01/ 0,0316/ 0,1/ 0,316/ 1%	Not phototoxic
ABS (002-2014)	Skin Sensitization 3D Model	Sensitization	0,0003/ 0,003/ 0,03/ 0,3/ 3/ 30%	Sensitizer
EN4546-14	HRIPT (Raw Material)	Sensitization	60%	Not sensitizing
EN5202-15	Photopatch test (Raw Material)	Phototoxicity	60%	Not phototoxic
All-S- RIPT-052907-01-06-15-RF01-Rev01	HRIPT (Final product)	Sensitization	0,0036%	Not sensitizing
All-S-FA-FT-052907-01-06-15-RF01-Rev02	Photopatch test (Final product)	Phototoxicity	0,0036%	Not phototoxic

The safety of this aromatic extract for cosmetic use was confirmed with clinical tests, where no irritation, sensitization or phototoxic reactions were observed in human subjects, in the intended use concentrations.

Conclusions

The present case-study demonstrated that *in vitro* testing combined with exposure based scenario, DST principles, and clinical studies can be used as integrated strategies to make feasible the use of new botanical extracts.

References

Safford, RJ, Epi AM, Roberts DW, Lalko JF. *Extension of the Dermal Sensitisation Threshold (DST) approach to incorporate chemicals classified as reactive*, Regulatory Toxicology and Pharmacology 72 (2015) 694–701
SCCS/1564/15, Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 9th revision, 2015.
Api, AM, et all. *Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients*, Food and Chemical Toxicology, 2015

Working Together

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 Concea normatives;

_ Contribute for the scientific progress with training, Workshops for companies.

ABIHPEC
Brazilian Association of the Cosmetic, Toiletry and Fragrance Industry

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

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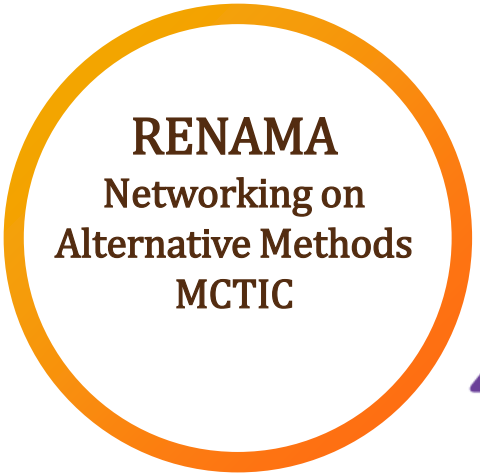


_Associated Laboratory

_LNBIO 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).

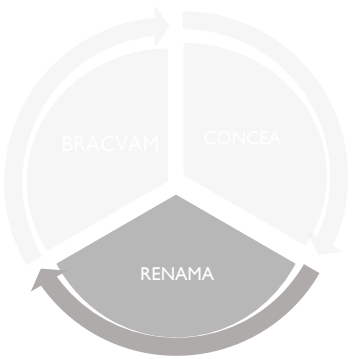
_Natura and USP represents the associated laboratories

Working Together

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

30 participants

MAPPING WHAT WAS IMPLEMENTED



24 Methods (Normative 18th and 31st)

Skin Irritation and Corrosion	
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	✓
Ocular Irritation and Corrosion	
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) Ch	✓
OECD 492 - Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring clas	✓
Phototoxicity	
OECD 432 - In Vitro 3T3 NRU Phototoxicity Test	✓
Skin Absortion	
OECD 428 - Skin Absorption: In Vitro Method	✓
Skin Sensitization	
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
Genotoxicity	
OECD 487 - In Vitro Mammalian Cell Micronucleus Test	✓
Acute Toxicity	
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	✓
Reproductive Toxicity	
OECD 421 - Reproduction/Developmental Toxicity Screening Test	X
OECD 422 - Combined Repeated Dose Toxicity Study with the reproduction/Developmental Toxicity Screening Test	X
Pyrogenic Contamination	✓
Bacteria Endotoxin Test -Brazilian Pharmacopeia	X

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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 Concea's Normatives and use of alternative methods

2. Training and qualification

3. Big projects including international partners

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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//

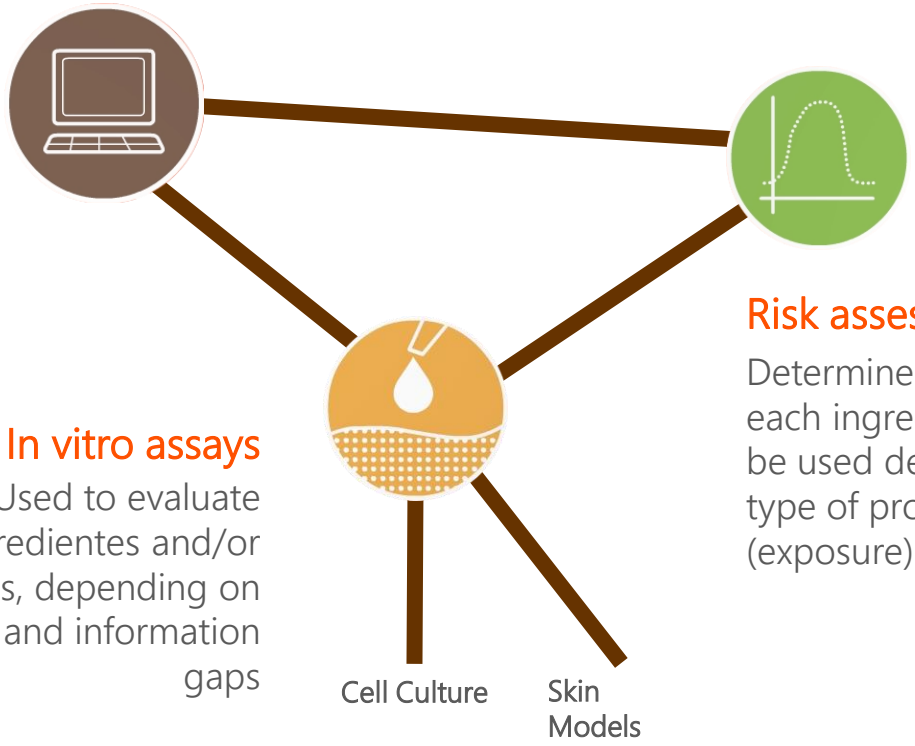

Obrigada
Thank you

ALTERNATIVE METHODS FOR SAFETY ASSESSMENT

Integrated testing Strategies

In silico

Tools to analyse hazard of ingredientes based on their structure



Risk assessment

Determine the amount of each ingredient that can be used depending on the type of products (exposure)

In vitro assays

Used to evaluate ingredientes and/or products, depending on the needs and information gaps

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