Vigilância Sanitária em Debate
ISSN: 2317-269X
INCQS-FIOCRUZ

De Vecchi, Rodrigo; Dakic, Vanja; Mattos, Guilherme; Rigaudeau, Anne-Sophie; Oliveira, Veronica; Garcia, Cristina; Alépée, Nathalie; Cotovio, José; Bouez, Charbel

Implementation, availability and regulatory status of an OECD accepted Reconstructed Human Epidermis model in Brazil

Vigilância Sanitária em Debate, vol. 6, no. 1, 2018, February, pp. 64-71
INCQS-FIOCRUZ

DOI: https://doi.org/10.22239/2317-269X.01055

Available in: https://www.redalyc.org/articulo.oa?id=570563069008

ARTICLE
https://doi.org/10.22239/2317-269x.01055

Implementation, availability and regulatory status of an OECD accepted Reconstructed Human Epidermis model in Brazil

Implementação, disponibilidade e contexto regulatório de um modelo de Epiderme Humana Reconstruída no Brasil aceito pela OECD

Rodrigo De Vecchi[I,&,*]
Vanja Dakic[I,&]
Guilherme Mattos[I]
Anne-Sophie Rigaudeau[III]
Veronica Oliveira[I]
Cristina Garcia[I]
Nathalie Alépée[II]
José Cotovio[II]
Charbel Bouez[IV]

[I] L´Oréal Research and Innovation, Rio de Janeiro, RJ, Brazil
[II] L´Oréal Research and Innovation, Aulnay-sous-Bois, France
[III] EPISKIN, Lyon, France
[IV] L´Oréal Research and Innovation, Clark, USA
[&] Equally contributed to this work

*** E-mail:** rvecchi@rd.loreal.com

Received: Oct 02, 2017
Approved: Jan 04, 2018

ABSTRACT

Introduction: In 2014, Brazil has joined the growing list of countries to ban cosmetic products from being tested on animal models. The new legislation comes into force in 2019. As a result, the interest for validated alternative testing methods for safety assessment has been increasing in academia, industry and associations. However, the lack of specific legislation on the use of biological material of human origin for toxicological tests makes the access to alternative *in vitro* models difficult. Furthermore, importation to Brazil is not possible on timely manner. **Method:** In this article, we report the implementation process of a Reconstructed Human Epidermis (SkinEthic™ RHE), an alternative model internationally accepted by OECD, through a technology transfer from EPISKIN® Lyon to Brazil. Regulatory evolution has been motivating the implementation and wide use of alternative methods to animal testing in several industry segments including cosmetic and pharmaceutical. **Results:** Protocol has been shown to be robust and highly reproducible. Quality control parameters (histological analysis, barrier function test and tissue viability) were performed on 24 batches assembled in Brazil. SkinEthic™ RHE model use allows the full replacement of animal test methods for skin hazards identification. It has regulatory acceptance for several toxicological endpoints, such as the Draize test for skin irritation and corrosion. It allows the reduction and refining of pre-clinical protocols through tiered strategies. Implementation of SkinEthic™ RHE protocol is just a first and important step towards a new approach of toxicological safety testing in Brazil. **Conclusions:** The implementation was successfully done and reported here. However, in order to follow completely the new legislation up to 2019, the availability of validated models is essential. Quality control tests done on RHE batches produced in Brazil demonstrate that the model met OECD acceptance criteria and therefore can be used for reliable prediction of irritation and corrosion classification.

KEYWORDS: Reconstructed Human Epidermis; SkinEthic™ RHE; Preclinical In Vitro Testing; Alternative Methods; Skin Irritation; Corrosion; Safety Assessment; Toxicological tests

RESUMO

Introdução: Em 2014, o Brasil aderiu à crescente lista de países a banir testes de produtos cosméticos em modelos animais. A nova legislação entra em vigor em 2019. Como resultado, o interesse em métodos de testes alternativos validados para avaliação de segurança tem aumentado na academia, indústria e associações. No entanto, a falta de legislação específica sobre o uso de material biológico de origem humana para testes toxicológicos dificulta o acesso aos modelos alternativos *in vitro*. Além disso, a importação no Brasil não é possível em tempo hábil. **Método:** Neste artigo, relatamos o processo de implementação de um modelo de Epiderme Humana Reconstruída (SkinEthic™ RHE) internacionalmente aceito pela OECD, através de uma transferência

tecnológica da Episkin Lion para o Brasil, bem como discutimos a evolução regulatória que tem motivado a implementação e a ampla utilização de métodos alternativos à experimentação animal em diversos segmentos além do cosmético e farmacêutico. **Resultados:** O protocolo de fabricação dos tecidos mostrou-se robusto e altamente reprodutível, considerando os parâmetros de controle de qualidade (análise histológica, função barreira e viabilidade tecidual) analisados em 24 lotes fabricados no Brasil. **Conclusões:** A implementação do modelo SkinEthic™ RHE é apenas um primeiro e importante passo em direção a uma nova abordagem para testes de segurança toxicológica no Brasil, realizada com êxito e aqui relatada. No entanto, para seguir plenamente a nova legislação até 2019, a disponibilidade de modelos validados é essencial. Os testes de controle de qualidade realizados nos lotes RHE produzidos no Brasil demonstram que o modelo atende aos critérios de aceitação da OCDE e, portanto, pode ser usado para uma previsão confiável de irritação e classificação de compostos corrosivos.

PALAVRAS-CHAVE: Epiderme Humana Reconstruída; SkinEthic™ RHE; Testes Pré-Clínicos *in vitro*; Métodos Alternativos; Skin Irritation; Corrosion; Safety Assessment; Toxicological tests

INTRODUCTION

Since 1986, the European Union (EU) has put in place specific legislation covering the use of animals for scientific purposes. On September 2010, the EU adopted the Directive 2010/63/EU, which has updated and replaced the Directive 86/609/EEC on the protection of animals used for scientific purposes, taking full effect on January 1st of 2013. The directive builds upon the 3Rs (Reduction, Refinement and Replacement) ethical framework from Russell and Burch in 1959[1], and commits to the "development, validation and uptake" of alternative methods[2]. This directive was supported by the European Citizens' Initiative "Stop Vivisection", which reinforced its implementation and expanded the movement to a continental scale. Apart of Europe, US and Japan also organized government agencies responsible for the regulation and recognition of *in vitro* tests[3]. In 2017, this initiative was raised to the global scale. To provide a central access point, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods developed the Integrated Chemical Environment (ICE) web resource, enabling access to high-quality reference data. ICE currently includes data about acute oral and dermal toxicity, skin irritation and sensitization, eye corrosion and endocrine disruption, as well as *in silico* predictions of test endpoints[4].

In cosmetic industry, animal testing in the EU is prohibited for cosmetic products since 2004, and for new ingredients since 2009[5]. Replacement of the rabbit Draize skin irritation test, for example, was motivated in Europe under the REACH chemical strategy and Cosmetics Directives. Various *in vitro* protocols, including three-dimensional (3D) skin models, have been developed to overcome model limitations. Academic community and industries like L´Oréal[6], Unilever[7], Procter & Gamble[8] and Henkel[9] have collaborated to conduct interlaboratory comparisons[10] and joined efforts to develop, validate and implement alternative methods in their routines, reducing the use of animal for safety assessment, with the support and acceptance of regulatory bodies. Therefore, the European Centre for the Validation of Alternative Methods (EURL ECVAM) established a list of *in vitro* validated cell-based test methods for predicting the safety and toxicity of ingredients and mixtures[11].

In this global taskforce, cell culture systems are essential tools used in a wide range of biomedical and clinical studies worldwide. Test methods based on 3D tissue-engineered epidermis for toxicological applications, including skin irritation and corrosion, use commercially available tissues such as EST-1000 (Cell Systems, St. Katharinen, Germany), EpiDerm™ (MatTek Corporation, Ashland, USA) and SkinEthic™ Reconstructed Human Epidermis (RHE) (EPISKIN, Lyon, France)[6,12,13]. Growing 3D reconstructed skin tissues approximate these models to the *in vivo* functionality, reducing the cost of drug development with an improving efficiency of preclinical trials, minimizing the failure rate in drug discovery and replacing the animal testing[14]. Moreover, the testing conditions are controlled and standardized, reducing the variability between experiments and project costs. With the advances in science and technology, the existing models are constantly being improved and becoming more complex, in order to reflect the interactions between cells, tissues and organs[15].

SkinEthic™ RHE is a fully differentiated epithelium obtained *in vitro* on an inert polycarbonate filter substrate by culturing normal human keratinocytes (NHK) in chemically defined medium. SkinEthic™ RHE presents apparently normal multilayered epidermis, with clearly visible basal layer, spinous layer, granular layer and corneal layer. It expresses the major protein and lipid differentiation markers like keratins 1, 10, 11, involucrin, ceramides[16]. Taken together, the architecture and ultrastructure of the cultured epidermis resembled closely to epidermis *in vivo*. SkinEthic™ RHE test method has been adopted within the context of OECD TG 431[17] for distinguishing corrosive and non-corrosive chemicals. EU classification and labelling (CLP/GHS) system requires subcategorization of corrosive chemicals into the three United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS) subcategories 1A, 1B and 1C. Previous studies have demonstrated the usefulness of the validated SkinEthic™ RHE test method to identify skin corrosive UN GHS subcategories to discriminate skin corrosive UN GHS subcategories[18]. More recently, the pharmaceutical industry has been also following and implementing alternative methods for the research and development of drugs in Europe[19] and Brazil[20].

The first Brazilian legislation on animal experiment has passed in 2008[21] creating the Arouca Law, based on the controlled use of animals following the ethical doctrine of the 3R´s and making sure that death by humanitarian means involves the expression "minimum physical or mental suffering". In 2012, Ministry of Science, Technology, Innovation and Communication (MCTIC) officially encouraged implementation of alternative methods by inviting the National Institute of Metrology, Quality and Technology (Inmetro), the Brazilian Biosciences National Laboratory (LNBio) and the National Institute for Quality Control in Health (INCQS) to organize the National Network on Alternative Methods (Renama). Renama works alongside with Brazilian Center for the Validation of Alternative Methods (BraCVAM) following the OECD GD 34[22] (Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment OECD, 2005). The evolution of GD 34 involved the efforts of several international validation bodies, including BraCVAM in Brazil, responsible for identifying and receiving requests from parties interested in submitting tests for validation[23]. The list of promising assays is passed to Renama, which helps with prioritization and contribution to the validation studies of the selected assays. Subsequently, a validation study is done under the supervision of BraCVAM, and the results are sent to the National Council for the Control of Animal Experimentation (Concea). Concea is in charge of registering the institutions for new validated test methods, following an open public consultation[23]. In 2014, Concea has recognized the first 17 alternative methods to animal testing in Brazil, based on international officially published studies and OECD guidelines[24]. In addition, a period of 5 years was established as a limit for the mandatory replacement of the *in vivo* method by the available alternative method, through Normative Resolution 18, based on the rules established by Normative Resolution 17. In 2016, 7 more alternative methods were recognized and recommended by Concea through the Normative Resolution 31[25]. Timeline in the Figure 1 illustrates the recent regulatory evolution related to alternative methods in Brazil since 2008.

For decades, EPISKIN has been distributing SkinEthic™ RHE tissues worldwide, shipping it from France to several countries in Europe, Asia and Americas. Normally, RHE kits are simply classified as a laboratory reagent, compliant with ISO full traceability and guidelines such as Food and Drug Administration[26] for *in vitro* products for Research Use Only (RUO). However, the lack of specific classification and agile process to release RHE kits in the customs makes importation not possible in a timely manner, as the living tissues survive around 3 days outside of the incubator.

This work presents a first possibility of how to deal with this dilemma, by implementing the OECD accepted SkinEthic™ RHE model in Brazil. We describe the quality control parameters measured on tissues fabricated in Brazil to guarantee its conformity with international defined criteria.

METHOD

SkinEthic™ RHE

SkinEthic™ RHE is an *in vitro* reconstructed human epidermis from normal human keratinocytes grown on an inert polycarbonate filter (0.5 cm^2) at the air-liquid interface, in a chemically defined medium[16]. The cells, donated from volunteers with Consent Term, are provided from EPISKINs cell bank. Viability, barrier function and morphology are evaluated for all SkinEthic™ RHE production batches. Tissues were transferred on nutritive agarose plates and enclosed for shipment.

Chemicals

3-(4.5-Dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT), phosphate buffered saline (D-PBS) without calcium and magnesium, Triton X-100 and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) was purchased from BIO-RAD (Hercules, CA, USA). Optimum cutting temperature (OCT) compound was purchased from Sakura Finetek (Torrance, CA, USA). Hematoxylin from Ral Diagnostics (Martillac, France) and eosin from Merck KGDA (Germany).

Histology

SkinEthic™ RHE was fixed in 4% paraformaldehyde for 30 min, embedded in OCT and frozen in liquid nitrogen. Six-micrometer thick vertical sections were cut using a cryostat and stained with hematoxylin-eosin for histological analysis of the tissues.

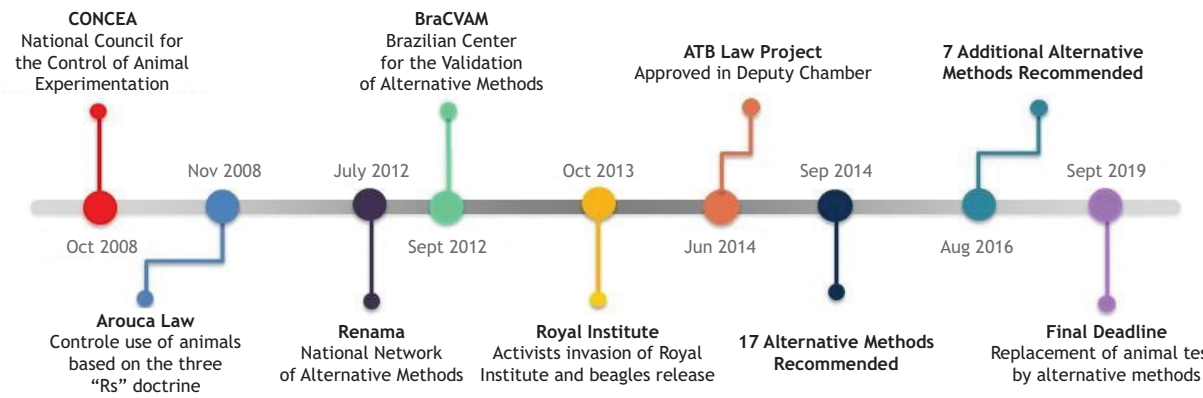


Figure 1. Regulatory evolution related to the control of animal experimentation and alternative methods recognition in Brazil. Since the creation of Concea in 2008, Brazil had significant regulatory advances and its counting down to ban animal testing for several endpoints on September 2019.

Viability test

The assay used for quantifying tissue viability was the MTT-assay. The mitochondrial dehydrogenase activity of the viable cells of the SkinEthic™ RHE tissues construct reduces the vital yellow dye of the MTT into a blue formazan precipitate[27], which is then extracted from the tissues using isopropanol.

Tissues were transferred into a 24-well plates containing 0.3 mL of 0.5 mg/mL MTT solution and incubated for 3 h at 37°C, 5% CO_2 and 95% humidity. After 3 h incubation, the tissues were placed in 1500 µL of isopropanol at room temperature for 2 h. Viability was measured on 24 independent productions, using always a minimum of two tissues per production. Optical density (OD) was measured by adding a three technical replicates of 200 µL of each sample into 96 flat bottom well plates for measuring the absorbance at 570 nm. The OD of the extraction solvent alone was small, i.e., OD < 0.1 for each batch of the tested RHE. Acceptability limit of OD ≥ 0.7 for viable tissues on day 17 of differentiation was established, according to EPISKIN France historical database.

Barrier Function

The barrier function property of the tissue was estimated by the exposure time required to reduce relative cell viability by 50% (ET-50) upon application of 1% Triton X-100.Three time points were used: 6, 4 and 2.5 h. For each condition we utilized 3 tissues, including control group, which was treated with water instead of Triton X-100. After treatment, the tissues were transferred into a 24-well plates containing 0.3 mL of 0.33 mg/mL MTT solution and incubated for 3 hours at 37°C, 5% CO_2 and 95% humidity. After 3 hours of incubation, the tissues were placed in 1500 µL of isopropanol at room temperature overnight. OD was measured by adding a triplicate of 200 µL of each sample into 96 flat bottom well plates for measuring the absorbance at 570 nm. Viability of Triton X-100 treated tissue at different time points was compared to the concurrent negative control tissues. An acceptability range (upper and lower limit) for the ET-50 Triton X-100 has been established in OECD test guidelines 439[28] and 431[17] ranging from 4 to 10 hours.

Irritation test

Irritation test was done using proficiency chemicals recommended in OECD test guidelines 439. We tested five non-irritant and four irritant substances, including liquids and solids. SDS 5% was used as reference irritant (positive control) and PBS (without Ca^{++} and Mg^{++}) served as negative control (NC), in each series of experiments. SD value is considered as valid if it is ≤18%. Positive control data meets the acceptance criteria if the mean viability, expressed as % of the NC, is < 40% and the standard deviation (SD) value is ≤18%.

RHE tissues were topically exposed to undiluted liquids (16 ± 0.5 µl) or solids (16 ± 2 mg) for 42 min, at room temperature. Prior applying solids, 10 ± 0.5 µl of distilled water was spread on the whole surface of RHE tissues. A nylon mesh (EPISKIN, France) was applied onto the test substance as spreading support for all liquid and viscous test substances. RHE tissues were then rinsed 25 times, with 1 mL each, of sterile PBS without calcium and magnesium. Treated tissues were incubated for 42 h at 37°C, 5% CO2, with 2 mL of growth medium. Cytotoxicity was determined by measuring the dehydrogenase activity of viable RHE tissues following a 42 h post-incubation. Each experiment was performed at least in triplicate of one tissue production batch. Each substance was tested on reconstructed tissues of three different cell batches.

After subtracting the blank OD from all raw data, mean OD values ± SD were calculated using nine measurements per test substance (three RHE tissues, three replicates/tissue) and the percentage of cell viability was expressed relatively to negative control as following: 100 x mean $OD_{treated}$/mean $OD_{control}$. Negative control value was set at 100%. For further details, please refer to OECD test guidelines 439.

RESULTS

RHE models were produced following rigorously the same quality specifications as the original tissues produced in EPISKIN Lyon, France. In order to confirm the reproducibility and robustness of the protocol in Brazil, viability, barrier function and histology were evaluated on each SkinEthic™ RHE batch generated. In this study, 24 independent productions were carried out, with 4 different primary keratinocytes cell batches. Quality controls were conducted in different stages of differentiation, and included histology, barrier function, and viability. A SkinEthic™ RHE tissue batch was considered as normal histologically if at least four viable layers of cells were present. Tissues histology analyzed during the second, third and fourth week of differentiation are shown on Figure 2. In the second week of protocol, the tissue is still not fully differentiated, however, at least 4 layers of live cells are already present and well organized with a very thin

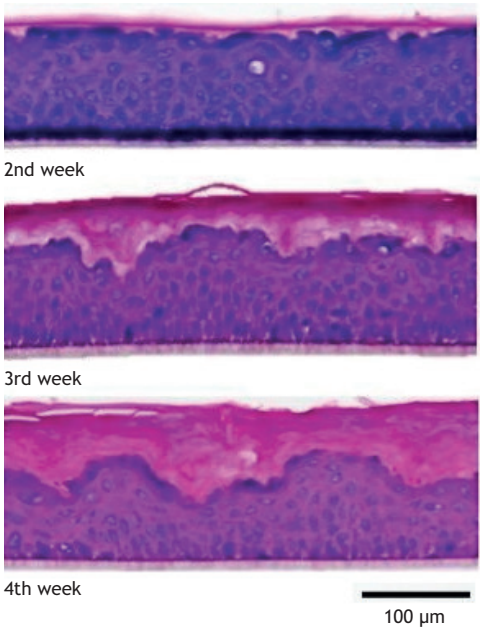


Figure 2. Histology of SkinEthic™ RHE using Hematoxylin/Eosin staining of one representative batch during 2nd, 3rd and 4th week of differentiation *in vitro*. Scale bar: 100 µm.

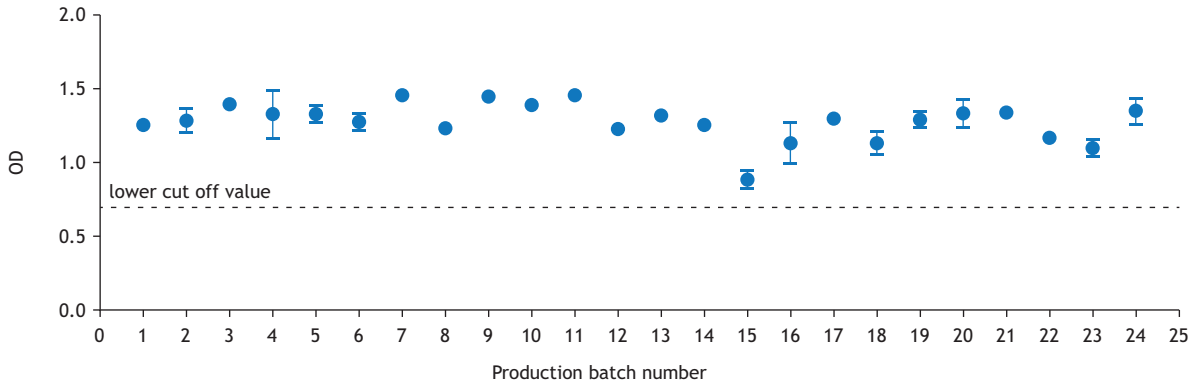
corneal layer. On the third week in culture, the tissue is fully-differentiated, consisting of basal layer, *stratum spinosum*, granular layer and multilayered *stratum corneum*. If we maintain the RHE for one additional week, the organization of tissue is preserved with increased corneal layer thickness. No histological alterations were observed in the experiments.

The MTT assay was used to measure cell viability. Mean OD value for 24 production batches (independent experiments) is 1.278 ± 0.050, having minimum of two tissues per production. Figure 3 demonstrates the reproducibility of the production batches over time. All batches generated in Brazil had the mean OD values above 0.7, which is the threshold acceptance limit established in OECD TG 431 and 439. The OD of the extraction solvent alone was minimal, i.e., OD < 0.1 for each batch of the SkinEthic™ RHE production.

Barrier function tests showed that batches generated in Brazil are in conformity with OECD Test Guidelines 431[17] and 439[28] standards. Values of ET-50 were inside the acceptability range, ranging from 4 to 10 h, for all produced batches. Mean value of 19 batches was 7.33 ± 1.45 h (Figure 4). This result prove that the RHE produces a functional barrier with robustness to resist rapid penetration of Triton X-100 to the viable tissue.

All chemicals from the proficiency list were well classified using the MTT endpoint. After the treatment with the group of non-irritants (NI) viability was always higher than 50% and after the treatment with irritants (I) always lower than 50% (Figure 5).

A



B

Production*	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Mean	1.250	1.284	1.395	1.326	1.328	1.275	1.456	1.230	1.451	1.392	1.458	1.229	1.315	1.249	0.888	1.130	1.300	1.136	1.293	1.329	1.338	1.166	1.096	1.346
SD	0.031	0.083	0.018	0.166	0.064	0.062	0.020	0.007	0.027	0.036	0.006	0.007	0.028	0.018	0.060	0.144	0.031	0.075	0.047	0.101	0.009	0.010	0.053	0.089

Figure 3. (A) Graph showing average ODs of tissue viability test (MTT) of 24 batches generated in Brazil, showing a high repeatability and reproducibility. Dots represent mean OD of two tissues from the same production. (B) Table with the mean and standard deviation values corresponding to graph A.

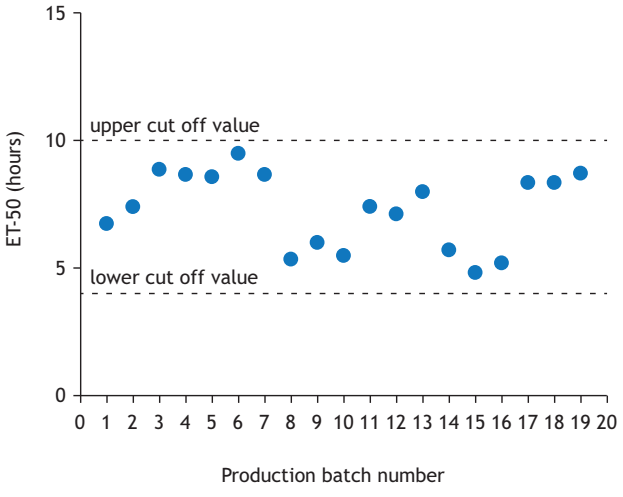


Figure 4. ET-50 values for 19 batches generated in Brazil, showing a high repeatability and reproducibility.

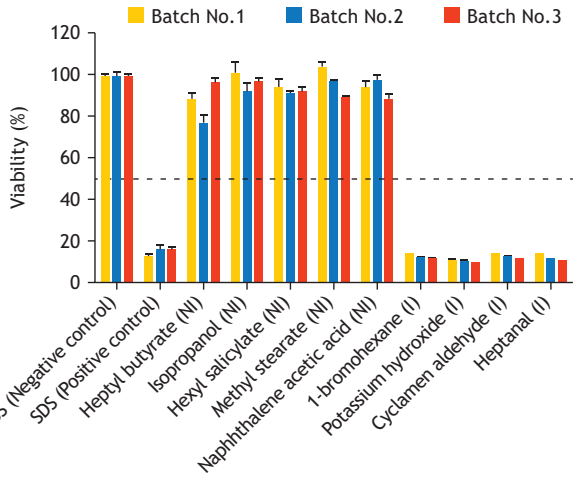


Figure 5. Cell viability after treatment with proficiency chemicals suggested in the OECD test guideline 439. Each substance was tested on RHE tissues reconstructed from three different cell batches. Bar represents mean with SEM.

DISCUSSION

The availability of validated alternative test methods depends on robust and reproducible protocols for testing systems, such as the Reconstructed Human Epidermis (RHE) model. These models have been recognized as technical foundation for scientific community and several industrial segments to maintain international competitiveness of Brazilian research, innovation and development activities.

Several human skin models use cultured primary cells or immortalized cell lines to produce artificially reconstituted 3D skin multilayered tissues[29]. These tissues, also known as Reconstructed Human Epidermal models, like SkinEthic™ RHE, EpiDerm and others, are commercially available and show reasonable similarities to the human skin in terms of morphology, lipid composition and biochemical markers. Although are not human skin fragments, these models are useful testing tools for safety assessment of new compounds for phototoxicity, corrosivity and irritancy, so several test protocols have been developed for toxicological and pharmacological evaluation[30]. For instance, recent versions of the OECD guidelines for skin irritation (TG 439) and skin corrosion (TG 431) have been released and internationally accepted[6].

Here we report the successful implementation of validated SkinEthic™ RHE model in Brazil. This RHE model is in conformity with EPISKIN global standards, based on evaluation of quality control parameters such as histology, barrier function and viability tests. Results presented here (Figures 2, 3, 4 and 5) confirm the robustness of SkinEthic™ RHE model and its high reproducibility. Considering results obtained with several RHE batches made in Brazil, following strictly the original production protocol practiced in France for decades, there was no need of formal re-validation. Test methods using SkinEthic™ reconstructed epithelia, mainly for irritation and corrosion, have been internationally validated and accepted by OECD member countries[28,17,31] (OECD Test Guidelines TG 439, 431, 492).

Recent regulatory advances in Brazil are not followed by legislative adaptations. The current law does not specify the distribution of engineered tissues, as it dates from 1988[32] (the Article 199, § 4 of the Brazilian Federal Constitution). By that time, the scientific advances from the last decades could not be foreseen, as well as the importance of alternative methods availability to the national industrial demand for research, development and innovation in a banning scenario. By not specifying engineered tissues, the Article 199 makes the availability and distribution of RHE tissues uncertain in the country, due to eventual misinterpretations of its paragraph 4.

Therefore, the absence of a specific framework in Brazil to confer legal safety for the wide use and availability of alternative methods, such as SkinEthic™ RHE model, is still one of the factors that hinder the development of this technology in the country and need to be clarified by legislators. On the other hand, the recognition of the 24 methods by Concea, covering 11 endpoints including 5 of which RHE model is useful and internationally recognized contributing to the reduction, replacement and refining of animal use in research activities and preclinical toxicological tests. However, the access, availability and deployment of alternative methods are still limited by the referred paragraph of Brazilian Constitution, dated from 1988[32]. Almost 30 years ago, it could not preview the coming scientific advances in the country and the importance of alternative methods availability to supply the industrial demand in the current banning scenario of nowadays. Thus, the referred article is not applied in the case of RHE, as it states specifically on the conditions and requirements for the removal, collection and processing of organs, tissues and human substances for transplant, research and treatment.

Although human epithelia artificially reconstructed *in vitro* do not record in these legal articles, RHE cannot be classified as human tissue or organ, once they are not removed or developed by the human body, but generated in laboratory from human epidermal keratinocyte cultures, which are legally imported and freely commercialized in the Brazilian territory. Furthermore, SkinEthic™ RHE model is structurally constituted of single cell type, which grows in a multilayered cell culture with air-liquid interface, differentiate and allows the formation of the *stratum corneum* mimicking the human skin barrier. The reproducibility of this reconstruction process, internationally accepted and recommended by several OECD test guidelines, is only possible following a specific and extensively manipulated protocol, developed and validated along decades, where the cells are plated on the polycarbonate insert, embedded in a chemically defined medium.

Besides its functional similarity with the human epidermis, which is only the most external layer of human skin, there is a consensus that RHE does not fit to the constitutional article 199 paragraphs since it is constituted of extensively manipulated cells, entirely generated in laboratory. These multilayered cell cultures present biochemical and morphological features that distinguish them from human tissues and organs, so they cannot be classified as human tissues or organs. This interpretation was confirmed in a Legal Advice n. 01/2017 issued by CGREG/Direg/Anvisa stating that RHE is a Research Use Only (ROU) product, exempt of Anvisa surveillance and control, according to the exception established in the Article 2°, subsection VIII of the Resolution RDC n° 36/2015[33]. Moreover, RHE model is a useful tool to replace animal testing methods, such as Draize *in vivo* rabbit irritation test[13], resulting data is internationally accepted by OECD. In Brazil, the use and implementation of validated alternative methods is recognized and recommended by Concea, and supported by Renama and MCTIC[34].

CONCLUSIONS

Implementation of the validated SkinEthic™ RHE model in Brazil opens the possibility of assessing several toxicological endpoints, from skin irritation and corrosion to other endpoints, such as skin sensitization, skin penetration, phototoxicity and genotoxicity. *In vitro* reconstructed human epithelia models reproduce the main features of human *in vivo* tissues. Their robustness, reproducibility and proximity to targeted human tissues makes it possible to overcome the animal testing, to

build *in vitro* screening architectures and predictive assessment of the effects in humans. Moreover, they have evidenced time and cost savings. For all these reasons, *in vitro* reconstructed human tissue models are massively used worldwide for safety and efficacy screening. Implementing OECD accepted alternative models in Brazil is one of the most effective ways to promote the implementation of alternative methods before the complete banning in 2019. Brazilian legislation on the use of biological materials of human origin makes *in vitro* epidermis model inaccessible to wide use. In order to implement completely the new legislation up to 2019, the availability of *in vitro* models is essential. Therefore, specific public documents conferring its legal safety are of great relevance to the country. Quality control tests done on RHE batches produced in Brazil show that the model met OECD acceptance criteria and therefore can be used for reliable prediction of irritation and corrosion classification. Quality control tests done on RHE batches produced in Brazil show that the model met OECD acceptance criteria and that therefore can be used for reliable prediction of irritation and corrosion classification.

REFERENCES

1. Russell WMS, Burch RL. The principles of humane experimental technique. Wheathampstead (UK): Universities Federation for Animal Welfare; 1992.
2. Germain, P, Chiapperino, L, Testa, G. The European politics of animal experimentation: from Victorian Britain to 'Stop Vivisection'. Stud Hist Philos Biol Biomed Sci. 2017;64:75-87. https://doi.org/10.1016/j.shpsc.2017.06.004
3. Araújo GL, Campos MA, Valente MA, Silva SC, França FD, Chaves MM et al. Alternative methods in toxicity testing: the current approach. Braz J Pharm Sci. 2014;50(1):55-62. https://doi.org/10.1590/S1984-82502011000100005
4. Bell SM, Phillips J, Sedykh A, Tandon A, Sprankle C, Morefield SQ et al. An integrated chemical environment to support 21st-Century toxicology. Environ Health Perspect. 2017;125(5):054501. https://doi.org/10.1289/EHP1759
5. Almeida A, Sarmento B, Rodrigues F. Insights on in vitro models for safety and toxicity assessment of cosmetic ingredients. Int J Pharm. 2017;519(1-2):178-85. https://doi.org/10.1016/j.ijpharm.2017.01.024
6. Alépée N, Grandidier MH, Tornier C, Cotovio J. An integrated testing strategy for *in vitro* skin corrosion and irritation assessment using SkinEthic™ Reconstructed Human Epidermis. Toxicol In Vitro. 2015;29(7):1779-92. https://doi.org/10.1016/j.tiv.2015.07.012
7. Basketter DA, Whittle E, Chamberlain M. Identification of irritation and corrosion hazards to skin: an alternative strategy to animal testing. Food Chem Toxicol. 1994;32(6):539-42. https://doi.org/10.1016/0278-6915(94)90111-2
8. Robinson MK, Cohen C, Fraissinette AB, Ponec M, Whittle E, Fentem JH. Non-animal testing strategies for assessment of the skin corrosion and skin irritation potential of ingredients and finished products. Food Chem Toxicol. 2002;40(5):573-92. https://doi.org/10.1016/S0278-6915(02)00005-4
9. Mewes KR, Fischer A, Zöller NN, Laubach V, Bernd A, Jacobs A et al. Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (phase I). Toxicol In Vitro. 2016;36:238-53. https://doi.org/10.1016/j.tiv.2016.07.007
10. Reus AA, Reisinger K, Downs TR, Carr GJ, Zeller A, Corvi R et al. Comet assay in reconstructed 3D human epidermal skin models—investigation of intra- and inter-laboratory reproducibility with coded chemicals. Mutagenesis. 2013;28(6):709-20. https://doi.org/10.1093/mutage/get051
11. European Parliament. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010. Protection of animals used for scientific purposes. Oficial J European Union. 2010 Oct 20.
12. Groeber F, Schober L, Schmid FF, Traube A, Kolbus-Hernandez S, Daton K et al. Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (phase II). Toxicol in Vitro. 2016;36:254-61. https://doi.org/10.1016/j.tiv.2016.07.007
13. Lee M, Hwang JH, Lim KM. Alternatives to *In vivo* draize rabbit eye and skin irritation Tests with a focus on 3D reconstructed human cornea-like epithelium and epidermis models. Toxicol Res. 2017;33(3):191-203. https://doi.org/10.5487/TR.2017.33.3.191
14. Amelian A, Wasilewska K, Megias D, Winnicka K. Application of standard cell cultures and 3D *in vitro* tissue models as an effective tool in drug design and development. Pharmacol Rep. 2017;69(5):861-70. https://doi.org/10.1016/j.pharep.2017.03.014
15. Vries RB, Leenaars M, Tra J, Huijbregtse R, Bongers E, Jansen JA et al. The potential of tissue engineering for developing alternatives to animal experiments: a systematic review. J Tissue Eng Regen Med. 2015;9(7):771-8. https://doi.org/10.1002/term.1703
16. Rosdy M, Clauss LC. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface. J Invest Dermatol. 1990;95(4):409-14. https://doi.org/10.1111/1523-1747.ep12555510
17. OECD Library. In vitro skin corrosion: reconstructed human epidermis (RHE) test method. Paris: OECD; 2016. (OECD Guideline for the testing of chemicals, Vol. 431).
18. Alépée N, Robert C, Tornier C, Cotovio J. The usefulness of the validated SkinEthic™ RHE test method to identify skin corrosive UN GHS subcategories. Toxicol In Vitro. 2014;28(4):616-25. https://doi.org/10.1016/j.tiv.2013.12.013
19. Goh JY, Weaver RJ, Dixxon L, Platt NJ, Roberts RA. Development and use of *in vitro* alternatives to animal testing by the pharmaceutical industry 1980-2013. Toxicol Res (Camb). 2015;4(5):1297-307. https://doi.org/10.1039/C5TX00123D

20. Agência Nacional de Vigilância Sanitária - Anvisa. Guia para a condução de estudos não clínicos de toxicologia e segurança farmacológica necessários ao desenvolvimento de medicamentos. Brasília, DF: Agência Nacional de Vigilância Sanitária; 2013.
21. Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. Acta Cir Bras. 2009;24(1):69-74. https://doi.org/10.1590/S0102-86502009000100015
22. OECD. Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. Paris: OECD; 2005. (OECD Series on testing and assessment, Vol. 34).
23. Presgrave O, Moura W, Caldeira C, Pereira E, Bôas MH, Eskes C. Brazilian Center for the Validation of Alternative Methods (BraCVAM) and the process of validation in Brazil. Altern Lab Anim. 2016;4(1):85-90.
24. Conselho Nacional de Controle de Experimentação Animal - CONCEA. Resolução normativa Nº 18, de 24 de setembro de 2014. Reconhece métodos alternativos ao uso de animais em atividades de pesquisa no Brasil, nos termos da Resolução Normativa nº 17, de 3 de julho de 2014, e dá outras providências. Diário Oficial União. 2014 set 25.
25. Conselho Nacional de Controle de Experimentação Animal - CONCEA. Resolução Normativa Nº 31, de 18 de agosto de 2016. Reconhece métodos alternativos ao uso de animais em atividades de pesquisa no Brasil. Diário Oficial União. 2016 ago 19.
26. U.S. Department of Health and Human Service. Food and Drug Administration - FDA. Distribution of in vitro diagnostic products labeled for research use only or investigational use only: guidance for industry and food and drug administration staff. Silver Spring: Center for Biologics Evaluation and Research; 2013.
27. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 16;65(1-2):55-63, 1983.
28. OECD. *In vitro* skin irritation reconstructed human epidermis (RHE) test method. Paris: OECD; 2015. (OECD Guideline for the testing of chemicals, Vol. 439).
29. Hewitt NJ, Edwards RJ, Fritsche E, Goebel C, Aeby P, Scheel J et al. Use of human in vitro skin models for accurate and ethical risk assessment: metabolic considerations. Toxicol Sci. 2013;133(2):209-17. https://doi.org/10.1093/toxsci/kft080
30. Netzlaff F, Lehr CM, Wertz PW, Schaefer UF. The human epidermis models EpiSkin, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. Eur J Pharm Biopharm. 2005;60(2):167-78. https://doi.org/10.1016/j.ejpb.2005.03.004
31. OECD. Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage Paris: OECD; 2015. (OECD Guideline for the testing of chemicals, Vol. 492).
32. Senado Federal (BR). Constituição da República Federativa do Brasil. Brasília, DF: Senado Federal. 1988.
33. Agência Nacional de Vigilância Sanitária ¤ Anvisa. Resolução - RDC N° 36, de 26 de agosto de 2015. Dispõe sobre a classificação de risco, os regimes de controle de cadastro e registro e os requisitos de rotulagem e instruções de uso de produtos para diagnóstico in vitro, inclusive seus instrumentos e dá outras providências. Diário Oficial União. 27 ago 2015.
34. Ministério da Ciência, Tecnologia, Inovações e Comunicações (BR). Portaria Administrativa Nº 3.856, 2017. Diário Oficial União. 12 set 2017.

Conflict of Interest

The authors are employees of L'Oréal Research & Innovation and EPISKIN.