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CRUELTY-FREE COSMETICS SAFETY GUARANTEES AND ETHICAL CONSUMPTION: IS IT ABOUT ALTERNATIVES TO ANIMAL TESTING?

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Trabalho de Conclusão do Curso de Farmácia-Bioquímica da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo.

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SUMMARY

ABSTRACT

The concern for animal welfare in the scientific research environment started with the 3Rs principle of “Replacement, Reduction, and Refinement”, which proposes methods, strategies, and modifications to processes to avoid or reduce the use of animal testing in research. This principle is the very root of great changes that have been occurring in many countries and regions since its publication, with bans and limits to animal use in testing and research, leading the way to a new concept of products called “cruelty-free”. Despite not yet having a legal definition, “cruelty-free” stands for products whose final products and/or its ingredients have not been tested in animals in any stage of development. There are currently several alternative methods to the tests usually performed in animal models, and it is important to discuss these methods, and if and how these alternatives guarantee the safety of “cruelty-free” cosmetics. The main objective is to compile and discuss information about how cruelty-free cosmetics are tested to guarantee the safety of these products and provide an overview about regulation related to use of animals for testing; as well as discuss how cosmetics are currently perceived in our society and how these topics are directly tied to ethical consumption. The starting point for the development of this project is a literature review of articles and textbooks available in online databases. The criterion for the search includes year of publication, relation to the theme, language, and accessibility. It’s expected to increase knowledge about the alternatives to animal testing and “cruelty-free” cosmetics and how the use of animal models is regulated across the world, as well as addressing the main questions raised in the theme.

Key-words: Animal testing; cruelty-free; cosmetics; alternative methods; ethical consumption

Introduction

The concern for animal welfare in the scientific research environment started with the 3Rs principle, introduced by Russel and Burch through *The Principles of Humane Experimental Technique* (1959). The 3Rs stand for "Replacement, Reduction, and Refinement" and address the reduction or complete removal of inhumane practices performed on animals used for research.

Due to the considerable growth of concern for animal welfare and deeply related to the 3Rs principle, several bans and/or limitations on animal testing and animal-tested products in many countries, paved the way for "cruelty-free" products. Even though there is no legal definition for the term "cruelty-free", it means that a certain product or ingredient does not contain any animal testing throughout its development.

The first region to take unfavorable action towards animal testing was the European Union (EU), in 2013, by fully banning the testing and marketing of cosmetic products tested in animals (European Union EUR-Lex, n.d.). This action was then followed by other countries, directly impacting the cosmetic market by banning sales and importation of final products tested in animals or products that contained animal-tested ingredients. This move taken by the EU forced other countries and regions to look for alternatives to guarantee production and marketing of their products, avoiding animal-testing.

Considering the most common tests performed in animals (skin irritation and corrosion; skin sensitization; eye irritation; genotoxicity and mutagenicity; chronic toxicity; and phototoxicity) (Díez-Sales et al, 2018), it is important to understand which alternatives there are for these methods, if safety of products tested and not tested in animals can be compared, and if safety can be guaranteed for cruelty-free products. In Brazil, there are currently around 40 recognized alternative methods, most of them involving *in vitro* and *in silico* techniques, as well as artificial tissue engineering and human volunteers.

In addition to discussing methods, procedures and safety related to cruelty-free products and how practices associated with animal testing are regulated around the world, this project intends to discuss the evolution of how cosmetics have been perceived in our society up until now and how it creates a grey area when it comes to discussing complete abolition of animal testing; as well as associate ethical

consumption with the spotlight under which we have cruelty-free cosmetics, alternatives to animal testing and animal welfare.

Research and study selection

The selection of scientific literature in online databases such as PubMed, Scielo, Google Scholar was performed considering year of publication being 2018 and onwards; defined keywords being animal testing, cruelty-free, cosmetics, alternative methods, and ethical consumption; and availability of full text in English and Portuguese. Additionally, relevance to theme and associated keywords (3Rs, ethical consumption, animal-testing ban) were also taken into consideration for selection.

A total of 52 scientific papers and books were selected for thorough evaluation and, out of these, 16 works were included in this review. The 18 works of selected literature were not included due to data discussed not providing enough relevant information for the theme of this review.

Complementary documentation, such as Regulatory Agencies' (and other authorities) guidelines and regulations were selected separately from the aforementioned inclusion criteria due to content and historical relevance, which were collected directly from their websites.

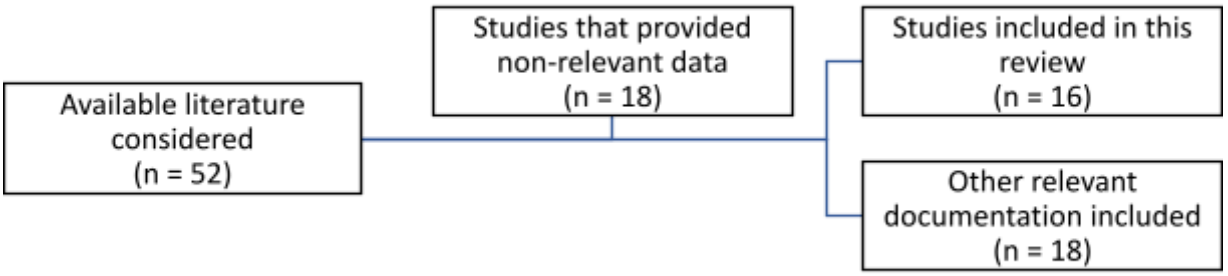

Figure 1 - Selection of literature on published in public databases from 2018 onwards on keywords animal testing, cruelty-free, alternative methods.

Replacement, reduction, and refinement

The concept of the 3Rs, which stands for Replacement, Reduction and Refinement, introduced by Russel and Burch in 1959 by the publication of the book

"*The Principles of Humane Experimental Technique*" is one of the greatest breakthroughs related to animal welfare and animal testing in the scientific field. The authors' intention with this new concept was to address ways in which inhumanity could be and was being diminished or removed (Russel and Burch, 1959).

While the definitions of Replacement and Reduction are considerably obvious, the first meaning to substitute the use of animals and the second to reduce the number of animals used to obtain the data expected, Refinement is not as clear. The last of the Rs addresses the minimization of pain and suffering by decreasing the among inhumane (ethically unacceptable) procedures and their severity on animals that would still be used. Presently, these primary definitions have been expanded as Reduction also applies to anything that can be reduced in the experiment, Replacement can be partial and cover aspects and parts of the experimental protocol and the Refinement includes all aspects related to the welfare of the animals involved and not only aspects related to pain and suffering (de Mori, 2019).

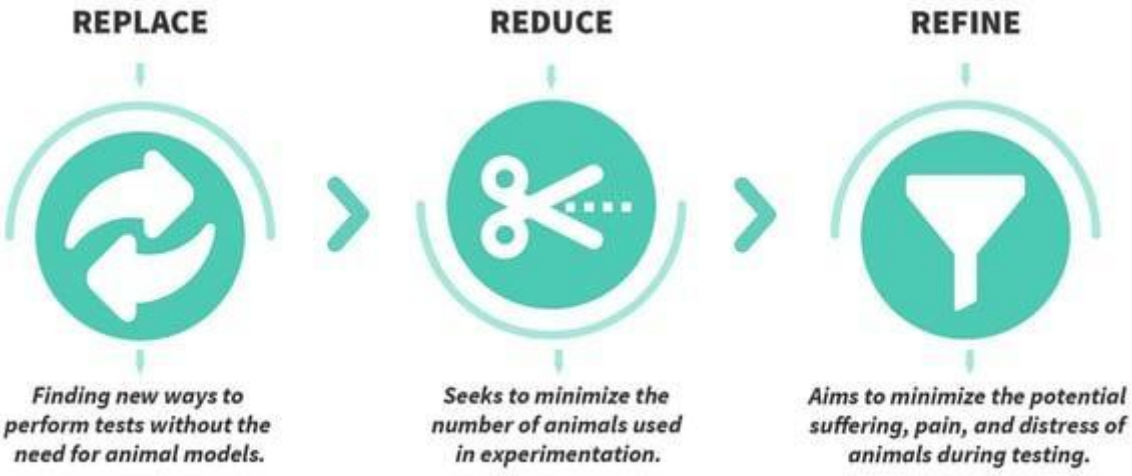

Figure 2 – Simple definition of 3Rs: replace, reduce and refine. (Biobide, n.d.)

However, an important discussion brought up by Herrmann and Jayne, in 2019, in their book "*Animal Experimentation: Working Towards a Paradigm Change*", highlights that even though the 3Rs have greatly contributed to animal welfare and animal law, it is possible that animal suffering has not decreased as much as expected considering all efforts to reduce it.

Alternative methods to animal testing

In addition to complying with replacement, reducing and refinement, the implementation of alternative methods also provides advantages associated with reduction of manpower, time consumed and financial resources (Mushtaq et al, 2018), as animal testing involves the cost of animal breeding/housing for scientific purposes, which reveals high expenses, can be time-consuming and require skilled and trained operators to perform specific experiments (Bédard et al, 2020).

Another great motivation to invest in the development of alternative methods reside in the lack of effective extrapolation, as the results obtained from animal testing must be extrapolated to humans. As animals have several differences in relation to humans, animal testing may fail to present relevant data due to factors such as discrepancies between species, lack of clinical translation, unsuitable methodology, inconsistencies, and publication bias (Silva et al, 2022).

The alternative methods most addressed in the literature reviewed are presented below.

Cell culture and tissue engineering

Cell culture or *in vitro* testing is a method that involves growing cells, obtained from animals or human biopsies, in an artificial environment. cells originating from animal or human biopsies are grown in an artificial environment, which has controlled conditions, including parameters such as pH, osmolarity, temperature, humidity, and gaseous atmosphere (Bédard et al, 2020). Additionally, to the controlled conditions, a suitable medium is also used to provide nutrients (amino acids, vitamins, inorganic salts, and carbohydrates) to the culture (Bédard et al, 2020).

This method is highly used, especially for testing efficacy and toxicity of topical cosmetics, drugs, and chemicals. A great example of use of this method is the substitution of the previously used Draize test, which was performed *in vivo* on rabbit eyes, and presently it is almost completely replaced by in vitro cultured bovine cornea (Mushtaq et al, 2018).

Presented below are two of the main cell culture methods. However, it is important to keep in mind that, whichever method is chosen, a cell culture is not able to completely substitute whole organisms, making animals still necessary to provide information on systemic interactions and physiological reactions (Bédard et al, 2020).

2D Cell culture

The traditional way to apply this method is 2D cell culture. In these systems, a monolayer of cells is grown into a flat dish made of plastic or glass, and a medium of nutrient fluid is used. The advantage of this technique is that it is a relatively easy and fast method to obtain information on cell behaviour, however it has some important limitations, such as not accurately mimicking natural 3D organization of cells and their extracellular matrix (ECM) (Bédard et al, 2020).

Therefore, by not being able to provide accurate cell-cell and cell-ECM interactions, the 2D cell culture method can lead to results that differ from in vivo responses (Bédard et al, 2020).

3D Cell culture

While the 2D cell culture system fails to mimic natural cell and tissue organization as seen in the human body, 3D cell culture overcomes that limitation. This method presents a three-dimensional organization, in which cells are surrounded by ECM, that can generate a more *in vivo*-like environment with heterogeneous access to soluble factors, nutrients and oxygen (Bédard et al, 2020).

Therefore, more accurate cell-cell and cell-ECM interactions are observed in 3D cell culture than 2D cell culture, which results in more reliable and predictable data for human application and may reduce the use of animal testing (Bédard et al, 2020).

In silico testing

This method involves using computer softwares that can assist making decisions, predictions, and hypotheses, that could reduce in vivo testing (Bédard et al, 2020) as these systems and mathematical equations are able to mimic the operation of a human body's structure, function, and metabolism (Mushtaq et al, 2018). The advantages presented by this method include scale-in the number of chemicals that can be tested quickly, the types of endpoints and biological pathways covered, and the range of conditions that can be rapidly simulated (Van Norman, 2020).

Figure 3 – Difference between *in silico*, *in vitro* and *in vivo* methods. (ZeClinics, 2022)

Microfluid chip testing

This technique uses a microchip that contains a series of tiny chambers and channels, and each chamber contains a sample of tissue from different parts of the body. The microchannels that connect the compartments of the chip are filled with a fluid that substitutes the blood flow and nourishes the sample tissues. The data collected from the sensor of the chip is then analyzed by a computer and provide better understanding of a tissue in a micro scale (Mushtaq et al, 2018; Van Norman, 2020).

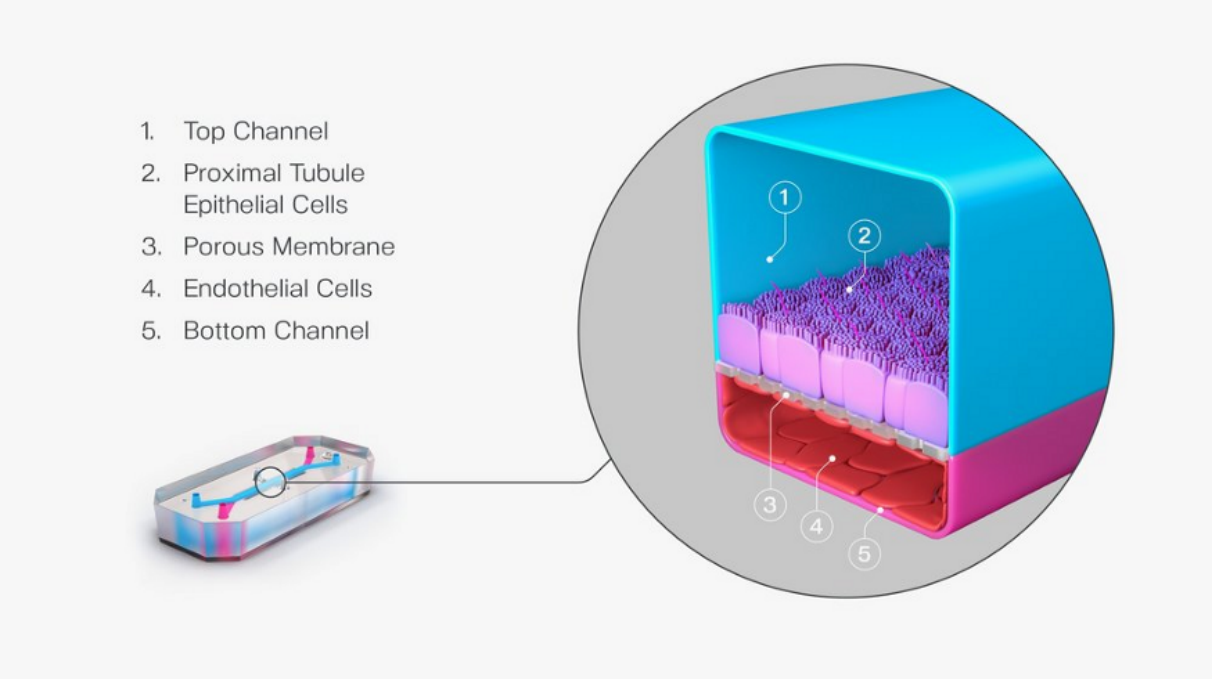

Figure 4 – Example of microfluid chip with kidney cells. (Axial, 2021)

Microdosing

Microdosing as an alternative method involves human volunteers who are given micro doses of drugs that allows for its metabolism in the human body to be studied (Herrmann et al, 2019). The drawback of this method is that, even though it substitutes animals for microdosing tests, those are still needed for full dose testing to provide final recommendations for humans (Mushtaq et al, 2018).

International and Brazilian scenarios

Considering the increase of awareness related to animal welfare, especially in terms of animal testing for cosmetics, many countries and regions have started to apply or reinforce animal testing regulations to either ban or limit the use of animal testing (Sreedhar, 2020).

Below, an overview of the scenarios for some countries is further discussed.

Europe

European Union (EU) was the first region to start animal testing ban, beginning in 2004 for finished cosmetics products, and expanding the ban to cosmetic ingredients in 2009. Still in 2009, EU also prohibited cosmetic products and their ingredients which had been tested in animals to be commercialized in the region Additionally, in 2013, EU extended the marketing ban to products that were exempted previously. (European Union EUR-Lex, n.d.).

Data and information resulting from animal testing which occurred before the bans can continue to be used to address safety of cosmetic products and the Union recognizes that using animal testing methods might still be required whenever alternatives methods are not available. However, if animal testing is required, EU legislation sets high standards for animal welfare and maintains the requirement for animal testing to be replaced, reduced, and refined whenever possible (European Union EUR-Lex, n.d.).

United States of America (USA)

In the United States, animal welfare is mainly regulated by Animal Welfare Act (AWA), which became a law in 1966, and the Policy on Humane Care and Use of Laboratory Animals, dated 1985. AWA addresses general standards for care and treatment of animals that are bred for commercial sale, sold sight unseen (Internet sales), exhibited to the public, used in biomedical research, or transported

commercially (U.S. Department of Agriculture, n.d.). The Policy on Humane Care and Use of Laboratory Animals further addresses animals used for research by establishing guidelines for proper care and treatment of these animals, and organization and operation of animal care committees in institutions that conduct biomedical and behavioral research (Office of Laboratory Animal Welfare, n.d.).

While Food and Drug Administration (FDA) is not the entity responsible for enforcing both Acts, it clearly states its support and adherence to regulation that guides animal welfare regarding testing, to consideration of alternative methods prior to animal testing and the development of those methods as well. However, as FDA is responsible for guaranteeing that products, such as cosmetics, are safe and properly labelled through the Federal Food, Drug and Cosmetic Act, the Agency does not prohibit animal testing if deemed necessary by manufacturers to guarantee safety of their product or ingredient (Food and Drug Administration, n.d.).

Also, each state in the USA is able to have its own regulation in addition to the aforementioned Acts, and some states, such as California, Nevada, Illinois New York and Virginia, already have regulations about animal testing, some which include marketing bans (Farrell, 2021).

Australia

In Australia, guidance on care and use of animals for scientific purposes has been provided by the National Health and Medical Research Council (NHMRC) through the so-called “Code”, which is the Australian code for the care and use of animals for scientific purposes, since 2013. The Code promotes ethical, humane, and responsible care and use of animals for scientific purposes by providing an ethical framework and governing principles when making decisions and actions to all involved with these animals (Australian code for the care and use of animals for scientific purposes, 2013).

In 2021, the Code was amended to address the ban of animal testing for cosmetic chemical ingredients and finished products (National Health and Medical Research Council, n.d.).

China

China is a country of great discussion when it comes to animal testing as those were allowed until recently. In 2014, so called “ordinary cosmetics” – (that

could include shampoo and mascara, for example) manufactured and sold inside China were allowed to avoid animal testing (Farrell, 2021).

Only in 2021, the National Medical Products Administration (NMPA) allowed animal testing not to be mandatory for imported products that were in accordance with certain requirements, such as: proving product country-of-origin, that the product is not for children and that certain raw materials were are not present in the product (Farrell, 2021).

New Zealand

In New Zealand, the ban of the use of animals for developing and producing products, or testing ingredients intended only for cosmetic use, occurred under the Animal Welfare Act in 1999. The Act has been amended ever since and recently updated to a 2022 (Sreedhar, 2020; New Zealand Parliamentary Counsel Office, n.d.).

Norway

The ban of cosmetics tested on animals in Norway occurred in 2019, after the EU (including Norway) banned the selling of these products. Therefore, in addition to banning tests on animals for new products, the new rules also applied to development of products already in the market. Pharmaceutical products, however, were not included in the ban (Sreedhar, 2020; Pereira, 2018; The Norway Post, 2019).

Israel

Israel was the third country to prohibit animal tests for production, sale and promotion of cosmetics, toiletries, and household cleaners. The ban occurred within the country in 2007 and, additionally, in 2013 imported products that have been tested on animals in other countries were also banned (Sreedhar, 2020; Pereira, 2018).

India

In 2014, a new rule was added to the existing *Drugs and Cosmetics Act* of 1945 by the Ministry of Health and Family Welfare, banning cosmetic testing on animals. Later that year, the import of products tested on animals was also banned (Sreedhar, 2020).

Brazil

The first breakthrough of Brazilian regulation regarding animal welfare and experimentation was Federal Decree n° 24.645, in 1934, which foresaw imprisonment and fines to any act of cruelty towards animals, including also scientific practices, even if addressed in a subjective manner. Later, in 1998, Federal Law nº 9.605 established imprisonment and fines to any individual to perform experiments that implies suffering of living animals when alternative resources are available.

In 2008, one of the most important federal laws was created. Federal Law nº 11.794 was the first law that specifically addressed animal testing, which created the National Council for the Control of Animal Experimentation (CONCEA - *Conselho Nacional de Controle de Experimentação Animal*, and obligation that institutions have Ethical Commissions for Animal Use (CEUAs - *Comissões de Ética no Uso de Animais*). Additionally, Federal Law nº 11.794 prohibits an animal to be used multiple times for different research, requires use of anesthesia and any practice that implies animal suffering.

2014 was a productive year for Brazilian regulation related to animal welfare as CONCEA published two Normative Resolutions, nº 17 and nº 18, which addresses alternative methods. Additionally, in 2018, Normative Resolution nº 38 addressed the use of animals in observational activities.

Presently, Normative Resolutions nº 18/2014, nº 31/2016, nº 15/2019 and nº 56/2022, recognize and make official around 40 alternative validated methods that aim to substitute, reduce, and refine the use of animals in research settings (Ministério da Ciência, Tecnologia e Inovações/Conselho Nacional de Controle de Experimentação Animal, 2014, 2016, 2022).

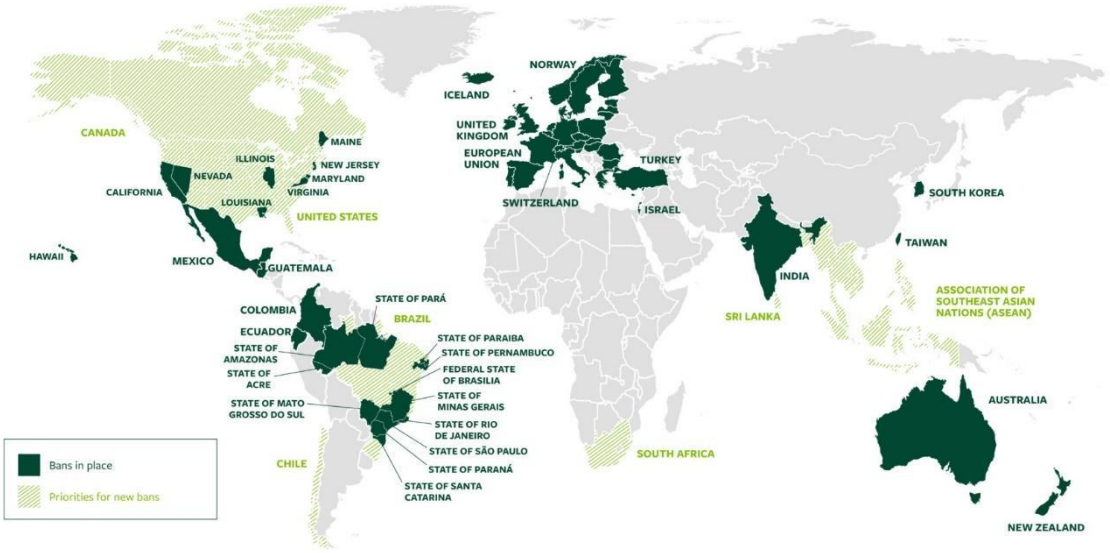

Figure 5 – Map of countries related to animal testing ban. (Humane Society International, n.d.)

Cruelty-free – definition and certifications

Essentially, cruelty-free refers to products and ingredients that have not been tested in animals throughout their development process (Sarmento, 2019; Pülm, 2021). Legally, however, when discussing cosmetics, there isn't a clear definition for the cruelty-free term (Farrell, 2021).

In agreement with the global growth of animal testing bans and limitations and increase of concern for animal welfare, certification systems, campaign and training programs are of great importance to further progress of cruelty-free cosmetics. (Farrell, 2021). Examples of certifications are Beauty Without Bunnies, created by nonprofit organization People for the Ethical Treatment of Animals (PETA), and the Leaping Bunny cruelty-free logos and the Leaping Bunny program, created by Coalition for Consumer Information on Cosmetics (CCIC). By having certified products, companies can include cruelty-free logos in labels which function as shopping aids (Pülm, 2021) so customers can easily spot cruelty-free products, besides explaining the fact that those were not tested in animals.

However, the absence of legal definitions and guidelines associated with the cruelty-free term, as well as the existence of important animal testing bans, many companies around the world still engage in the use of animal testing methods and

are able to label their products as cruelty-free, while they actually are not (Pülm, 2021). Additionally, an example of this kind of misuse of the cruelty-free label could be cosmetic products, and respective ingredients, not being tested in animals but still being commercialized in China, a country where testing of imported products was mandatory until recently, as mentioned in the previous section.

Figure 6 – The Leaping Bunny cruelty-free logo. (Leaping Bunny, n.d.)

Figure 7 – PETA cruelty-free logos. (PETA, n.d.)

Safety guarantee by alternative methods

Multiple tests are available to manufacturers to perform safety tests and assessments. Some of the most relevant categories, which have presented important advances (Díez-Sales et al, 2018), are described below:

Acute toxicity

Acute toxicity testing is the first stage of safety assessment and aims to obtain results related to a substance's intrinsic toxicity by estimating the lethal dose at which a substance will lead to the death of 50% of the test population (LD_{50}). As this kind of test depends considerably on *in vivo* experiments, the proposed alternative methods, such as Organisation for Economic Co-operation and Development (OECD) test OECD Guidance Document (GD) 129, cannot be the only testing method considered. However, a testing strategy associated with *in silico* methods may be able to provide an assessment compliant with the 3Rs. The use of microfluid chips to simulate human tissues and organs is considered a good option for the future, as several validation procedures are required of such a cell based assay (Silva et al, 2022).

Skin corrosion/irritation

Skin corrosion and irritation tests evaluate substances that generate local effects, meaning that it is able to produce alteration at first point of contact. A substance is considered corrosive if it leads to irreversible destruction of a tissue, and irritant if the effects are reversible (Silva et al, 2022).

Alternative methods available, such as OECD Test Guideline (TG) 431 and OECD TG 439, consist of multiple tests using three dimensional systems that mimic human skin (reconstructed human epidermis) (Silva et al, 2022).

Eye irritation

Such as skin irritation, eye irritation consists of reversible damage caused to the eyes by a certain substance, however considering a 21-days period of exposure. If a substance generated irreversible damage to the ocular tissue or deterioration of vision, within the same 21-days period of exposure, then the damage is considered as severe/serious (Díez-Sales et al, 2018; Silva et al, 2022).

The main test used to determine eye irritation is the Draize test, which is an animal model test performed in rabbits' eyes. Even though there isn't, until now, a single alternative test that is able to substitute the Draize test, some tests were included in the international recommendations, such as OECD TG 437 and OEDCF TG 438 (Díez-Sales et al, 2018; Silva et al, 2022).

Skin sensitization

A skin sensitizer consists of a substance that causes an allergic response; however, skin sensitization (or allergic contact dermatitis) may not initially generate a

skin reaction. Subsequent contact with the substance is mostly when the reaction is provoked and observed (Díez-Sales et al, 2018; Silva et al, 2022).

As skin sensitization is a complex biological process, which involves multiple factor and events, until recently there wasn't a suitable replace to animal testing. In 2021, the OECD 497 was published, defining alternative approaches for this kind of test (Díez-Sales et al, 2018; Silva et al, 2022). Within OECD 497, other OECD testing guidelines such as testing guidelines 442C, 442D and 442E (Silva et al, 2022).

Repeated dose toxicity

Long-term exposure to a substance can lead to chronic toxicity, which occurs due to persistent or progressively dysfunction from cell-level to whole organ systems. OECD tests to address chronic toxicity are available, such as OECD TG 407 to OEC TG 413 (Díez-Sales et al, 2018).

Genotoxicity/mutagenicity

While the term genotoxicity is more general, and consists of modification of the structure, information content or segregation of genetic material caused by an agent or conditions; mutagenicity refers to permanent changes to genetic material (Díez-Sales et al, 2018; Silva et al, 2022).

Guidelines already made available by OECD are bacterial reverse mutation test (OECD TG 471, also known as the Ames test), in vitro mammalian cell gene mutation test (OECD TG 476), in vitro micronucleus test (OECD TG 487) and in vitro mammalian chromosome aberration test (OECD TG 473) (Díez-Sales et al, 2018; Silva et al, 2022). Even though multiple alternative methods are available, animal testing is still required to guarantee that substances do not cause *in vivo* mutagenicity (Silva et al, 2022).

UV-induced toxic effects (specifically phototoxicity)

Phototoxicity (or acute phototoxicity) occurs when skin irritation and sensitization is observed after a substance gets in direct contact with the skin or reaches the skin after being systemically absorbed and is then activated by incident radiation (Díez-Sales et al, 2018; Silva et al, 2022). Alternative methods presently available, such as OECD TG 432, are able to substitute animal testing (Díez-Sales et al, 2018).

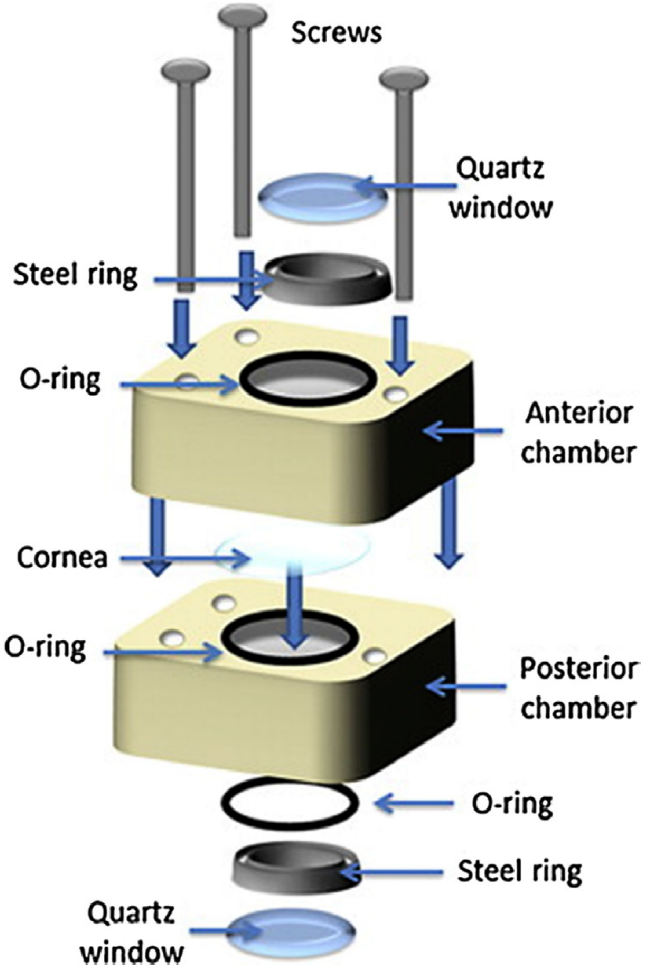

Figure 8 - Testing chamber utilized in bovine corneal opacity permeability test. (Wilson et al., 2015)

Even though a general scenario was addressed up until this point, the following paragraphs will address Brazilian safety testing only as it is the most relevant for this review.

CONCEA is the entity responsible for regulating alternative methods to animal testing in Brazil and, the validation of these methods is under the responsibility of the Brazilian Center for Validation of Alternative Methods - braCVAM (BRACVAM, n.d).

The methods recognized by CONCEA in the Resolutions mentioned previously are in accordance with the Organisation for Economic Co-operation and Development, as Brazil is one of the accession candidates and key partners of the Organisation (OECD, n.d.).

All OECD methods recognized by CONCEA within Normative Resolutions nº 18/2014, nº 31/2016, nº 15/2019 and nº 56/2022 are compiled in Appendix A. The test categories addressed so far are skin corrosion and irritation, ocular corrosion and irritation, phototoxicity, skin absorption, skin sensitization, acute toxicity,

genotoxicity, reproductive toxicity, estrogenic effects, endocrine effects, androgenic effects, mutagenicity, photoreactivity, effects on biotic systems, pyrogenic contamination in injectables.

Ethical consumption

As cosmetics are of great importance in people's daily lives, the cosmetic industry and its products would not be able to escape the increase in awareness of environmental impact. Thus, a demand from customers to acquire more natural products, with no to low environmental impact has been leading companies of the cosmetic industry towards production of such items (Rawolf, 2021).

Even though there isn't a clear and final definition of sustainability, in the cosmetics industry it is associated with products that meet customer needs without causing harm to the environment, animal suffering and negatively impacting natural resources (Rawolf, 2021).

In alignment with that definition, ethical consumption occurs when a customer actively purchases a product considering companies' social responsibility and avoids those that maintain unethical practices. Alaouir et al (2019) brings up some of the decisions made by customers when purchasing products, such as concern for fair trade goods, environmental-friendly, animal welfare and labour conditions.

Discussion

All discussions and current international scenario surrounding animal testing, alternative methods and animal welfare started with Russel and Burch, with the publication of "The Principles of Humane Experimental Technique", which brough to the world the 3Rs - Replacement, Reduction and Refinement.

The path towards development of methods to change the animal testing scenario, by implementing practices aiming to replace animal models, reduce the use of animals and refine the current methods using animals, started to be traced firstly by Europe Union. The EU was the first region to establish testing and marketing bans, which was followed by several other countries. Even though, a great part of the world still does not have animal testing or marketing bans to products tested in animals in place or even under discussion, great progress is observed as countries such as United States of America, Australia, New Zealand, and some parts of South America are moving towards animal welfare awareness.

As development of alternative methods grow, great motivators and advantages are noticed when comparing those to animal testing. Important factors as reduction of manpower, less time-consuming tests and requirement of less financial resources are the initial main motivators; however, it is important to also highlight the limitation of animal testing, such as differences between humans and animals can interfere with generation of relevant data in animal studies that can accurately be extrapolated to human.

Presently, the main alternative methods consist in cell culture, which can be 2D or 3D, and tissue engineering; *in silico* methods; microfluid chips; and microdosing. In addition to those, other kinds of alternative methods are also available, but were not discussed in this review. Those tests are of great importance to guarantee the safety of cosmetic products that aim to reach the market, as regulation across the globe has been tightening and requiring more and more the replacement of animal models. The categories of alternative tests mostly discussed include skin irritation and corrosion; skin sensitization; eye irritation; genotoxicity and mutagenicity; chronic toxicity; and phototoxicity.

Currently, in Brazil, which is the most relevant country for this review, around 40 alternative methods validated by the OECD are recognized by CONCEA, the entity responsible for overseeing and enforcing the development and application regulation related to alternative tests.

In alignment with the discussion regarding alternative methods to animal testing, it is important to recognize the ascension of cosmetic products labelled as “cruelty-free”, which refer to products (and ingredients as well) that have not been in animal throughout its development process. Still, as this term does not have a legal definition, organizations have created certifications to assist identification of cruelty-free products and promoting alternative testing, such as Beauty Without Bunnies, created by nonprofit organization People for the Ethical Treatment of Animals (PETA), and the Leaping Bunny cruelty-free logos and the Leaping Bunny program, created by Coalition for Consumer Information on Cosmetics (CCIC). Another matter related to the absence of a legal definition lies with the fact that it allows companies to label their products as cruelty-free without being.

Finally, another great discussion that follows along with the topics presented previously, is ethical consumption. The increased concern of customers to purchase

environmental-friendly products, in which animal welfare is a highly considered factor, impacted the cosmetic industry to adapt to new demand and work towards producing products that refrain from causing harm to the environment, animal suffering and negatively impacting natural resources.

Conclusion

Addressing the question raised by the theme of this review about alternative testing reveals, indeed, a work in progress. Moving towards complete replacement of animal testing relies on animal welfare as a main motivator, but relies also on economics, time saving testing, and products safety guarantees. It is about the possibility of providing accurate safety results through tests that, aside from not promoting animal suffering, should properly reflect interactions and reactions of the human body.

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APPENDIX

Appendix A – Table containing compilation of alternative methods recognized by CONCEA.

Alternative Method	**Category**
OEDC TG 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)	Skin Corrosion and Irritation
OECD TG 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method	
OECD TG 435: In Vitro Membrane Barrier Test Method for Skin Corrosion	
OECD TG 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method	
OECD TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Ocular Corrosion and Irritation
OECD TG 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	
OECD TG 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants	

OECD TG 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	
OECD TG 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage	
OECD TG 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	
OECD TG 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	
OECD TG 432: In Vitro 3T3 NRU Phototoxicity Test	Phototoxicity
OECD TG 428: Skin Absorption: In Vitro Method	Skin Absorption
OECD TG 429: Skin Sensitization	Skin Sensitization
OECD TG 442A and 442B: Skin Sensitization	
OECD TG 442C: In Chemico Skin Sensitization	
OECD TG 442D: In Vitro Skin Sensitization	
OECD TG 442E: In Vitro Skin Sensitization	
OECD TG 420: Acute Oral Toxicity - Fixed Dose Procedure	Acute Toxicity
OECD TG 423: Acute Oral toxicity - Acute Toxic Class Method	
OECD TG 425: Acute Oral Toxicity: Up-and-Down Procedure	

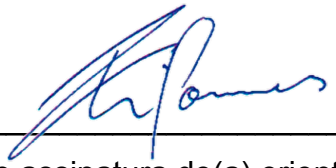
OECD GD 129: Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systematic Toxicity Tests	
OECD TG 487: In Vitro Mammalian Cell Micronucleus Test	Genotoxicity
OECD TG 421: Reproduction/Developmental Toxicity Screening Test	Reproductive Toxicity
OECD TG 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	
OECD TG 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists	Estrogenic effects
OECD TG 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity	
OECD TG 456: H295R Steroidogenesis Assay	Endocrinal effects
OECD TG 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals	Androgenic effects
OECD TG 471: Bacterial Reverse Mutation Test	Mutagenicity
OECD TG 473: In Vitro Mammalian Chromosomal Aberration Test	
OECD TG 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes	
OECD TG 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene	
OECD TG 495: Ros (Reactive Oxygen Species) Assay for Photoreactivity	Photoreactivity
OECD TG 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	Effects on Biotic Systems

OECD TG 236: Fish Embryo Acute Toxicity (FET) Test	
OECD TG 319-A: Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)	
OECD TG 319-B: Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)	
Bacterial Endotoxin Test	Pyrogenic Contamination in Injectables
Monocyte Activation Test	

São Paulo, 21 de outubro de 2022.

Tainá Lourenço

Data e assinatura do(a) aluno(a)



Data e assinatura do(a) orientador(a)