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Historical Analysis of Cosmetics Testing Development and Alternatives

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Abstract: The cosmetics industry is diverse, encompassing products aimed at cleansing, beautifying, and altering appearance. Within this realm, the concept of cruelty free cosmetics has gained prominence, denoting products that eschew animal testing throughout development and production. While certifications like the Leaping Bunny Program offer assurance, discrepancies in interpretation and enforcement persist, leading consumers to scrutinize labels for authenticity. Moreover, distinctions exist between cruelty free, vegan, vegetarian, and organic cosmetics, each reflecting varying degrees of animal derived ingredients and production standards. Despite the functional necessity of animal testing for assessing safety and efficacy, ethical concerns have fueled debates on its justification. While some argue for its scientific validity and regulatory utility, others criticize its ethical implications and advocate for alternative testing methods, citing issues of reproducibility and relevance. The emergence of the 3Rs principle: Replacement, Reduction, and Refinement; proposed by Russell and Burch in 1959, underscores efforts to minimize animal usage while advancing scientific progress.

Historically, public outcry and activism have shaped regulations and practices surrounding animal experimentation, prompting initiatives like the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) to enforce ethical standards. Despite acknowledgment of the necessity of animal research for human welfare, calls for innovation and collaboration underscore the pursuit of alternative methods and the enhancement of animal welfare standards in laboratories. Advancements in biotechnology have spurred the development of in vitro models, offering promising alternatives to animal testing. These methods, ranging from organ and tissue cultures to computer simulations, present cost effective and scientifically robust options for toxicity testing and research. Acknowledgment of the biological complexities inherent in in vitro models underscores the importance of careful preparation and maintenance for reliable research outcomes.

In summary, the cosmetics industry stands at a crossroads, balancing scientific innovation with ethical considerations regarding animal testing. As stakeholders continue to advocate for cruelty free practices and alternative testing methods, collaboration and innovation offer avenues for advancing both scientific excellence and animal welfare.

Keywords: Alternative, Animal testing, Cosmetics, Cruelty free.

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INTRODUCTION

Any item meant to be applied to, poured, sprayed, sprinkled, or otherwise applied to the human body or any portion of it in order to clean, beautify, enhance attractiveness, or change appearance is referred to as "cosmetic," and this definition includes any item meant to be used as a component of cosmetics.[1] Cruelty-free cosmetics refer to products that haven't undergone animal testing, although interpretations can vary. While a final product might not be tested on animals, its ingredients could have been. Different certifying bodies have varying levels of tolerance for animal testing throughout production.[2] The Leaping Bunny Program, recognized in the United States and Canada, certifies products free from animal testing in all development stages.[3] Consumers should scrutinise what the cruelty-free label truly means, as some companies exploit loopholes. For example, major brands like Makeup Art Cosmetics (hereinafter M.A.C), Maybelline, and Estee Lauder may still test on animals if mandated by law. People for Ethical Treatment to Animals (hereinafter PETA) certified cruelty-free companies based on signed assurances or statements confirming no animal testing now or in the future.[4] As of 2020, no legal definition exists for "cruelty-free," making the PETA logo a reliable indicator.[5] Natural cosmetics are devoid of ingredients deemed unacceptable by a company, with exclusions varying among companies. Ethical products ensure fair treatment of the community, including customers, employees, and partners involved in product development and supply. Vegan cosmetics contain no animal products or by-products and can be certified by organisations like PETA, contingent on brand transparency and accuracy in disclosure during certification.[6] Vegan products exclude any animal-derived ingredients, while 100% vegetarian products refrain from using ingredients derived from animals, though they may include substances produced by animals like honey, beeswax, or milk.[7] Organic cosmetics are certified by bodies like the USDA and differ in the standard percentage of organic ingredients required.

Animal testing is to be functional because of certain factors such as to analyse and evaluate the safety and efficacy of cosmetics, exposure risk to chemical ingredients, development and testing of cosmetics, drugs, vaccines, medicines etc. As well as to stretch the medical knowledge. Few of these experiments were conducted because of global regulations significantly desired animal testing. The products to be sold should firstly have their safety and security continuously evaluated; this was the requirement accomplished by the nationalisation of animal experiments. Some people argue with the principle that no one wants to unimportantly harm their life with the purchase of a new product. Several scientists argue that animal testing remains an authentic key in safety assessment of cosmetics along with the modern alternative testing methods. At the same time, the antagonists argue that it figures out poor science and often down the conventional criteria of reproducibility, reliability and relevance.[8] Animal testing generally functions based on the Miners Canary principle. Animal experiments may also give a peaceful mind for the regulatory bodies while approving new cosmetics or drugs. Due to the advancements in knowledge and problem solving and eradicating the medical issues and new innovations, demand increases. Furthermore, there is a need to make sure such products and technologies are safe as far as possible. Moreover, even though there has been huge advancement in scientific and

[1] Ministry of health and family welfare, 'The Drugs and Cosmetics Act and Rules' (1964).
[2] Katelyn Urban, Rachel Giesey and Gregory Delost, 'A Guide to Informed Skincare: The Meaning of Clean, Natural, Organic, Vegan, and Cruelty-Free' (2022) 21 Journal of Drugs in Dermatology 1012.
[3] 'The Leaping Bunny Difference | Leaping Bunny' (*www.leapingbunny.org*) <https://www.leapingbunny.org/leaping-bunny-difference>.
[4] Sakshi Yadhav, 'Cruelty in Cruelty-Free Cosmetics: Is an Indian Consumer Contributing towards Cruel, Unethical Cosmetic Companies?' (2020) 8 International Journal of Creative Research Thoughts (IJCRT)1111 <www.ijcrt.org>.
[5] Peter Singer, 'Animal Liberation: A Personal View' (1986) 2 Between the Species: An Online Journal for the Study of Philosophy and Animals.
[6] Gary L Francione, 'Animal Rights Theory and Utilitarianism: Relative Normative Guidance' (2003) 13 Between the Species: An Online Journal for the Study of Philosophy and Animals.
[7] Peter Singer (n 5).
[8] Dr. O P Sood and Dr. Ashok Rattan, *Ethics in Animal Experimentation:Proceedings of the 13th Round Table Conference Held at New Delhi*, vol. 13 (Ranbax science foundation 2004).

medical knowledge has led to the progress in the health system, scientists should evaluate themselves whether they are ethically and morally allowed to let animals suffer for our well-being. In addition to the ethics, there also exists the use of 3R models. Most of the models are cheaper and scientifically good and fit to answer the raises about the effect on the health of humans.[9] Utilisation of animals for the development of cosmetics is an important issue for the extremist of animal experimentation, as the cosmetics industry probably harms and exploits the huge scales of animals in the screening process of ingredients for probable safety and efficient value.[10] During the mid 70s centuries in Europe and America there seems to be an increase of public interest in environmental concerns and use of animals in experimentation. The questioning of discourse of progress then revealed the new hierarchy between sanctity of biological life and scientific method materialism and this paved the way for awareness about the ethics of inflicting pain. There is great letter writing, campaigns; protests and riots against the use of animals in biomedical research.[11] A civil right activist known as Henry Spira who was a former union organiser conducted a humane protest against experiments on animals and managed to extract $750,000 from Revlon to fund the research of alternative methods to the Draize test. Even the dangerous analgesics and Draize test were practised by the sophisticated multinational pharmaceutical companies.[12] Prior to 1996 the general consensus that there were many industries in the country which were not keeping their experimental animals under the suitable conditions and the required ethical legislations for animal testing were not observed and that the formulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (hereinafter CPCSEA) and the rules enforced between 1996 to 2002 has updated much fruitful information's of the importance of "Ethics of Animal Experimentation" that it was important to enforce the system for regulating the ethical use of animal testing. It might be noted that the use of animals in research is inevitable in the interest of animal welfare and humans and cannot be abandoned in the name of prevention of cruelty to animals. In 1959, William Russell, a zoologist, psychologist, and classical scholar, and Rex Burch, a microbiologist, proposed the three R's of animal research: Replacement, Reduction, and Refinement. These principles form the basis of most animal research policy and practice.[13] We need strategies and ideas to foster agreement rather than disagreement. The beauty of the three 'R's is that they offer a way for all parties to collaborate in order to advance the cause of both animals and humans. They begin with the assumption that there will be animal research, but they leave open the possibility that science may advance to the point where it would no longer be necessary.[14] There is no benefit to forcing laboratories to close, as this will only hinder worthwhile research and development.[15] Thus, the necessity of the hour is ingenuity rather than confrontation, which will lead us nowhere. All experiments must be guided by the principle of proportionality: the advantages and disadvantages of vivisection must be balanced; the morality of sacrificing a baby to save the mother's life is likewise demonstrated by the fact that inflicting a small amount of pain and suffering on a small number of animals in order to relieve millions of humans is also perfectly acceptable.[16] Scientists and experts on animal welfare both agree that the ultimate goal of both groups is the welfare of society as a whole, which includes the welfare of animals. Starting with this shared objective would be one way to find a solution. An open and efficient exchange of information is necessary to address the concerns raised by experts in animal welfare, and a concerted effort is needed to enhance both the housing conditions and the quality of the animals used in experiments. The development of alternative methods and research into them should be actively encouraged. Relevant projects should be quickly reviewed and approved, and scientists should receive priority training in humane

[9] Sakshi Yadhav (n 4).
[10] Nick Jukes, Mihnea Chiuia and Interniche, *From Guinea Pig to Computer Mouse : Alternative Methods for a Progressive, Humane Education* (Interniche 2003).
[11] Bernard E Rollin, *The Unheeded Cry* (Ames Lowa University Press 1989) 330 330.
[12] Peter Singer, *Ethics into Action: Henry Spira and the Animal Rights Movement* (Rowman & Littlefield Publishers, Cop 2000).
[13] William Moy and Rex Leonard Burch, *The Principles of Humane Experimental Technique* (1959).
[14] R Smith, 'Animal Research: The Need for a Middle Ground' [2001] Selection from BMJ South Asia Edition 205.
[15] Nick Jukes, Mihnea Chiuia (n 10).
[16] Magendira Mani Vinayagam, 'Ethics for Animal Experimentation' www.academia.edu <https://www.academia.edu/4768544/Ethics_for_animal_experimentation>.

animal experimentation.[17] Several in vitro methods can be utilised to minimise or replace the experimentation in animals. These methods may be the living or non-living methods. The living methods are organ culture and tissue culture and in certain rare limited cases, human volunteers, microorganisms and lower animals were included. The chemicals, computer simulations, recombinant DNA technology, mathematical models, mechanical models… can be included in non-living methods as an alternative for animals.[18] In vitro toxicology generally refers to the study of toxicological phenomena in non-whole animal models, despite the fact that the term literally means in glass. This wider meaning of "in vitro" encompasses investigations using tissue slices, isolated primary cells, isolated organs, cell lines, and subcellular fractions (e.g. g. mitochondria, vesicles in the plasma membrane, microsomes, etc.). These model systems have significantly advanced the field of toxicological sciences, especially in terms of our comprehension of toxicity mechanisms.

Xenobiotic metabolism and variations in toxicity expression among species, to name a few. The usefulness of in vitro systems in toxicity testing has been extensively acknowledged and investigated during the past 20 years.[19] New in vitro models for toxicological research are being developed quickly as a result of the recent focus on in vitro models brought about by the quick advancements in biotechnology, the economic costs of conducting an adequate toxicological evaluation of novel industrial chemicals and commercial products, and societal concerns about animal welfare.[20] The biological system is an essential component of the in vitro approach, regardless of whether it is used as a model for investigative research or as a toxicity test for risk assessment. A satisfactory research outcome depends on the successful preparation and maintenance of the appropriate cell, tissue organ, or embryo for a given scientific objective.[21] In 1959, Russell and Burch argued that there is a close relationship between human use of laboratory animals and scientific excellence. They begged that the three R's be applied, i. e., Minimization, Enhancement, and Substitution of animal testing. Alternatives are required due to social and economic problems. Small institutions cannot afford the significant expenditures required to maintain a laboratory animal house in accordance with standard guidelines. A few potential alternatives include: enhancing test design; utilising in vitro methods; employing non-vertebrate animals; simulating physiological and biochemical processes mathematically; and enhancing the dissemination of research findings.[22]

HISTORY OF ANIMAL TESTING

Animal testing, also known as animal experimentation, played a key role in medical knowledge and scientific advancements which contributed to various innovations which have moulded healthcare. Animal studies have been constitutive for the discoveries such as from the initiation of insulin therapy and progress of antibiotics for diabetes to progress in anaesthesia, advancements in vaccines and development of mental health treatment and medical attention and therapeutics using lithium. Furthermore, the investigation as well as examination on animals give rise to progress in considering the biological processes and hormones, rectification of surgical techniques and modelling of medical devices. Regardless of these ethical concerns and endowments adjoining experiments on animals, critics disagree that

[17] Sakshi Yadhav, (n 4).
[18] 'Guideline for Care and Use of Animals in Scientific Research. Indian National Science Academy, New Delhi, First Edition:1992, Revised Edition: 2000'.
[19] *Bhonde, R.R. (1989). Differential Virus Susceptibility of Reptilian Organ Cultures to Human Viruses. In: Ahne, W., Kurstak, E. (Eds) Viruses of Lower Vertebrates. Springer, Berlin, Heidelberg. Https://Doi.org/10.1007/978-3-642-83727-2_13.*
[20] RR Bhonde, UV Wagh and NP Gupta, 'Replication of Non-Respiratory Viruses in Tracheal Organ Cultures.' (1983) 64 PubMed 1.
[21] Y.M Shewade, M Umrani and R.R Bhonde, 'Large-Scale Isolation of Islets by Tissue Culture of Adult Mouse Pancreas' (1999) 31 Transplantation Proceedings 1721.
[22] Kook Hyun Lee, Dong Won Lee and Byeong Chul Kang, 'The "R" Principles in Laboratory Animal Experiments' (2020) 36 Laboratory Animal Research.

animals undergo feasible injuries, sufferings and confinement raise objections to the scientific validity of making use of non-human subjects to anticipate human responses precisely.[23]

The dominant humanist perspectives about animals throughout the era under consideration were significantly impacted by Christian doctrine and Cartesian philosophy. According to the Christian perspective, animals were seen to be morally neutral and lacking in reason, whereas humans were special since they possessed both a soul and reason. Centuries before, St. Thomas Aquinas expressed a similar viewpoint, highlighting how superior humans are to other animals. Even though they were acknowledged as valuable members of God's creation and worthy of respect, animals were ultimately seen as things meant to serve human needs.[24] The Christian church did not, however, support needless animal abuse. Respect for some animals—like the dove in Christian devotion—and appreciation for people—like St. Francis of Assisi—who cared for animals—showed that they understood their importance. But if it accomplished a greater good, even inadvertent animal suffering was not seen as intrinsically bad. Under this viewpoint, animal suffering might be tolerated when necessary for knowledge acquisition, such as during experiments.[25] Anthropocentric views towards animals were further strengthened with the advent of Cartesian philosophy.[26] René Descartes put out a mechanistic theory that described animals and humans as intricate devices subject to the rules of mechanics. Descartes claimed that only humans were superior to other animals while acknowledging the physical similarities between humans and other animals.[27] These characteristics included awareness, logical reasoning, free choice, and real language. Animals were reduced to being inert machines that were not capable of feeling true pain or awareness. When it came to interpreting animal screams and behaviours as mechanical reactions rather than genuine indicators of pain, vivisectionists could ignore the suffering of their subjects since Descartes' theory justified it.[28] The scientific community adopted this mechanical perspective, which influenced attitudes towards animals and provided justification for their use in research. Scholars have argued for a re-evaluation of Descartes' ideas on animal awareness, despite the fact that interpretations of his position have changed. John Cottingham[29], for example, offered a more complex interpretation of Descartes' philosophy by refuting the idea that animals lack consciousness altogether. The antagonists argue that the circumstances that animals undergo during experiments cannot be legitimate despite the inherent welfare. The universal outlook of animal testing looks at obstacles, for instance lower contributions of lab monkeys because of export restrictions by several countries. This shortage has occurred simultaneously with high stipulations throughout the time of Covid 19 vaccine research, focusing attention on the ethical conundrum of acquiring research animals and convolutions. Animal welfare Act in the United States implemented by the Department of Agriculture lays the foundations of directives for the handling of animals in laboratories.[30]

There is an emerging observation of alternatives in several institutions inclusive of enlargement of micro devices which makes use of cell culture. These devices put forward a greater extent of compassionate attitude, presuming accurate details about how divergent products might influence human physiology, probably diminishes the ethical dilemmas correlated with animal testing. The ethical dilemmas of animal testing keep going, but its literal involvement to medical and scientific progress are unquestionable. As technology moves forward, the researchers endeavour to assault an equilibrium state between ethical deliberations and scientific developments by investigating alternative methods that reduce or minimise the use of live animals in experiments.

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[23] 'Animal Experimentation | Databases Explored from Gale' (*www.gale.com*2019) <https://www.gale.com/intl/databases-explored/social-issues/animal-experimentation>.
[24] Andrew Linzey and Paul AB Clarke, *Animal Rights : A Historical Anthology* (Columbia University Press).
[25] Vaughan Monamy, *Animal Experimentation : A Guide to the Issues.* (3rd edn., Cambridge University Press 2017) 11 11.
[26] *Bacon, F. 1605/2001. The Advancement of Learning (1605), Gould S.J. (Ed.). New York: Random House.*
[27] *Descartes, R. 1637/1984. Discours de La Méthode, Trans. Curtis, D. London: Grant and Cutler.*
[28] Leonora Cohen Rosenfield, *From Beast-Machine to Man-Machine. The Theme of Animal Souls in French Letters from Descartes to La Mettrie, by Leonora Cohen Rosenfield. With a Preface by Paul Hazard,... Submitted... In the Faculty of Philosophy, Columbia University* (1940).
[29] John Cottingham, '"A Brute to the Brutes?": Descartes' Treatment of Animals' (1978) 53 Philosophy 551.
[30] Nuno Franco, 'Animal Experiments in Biomedical Research: A Historical Perspective' (2013) 3 Animals 238.

Certain animal right protests be convinced by the fact that the well being of animal testing never be give a justification for barbarism associated with, without being affected by the attempts to minimise the sufferings and discomforts that animal subjects undergo and decrease the scales of animals pre-owned in scientific studies. To exclude animals from being using in researches, certain fundamentalist clusters of promoters summons to terminate all experiments on animals that have taken part in misconduct.[31] During the latter part of 1970s the animal right evolutionists began targeting the research facilities and companies utilising radical plan of action to disorganised these industries and support their activists tenets. These extremists were occasionally known as "Eco terrorists" by associates of Animal liberation front (hereinafter ALF) like revolutionists groups and federal authorities.[32]

EVOLUTION OF COSMETICS INDUSTRY

The historical background of modern cosmetics and cosmetology originated from several branches of pure and applied science such as chemistry, bacteriology, dermatology, physiology, biology etc. Based on several factors historical development of cosmetics industry can be classified into five phases:[33]

1. Religious phase
2. Medical phase
3. Disorganisation period
4. Rehabilitation and revival
5. Cosmetics: art to science

Religious phase (To 5th century BC)

In the earliest historical phases, cosmetics were intricately linked with religious practices, with the origins of cosmetic knowledge believed to stem from the Jewish people during their exile in Egypt. The Old Testament provides substantial evidence that the Jewish people not only acquired this knowledge but also elevated it during their early development. A comprehensive understanding of this period requires an exploration of the history of cosmetics in conjunction with the history of medicine.[34]

A scrutiny of historical records reveals the use of unique substances such as the 'blood of ibis' (derived from cats and crocodiles), 'tail of scorpion,' and 'nails of rats' in both medical and cosmetic formulations. Ancient scholars like Dioscorides and Zozimos have meticulously documented many of these ingredients. Cosmetic artefacts discovered in tombs across various countries from this era exhibit notable similarities, indicating a widespread cultural adoption of cosmetic practices. Skin colouring found diverse applications through the use of henna and litmus, while white lead was employed for skin whitening. Hair dyeing in societies worldwide utilised substances like henna, indigo, and sticks, with kohl being a favoured choice for eye adornment.[35]Historical accounts point towards a convergence of cosmetic practices across civilizations, with Persian contributions standing out prominently due to their active embrace of new substances and treatments, significantly enriching their cultural heritage. A detailed examination of cosmetics during this period unveils not just beauty practices but also reflects the medical knowledge of the time, intricately intertwining aesthetics and healthcare.[36]The adoption of various substances and techniques underscores a

[31] Saskia Stucki, 'Towards a Theory of Legal Animal Rights: Simple and Fundamental Rights' (2020) 40 Oxford Journal of Legal Studies.
[32] JM Loeb, 'Human vs Animal Rights. In Defense of Animal Research' (1989) 262 JAMA: The Journal of the American Medical Association 2716.
[33] MS Balsam and Edward Sagarin, *Cosmetics*, vol. 3 (2nd edn., John Wiley & Sons 1972).
[34] 'Nayak M, Ligade vs. History of Cosmetic in Egypt, India, and China. J Cosmet Sci. 2021 Jul-Aug;72(4):432-441. PMID: 35262483.'
[35] Ibid.
[36] SK Chaudhri and NK Jain, 'History of Cosmetics' (2019) 3 Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm <http://asiapharmaceutics.info/index.php/ajp/article/view/260>.

shared human fascination with enhancing appearance, transcending cultural and geographical boundaries. Through the historical perspective of cosmetics, the interconnectedness of diverse civilizations becomes apparent, as they independently discovered and incorporated similar practices into their beauty and medical rituals. This era serves as a testament to the enduring human desire for aesthetic enhancement, highlighting the timeless pursuit of beauty that spans across ages.[37]

Medical phase (5th B.C to 7th A.D)

The Hellenistic period, extending from the late 4th century B.C., marked a significant era of cultural advancements in medicine and cosmology. This phase, encompassing the Hellenistic period, Arabian period, and Renaissance, witnessed notable contributions to modern cosmetics and cosmetology. The study of dermatology by Hippocrates and his associates during this time laid the foundation for understanding skin health. Aristotle of Stagira, from 384 to 322 B.C., made invaluable contributions to biology and physical science, influencing the arts of cosmetics and cosmetology for the subsequent three centuries.[38]

During the Arabian period from the 7th to the 12th century, a wealth of cosmetic knowledge spread through interactions with travellers to and from India, China, and Japan. Early Indian practices during the Gupta period (3rd to 5th centuries A.D) left an indelible mark on perfumery. Ingredients like musk, saffron, camphor, and sandalwood, imported from India to Rome via Arabia during the Roman Empire, showcased the international influence on cosmetics. The Gupta period in India stood out for its advanced cosmetics and hygiene practices, establishing the region's supremacy in the industry.[39]

A subsequent period of disorganisation paved the way for the rise of trades, followed by a revival and rehabilitation phase in the 18th century. This period witnessed contributions from chemistry, industrial growth, international promotions, public acceptance, educational movements, and literary growth, all of which significantly propelled the cosmetics industry. [40] The 18th-century advent of newspapers and organised pharmacies contributed to the expansion of the fragrance and cosmetics industries. Scientific and technological developments by the 19th century had further sped up this growth, but for a variety of reasons, the use of cosmetics fell off.[41] Fortunately, a significant shift in public perception occurred in the 1920s, increasing acceptance and advocating for cosmetic procedures and products. Cosmetology and cosmetics underwent significant scientific advancements, with researchers from various disciplines studying recurring issues, particularly those related to maintaining skin health. The initial definition proposed for the New Food and Drug law, categorising cosmetics based on their external applications to cleanse, alter appearances, or promote attractiveness, underscores the broad scope of the sector. Looking forward, the future of cosmetics science and technology hinges not just on the longevity of products on shelves but on their effectiveness and utility for users, shaping the trajectory of this dynamic industry.[42]

Changes in attitudes and practices over time:

Over time, the ethical and scientific debates over animal testing in cosmetics have evolved. Animal testing was initially widespread, especially after the thalidomide tragedy. The incident highlighted the need for stricter

[37] MS Balsam and Edward Sagarin,(in 33).
[38] WA Littler, 'Death by Vanity in the 18th Century' (2019) 113 QJM: an International Journal of Medicine.

[39] A Diamandopoulos, L Kolonas and M Grapsa-Kotrotsou, 'Use of Lead Cosmetics in Bronze-Age Greece' (1994) 344 The Lancet 754.
[40] Irene Dini and Sonia Laneri, 'The New Challenge of Green Cosmetics: Natural Food Ingredients for Cosmetic Formulations' (2021) 26 Molecules 3921 <https://www.mdpi.com/1420-3049/26/13/3921>.
[41] Bruno Fonseca-Santos, Marcos Antonio Corrêa and Marlus Chorilli, 'Sustainability, Natural and Organic Cosmetics: Consumer, Products, Efficacy, Toxicological and Regulatory Considerations' (2015) 51 Brazilian Journal of Pharmaceutical Sciences 17 <https://www.scielo.br/j/bjps/a/TDpKrSLYxqM8yrJq5SwvJZH/>.
[42] Roger L McMullen and Giorgio Dell'Acqua, 'History of Natural Ingredients in Cosmetics' (2023) 10 Cosmetics 71 <https://www.mdpi.com/2079-9284/10/3/71>.

regulations and emphasised the role of animal models in the security of products.[43] As the 1900s drew to a close and a new millennium began, several nations including the United Kingdom and countries that make up the European Union implemented regulations prohibiting the testing of cosmetics on animals.[44] The personal care products industry was compelled to adopt ethical guidelines and alternative assessment techniques due to these lawful actions as well as public advocacy efforts. In many densely populated regions today, the utilisation of animals in beauty item testing is currently forbidden or subject to oversight. The requirement for safe cosmetics has grown worldwide owing to consumers' increasing desire for items produced responsibly and ethically without harming animals unnecessarily. While certain nations still permit animal testing, it is being entirely prohibited elsewhere. The utilisation of creatures in beauty care products testing keeps on being a questionable issue.[45] A few individuals raise worries about potential shopper peril since they accept substitute[46] routes have not been totally investigated and affirmed yet. In any case, it is turning out to be progressively conceivable that beauty care products won't expect testing on creatures later on because of developing backing for cruel free strategies and the proceeding with headway of substitute testing strategies. As more substitute testing techniques create and support for compassionate choices develops, the requirement for creature testing in magnificence items will probably diminish over the long run. In the interim, open discussion on this delicate issue will presumably proceed as we search for approaches to ensure well being without relinquishing our humanity.[47]

In 1966, Life magazine featured a striking image of a malnourished dog with the headline "Concentration Camp for Dogs"[48]. The horrific account of the life of research animals in the accompanying article sparked a public uproar against the use of pound animals in research. As a result of the overwhelming amount of correspondence that Congress received, the Laboratory Animal Welfare Act, the first federal law aimed at enhancing the conditions of research animals was approved.[49] As the new millennium dawns, public opinion towards animal welfare is still shaped by public collective beliefs. However, there are differences in popular opinion in the US about the status of nonhuman. Animal advocates make a strong case that it is immoral and causes significant pain for animals to engage in some activities, such as using animals in scientific research or eating animal meat. Just as ardently opposed to those opinions are a sizable portion of Americans. It is evident that within the past 25 years, there have been substantial changes in public perception, even if there does not yet appear to be a consensus in society about the moral standing of animals. A shift in public opinion towards better animal protection has led to the passage of laws like the Animal Welfare Act, a reduction in the use of animals in consumer product testing, a decline in support for the fur trade, and a sharp rise in the number of Americans who belong to animal protection groups.[50]Writing questions that ask about contentious matters is one of the main challenges social scientists confront when trying to gauge public opinion. It's ideal to formulate questions to reduce prejudice. As an illustration, Reader's Digest conducted a study in 1992 that surveyed

[43] Botting and Jack Howard, 'The History of Thalidomide In: Animals and Medicine: The Contribution of Animal Experiments to the Control of Disease.'
[44] Martin Parlasca and others, 'How and Why Animal Welfare Concerns Evolve in Developing Countries' (2023) 13 Animal Frontiers 26.
[45] Sreedhar D. and others, 'Ban of Cosmetic Testing on Animals: A Brief Overview' (2020) 12 International Journal of Current Research and Review 113.
[46] 'Search for Cruelty-Free Companies, Products, and More | PETA' (*Beauty Without Bunnies*) <https://crueltyfree.peta.org/>.

[47] Jon Hamm and others, 'Alternative Approaches for Identifying Acute Systemic Toxicity: Moving from Research to Regulatory Testing' (2017) 41 Toxicology in vitro: an international journal published in association with BIBRA 245 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5479748/>.
[48] 'Wayman, S. 1966. Concentration Camps for Dogs. Life Magazine, 4 February, Vol. 60, No.5: 25–28. Yankelovich, Clancy, Schulman. 1992. Vegetarian Times. October.'
[49] 'Herzog, H., Rowan, A., & Kossow, D. (2001). Social Attitudes and Animals. In D.J. Salem & A.N. Rowan (Eds.), the State of the Animals 2001 (Pp. 55-69). Washington, DC: Humane Society Press.'
[50] Nayak M, Ligade (in 34).

over a thousand persons about their opinions of the statement, "It is unethical to do medical research on animals in lab settings".33 percent of respondents, according to the findings, were somewhat against animal experimentation.[51] Five thousand members of the American Psychological Association were selected at random by Plous. Just 14% of the 3,982 psychologists who responded were against animal research, however the degree of support varied greatly depending on the kind of study being done.[52]

While almost 60% of the psychologists stated that they seldom or never used the results of animal research, just 10% claimed to use the findings of animal study regularly in their own work. In comparison to female psychologists, men psychologists were more inclined to favour animal research, while new Ph.D. graduates were less likely to do so than older respondents.[53]

The general public's view of using animals in medical research has changed dramatically over the past few decades. Only a small minority of poll respondents in the late 1940s opposed animal experimentation, with the majority supporting it. Support for these techniques has, however, significantly decreased in recent years. Public support for animal research has decreased, especially since the late 1940s when it was over 80%, despite attempts by scientific interests to highlight its significance.[54] However, more recent polls reveal a fall in public support, with only 60–65% of respondents for animal research and 30–40% against it.[55] Furthermore, the public's support has continued to diminish as a result of the particular mention of employing chimpanzees and dogs in research that causes pain or harm. For instance, just 35 percent of people in the UK agreed with such claims. Compared to Americans, Europeans generally have a more unfavourable opinion of animal experimentation.[56]The type of animal involved also affects public perception, with higher-profile species like dogs and chimpanzees receiving more attention. In comparison to earlier decades, there has been a discernible increase in public support for animal protection problems, which is indicative of a larger cultural movement towards more care for animal welfare.[57]

Recently Covid 19 like pandemic has an influence on customers' perceptions of attractiveness. While the importance of cosmetics decreased following the outbreak, people around the world still saw skincare as crucial throughout the pandemic. Knowledge about skincare and cosmetics has increasingly altered. With the exception of eye makeup products, which have benefited from the pandemics Standard Operating Procedures (hereinafter SOP), skincare items have benefited from the phenomenon.[58] The interest of consumers in masks was shown to have a positive correlation with interest in skincare items, such as cleaning products, but a negative correlation with interest in cosmetic products. The researchers conclude that the SOP had no significant impact on customers' interest in masks. While pandemics undoubtedly affect the perceptions of consumers throughout the world the interest in skincare items rises as a pandemic spread, whereas interest in cosmetics products falls. It can serve as a standard source of information for developing marketing plans in the event of a pandemic in the future.[59]

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[51] 'Reader's Digest. Reader's Digest Poll: March 1992, 1992 [Dataset]. Roper #31105863, Version 1. Wirthlin Group [Producer]. Cornell University, Ithaca, NY: Roper Center for Public Opinion Research [Distributor]. Doi:10.25940/ROPER-31105863'.
[52] S Plous, 'An Attitude Survey of Animal Rights Activists' (1991) 2 Psychological Science 194.
[53] S Plous, 'Attitudes toward the Use of Animals in Psychological Research and Education: Results from a National Survey of Psychologists.' (1996) 51 American Psychologist 1167.
[54] 'National Opinion Research Center (NORC). NORC Survey: Animal Experimentation, 1948 [Dataset]. Roper #31095033, Version 0. National Opinion Research Center (NORC) [Producer]. Cornell University, Ithaca, NY: Roper Center for Public Opinion Research [Distributor]. Doi:10.25940/ROPER-31095033'.
[55] 'National Science Board (1985–1998). Science and Engineering Indica- Tors—1989. Washington, D.C.: U.S. Government Printing Office.'
[56] Ralph Pifer, Kinya Shimizu and Linda Pifer, 'Public Attitudes toward Animal Research: Some International Comparisons' (1994) 2 Society & Animals 95.
[57] Elisabeth Ormandy and Catherine Schuppli, 'Public Attitudes toward Animal Research: A Review' (2014) 4 Animals 391 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4494309/> accessed 6 May 2019.
[58] Yeong-Hyeon Choi, Seong Eun Kim and Kyu-Hye Lee, 'Changes in Consumers' Awareness and Interest in Cosmetic Products during the Pandemic' (2022) 9 Fashion and Textiles.
[59] ibid.

DEVELOPMENT OF ALTERNATIVE TESTING METHODS

Tests on lab animals are pretty important. Not just figuring out diseases, but also te-sting how safe things are? The Food and Drug Administration (hereinafter FDA) is the one watching over testing for medicine-s, vaccines, food extras and makeup safety. But other groups handle different tests. The Occupational Safety and Health Administration, the Consumer Product Safety Commission and the Environment Protection Agency have other areas they oversee.[60] In 1933, a certain mascara calle-d Lash Lure led to tragedy. Over a large number of women became-blind and only one woman even lost her life. It contained a chemical name-d p-phenylenediamine- that at the time was unchecked. Back then, no safety rules e-xisted for these kinds of products. The- dreadful results of this chemical were extreme blisters, abscesses, and ulcers, particularly on the face. Eyelids and eyes suffered the- brunt of it. Some women lost their sight. In an extreme case, a woman's ulcer turned lethal due to a bacte-rial infection. The present safe-guards prevent such nightmares.[61] The FDA insists that every cosmetic make-r ensures product safety. This rule-is not just for some cosmetics. It applies to a wide- variety of products like makeup, pe-rfumes, shave cream, hair sprays and colours, shampoos and soaps. All product safety testing required animals in the past. However, things were altered in the 1980s. Animal free tests started to appear. These reduced cosmetics testing on animals by 90%! This is quite a reduction. However, some products still need to be tested on animals, including sunscreens, dandruff shampoos, fluoride toothpaste, and acne treatments. These goods include substances that alter the chemistry of the body. This might turn out poorly. These tests are necessary to guarantee that these products are safe for you to use.[62] The development of alternative testing techniques is underway to limit and improve the use of animals in testing, with the goal of improving test accuracy for predicting dangers to human health or the environment. An assay that employs in vitro cell cultures to detect if chemicals may burn or harm skin is an example of an alternative testing approach. Scientists working in government, university, and commercial facilities have created these substitute exams. Subsequently, the "Interagency Coordinating Committee on the Validation of Alternative Methods (hereinafter ICCVAM)" assesses them to guarantee the alternative test's ability to precisely ascertain a product's level of risk.[63] Updates to testing standards and guidelines are made by federal agencies based on the suggestions made by ICCVAM on alternate techniques of toxicological testing. An analogous organisation, the European Centre for the Validation of Alternative Methods, is part of the European Union and works to create and assess novel substitutes for animal experimentation. [64]

Before being made available for general use, products (mostly chemicals, pharmaceuticals, and cosmetics) are frequently subject to testing requirements by government regulatory authorities.[65] Animals are still utilised in these tests, for example, in the following procedures:

- Tests for acute toxicity involve giving a chemical a single dosage at a concentration high enough to cause toxic symptoms and death. The Lethal Dose 50 (hereinafter LD) test is an illustration of this kind of experiment in which 10% of the individuals in an experimental sample were predicted to die. To ascertain the biological activity of organic substances in experimental animals, biological screening techniques are used.

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[60] 'National Research Council (US) Committee to Update Science, Medicine, and Animals. Science, Medicine, and Animals. Washington (DC): National Academies Press (US); 2004. Safety Testing. Available From: Https://Www.ncbi.nlm.nih.gov/Books/NBK24645/ ' (2014).

[61] Leonora Cohen Rosenfield (n 28).

[62] ibid.

[63] National Research Council, *Science, Medicine and Animals* (Washington, DC National Academies 2003).

[64] Leonard M Schechtman, 'Implementation of the 3Rs (Refinement, Reduction, and Replacement): Validation and Regulatory Acceptance Considerations for Alternative Toxicological Test Methods' (2002) 43 ILAR Journal.

[65] Center for Food Safety and Applied Nutrition, 'Draft Guidance for Industry: Cosmetic Good Manufacturing Practices' (*U.S. Food and Drug Administration*9 February 2019) <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-cosmetic-good-manufacturing-practices>.

- Tests for carcinogenicity in which suspected carcinogens (agents that cause cancer) are regularly exposed to animals throughout the course of their lifetime, typically rats.[66] Various approaches used in developmental and reproductive toxicity testing to evaluate a substance's potential to cause birth abnormalities, infertility, or miscarriages in rodents and rabbits. The severity of toxic effects on the nervous systems of vertebrates is assessed by neurotoxicity testing. Animal behaviour is examined to look for any gross behavioural changes, learning disabilities, motor disorders, or lack of coordination. Rodents are frequently used in repeated dose chronic toxicity experiments to examine the results of prolonged (weeks to months) exposure to certain substances.[67]
- Study on Genotoxicity: When evaluating the safety of cosmetic compounds, genotoxic potential must be considered. For the assessment of chromosomal damages and for determining the potential for mutagenicity the Scientific Committee on Consumer Safety (hereinafter SCCS) 10th revision suggests two in vitro tests such as Ames test, in vitro micronucleus test and bacterial reverse mutation test. The two tests worked together to identify all relevant genotoxic carcinogens.[68]
- Study on skin sensitization: Skin sensitizers like chemicals will be able to make people hypersensitive and may lead to Allergic contact dermatitis (hereinafter ACD) after repeated contact. This chronic disease can only be avoided by excluding exposure to the inducing factor.[69] It involves adaptive immune response activation and immunological memory priming. So for assessing the safety of the chemicals, proactive assessment of skin sensitization potential becomes crucial and serves as an influential sociological point. Not least for cosmetics, dermal application is frequently a desired exposure route in a variety of industries among the regulatory bodies.[70]
- Study on dermal absorption: Unlike medications, which usually always enter the body through other routes, cosmetic products and ingredient safety assessment of skin absorption is a critical component. The most reliable technique for determining skin pharmacokinetics and forecasting human dermal absorption is believed to be in vitro dermal absorption research.[71] Dermal absorption tests are conducted to measure the amount of a drug that passes through the skin and determine whether or not it may get into the blood. Dermal irritation, or potentially irreversible skin damage, can occur for up to four hours after a test chemical is administered to the anterior surface of the eye Organization for Economic Cooperation and Development (hereinafter OECD 404). Ocular irritation, on the other hand, is the result of changes in the eye.
- Skin and eye irritation study: The most crucial component of ingredients safety assessment is assessment of a cosmetics ingredient potential for causing skin and eye irritation.[72] Skin and ocular irritations are evaluated based on rebuilt human tissues using test methods. Using commercially available 3D models based on Human epidermis reconstruction (hereinafter RHE), skin irritancy is measured (OECD test method 439). Whereas commercially available 3D models based on Human cornea-like epithelium reconstruction

[66] Enegide Chinedu, David Arome and Fidelis Solomon Ameh, 'A New Method for Determining Acute Toxicity in Animal Models' (2013) 20 Toxicology International 224 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3877490/>.
[67] 'Developmental & Reproductive Toxicity' (*National Toxicology Program*) <https://ntp.niehs.nih.gov/whatwestudy/testpgm/devrepro>.
[68] Manon Barthe and others, 'Safety Testing of Cosmetic Products: Overview of Established Methods and New Approach Methodologies (NAMs)' (2021) 8 Cosmetics 50.
[69] Patrick B Murphy and others, 'Allergic Contact Dermatitis' (*Nih.gov*26 July 2019) <https://www.ncbi.nlm.nih.gov/books/NBK532866/>.
[70] Iwona Bialas, Sandra Zelent-Kraciuk and Kamil Jurowski, 'The Skin Sensitisation of Cosmetic Ingredients: Review of Actual Regulatory Status' (2023) 11 Toxics 392 <https://www.mdpi.com/2305-6304/11/4/392> accessed 17 November 2023.
[71] 'JOINT MEETING of the CHEMICALS COMMITTEE and the WORKING PARTY on CHEMICALS, PESTICIDES and BIOTECHNOLOGY GUIDANCE NOTES on DERMAL ABSORPTION Series on Testing and Assessment No. 156 JT03305971' (2011) <https://www.oecd.org/chemicalsafety/testing/48532204.pdf>.
[72] Vinardell, M.P. and Mitjans, M. (2008), Alternative methods for eye and skin irritation tests: An overview. J. Pharm. Sci., 97: 46-59. https://doi.org/10.1002/jps.21088.

(hereinafter RHCE) are used to test eye irritancy (OECD test method 492).[73] While the RHCE model is the focus of most research, there are additional in vitro models that address serious ocular damage and identify compounds that do not lead to a classification for serious ocular irritation or injury. Since the human tissues used to create the 3D models have been reconstituted, their overall structure closely resembles the physiological and biochemical characteristics of the human eye and skin. A genuine human skin model is called RHE. An extremely distinct, multilayered epidermis has been generated from living human teratinocytes for the RHE skin model. The model has an extremely organised and functional basal cells and skin barrier respectively with a lipid composition similar to living organisms.[74]A differentiated, multi-layered corneal epithelium has been developed from human living cells to create the RHCE, a corneal model. Just like an average human corneal epithelium, this highly organised model having basal cells gradually flatten out as tissues apical surface. Cells are metabolically and mitotically active in both models. They also have a secret number of irritation and inflammation causing factors known as cytokinin.[75]

Currently, in vitro models are used to test the toxicity of cosmetics. These models include tests for mutagenicity, imitancy evaluation, reproductive toxicity, Quality Structure Activity Relationship (hereinafter QSAR) toxicity to target organs, immunotoxicity, hormonal toxicity, testing for neurotoxicity, as well as acute cytotoxicity. However cell culture systems are most probably considered as in vitro models for testing the toxicity of cosmetics.[76]

i. Astrocyte culture gliotoxicity assay: A useful and significant system for the mechanistic examination of neurotoxic substances and the prediction of their effects is provided by cultured astrocytes. Using cells drawn from a limited source, the culture processes enable relatively quick examination of various compounds or their metabolites over a range of concentrations.[77]
ii. Tests for nephrotoxicity: Cell culture with Cell Line of Rabbit Kidney (hereinafter LLC-RK1) cells are cultivated with test substances, whose cytotoxicity is then assessed using the Neutral Red technique as a potential nephrotoxicity indicator. The use of such cultures to assess nephrotoxicity may one day enable routine, quick, and highly repeatable testing of many substances.[78]
iii. Tests for thyroid toxicity using thyroid follicular cells: Human thyroid cells removed following surgery can be kept in culture for up to two months without losing their morphological or functional differentiation. It is mostly used to investigate morphological and biochemical alterations brought on by testing exposure to chemicals, cytotoxic agents, radiation, and toxicity in humans.[79]
iv. Hepatoma cell culture for assessing hepatotoxicity: Hepatoma cell lines may serve as an appropriate in vitro model for the evaluation of possible hepatotoxicity in vivo, according to certain research on hepatotoxicity testing.[80]
v. Rat alveolar macrophage culture for assessing dust toxicity: Macrophages can be isolated, cultivated, and subjected to suspensions of particulate matter. These cultures might serve as an effective in vitro screening

[73] Manon Barthe and others (n 68).
[74] ibid.
[75] Thomas Michael Shiju, Rodrigo Carlos de Oliveira and Steven E Wilson, '3D in Vitro Corneal Models: A Review of Current Technologies' (2020) 200 Experimental eye research 108213 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7655665/#R8>.
[76] Andreia Almeida, Bruno Sarmento and Francisca Rodrigues, 'Insights on in Vitro Models for Safety and Toxicity Assessment of Cosmetic Ingredients' (2017) 519 International Journal of Pharmaceutics 178.
[77] Uliana De Simone and others, 'Human Co-Culture Model of Neurons and Astrocytes to Test Acute Cytotoxicity of Neurotoxic Compounds' (2017) 36 International Journal of Toxicology 463 <https://pubmed.ncbi.nlm.nih.gov/29153031/> accessed 5 December 2022.
[78] W Pfaller and G Gstraunthaler, 'Nephrotoxicity Testing in Vitro--What We Know and What We Need to Know.' (1998) 106 Environmental Health Perspectives 559.
[79] Ayumi Arauchi and others, 'Functional Thyroid Follicular Cells Differentiation from Human-Induced Pluripotent Stem Cells in Suspension Culture' (2017) 8 Frontiers in Endocrinology.
[80] Guillermo Quintás, José V Castell and Marta Moreno-Torres, 'The Assessment of the Potential Hepatotoxicity of New Drugs by in Vitro Metabolomics' (2023) 14 Frontiers in Pharmacology.

system to determine whether inhaling particulate matter over a lengthy period of time is likely to be detrimental.[81]

vi. Islet culture: Using this approach, more than 2000 islets from a single mouse pancreas can be isolated. These islets serve as a great in vitro test subject for the evaluation of putative insulin secretagogues. The method minimises animal testing, is affordable, and reproducible.[82]

vii. Organ culture in the trachea: These cultures show ciliary activity as a sign of their functionality and vitality. Respiratory and non-respiratory viruses have both been grown in organ cultures of the trachea. They are excellent for testing antiviral medications because it is also feasible to establish persistent virus growth.[83]

viii. Culturing whole chick embryo: Developmental biologists frequently employ chick embryos as test subjects in their experimental research. It can be used to test for embryo toxicity, drug-induced developmental defects, and teratogenicity. Additionally, the shell-free chick embryo culture provides a superior approach for angiogenesis research.[84]

ix. Tests for gene mutation in mammalian cells: When compared to using whole animals, gene mutation tests in cultured mammalian cells are quick and easy to perform, and they can be used to measure the reaction of the mammalian genome to putative mutagens.[85]

In addition to in vitro models, in Silico models are always being used as an alternative cosmetics testing method in the cosmetics industry. The use of computer simulation, mathematical modelling, and audio-visual approaches for instruction and training are now non-biological, or in Silico, alternatives to trials involving vertebrates. Currently, mathematical modelling is used to actively develop medications for particular uses, simulate biochemical, toxicological, and physiological processes, and perform predictive modelling known as QSAR modelling. Quantitative structure-activity relationships, or QSARs, are used to evaluate the likely toxicity of chemical compounds based on their molecular structure. Since QSARS was introduced, the Draize rabbit eye irritation test has been used less frequently.[86]

The '3 R' principles:

As a response to mounting political and societal pressure to provide moral substitutes for animal testing in all industries, the "Three R's" refer to replacing, reducing, or improving the use of animals in research and testing. The notion was initially articulated more than 60 years ago. Testing techniques that take into account the Three R's are called "new alternative methods."[87]

❖ Alternative to Reduction

In order to ultimately use fewer animals to complete a given research project or test, the term "reduction alternative" refers to techniques that yield comparable levels of information from the use of fewer animals in scientific procedures or that yield more information from a given number of animals. There is proof that ineffective utilisation of animals and scientific resources arises from subpar experimental design and improper statistical processing of

[81] Yrjö Collan and Veli Matti Kosma, 'Dust Toxicity in Rat Alveolar Macrophage Cultures' [1995] Humana Press eBooks 43.
[82] Joshua C Neuman and others, 'A Method for Mouse Pancreatic Islet Isolation and Intracellular CAMP Determination' [2014] Journal of Visualized Experiments <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4205887/> accessed 23 January 2023.
[83] Brenda V Jones and Ruth M Hennion, 'The Preparation of Chicken Tracheal Organ Cultures for Virus Isolation, Propagation, and Titration' (2007) 454 SARS- and Other Coronaviruses 103 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7121057/> accessed 15 September 2023.
[84] Michael R Stark and Micah M Ross, 'The Chicken Embryo as a Model in Developmental Toxicology' [2019] Methods in molecular biology 155.
[85] Jesse J Salk and Scott R Kennedy, 'Next-Generation Genotoxicology: Using Modern Sequencing Technologies to Assess Somatic Mutagenesis and Cancer Risk' (2019) 61 Environmental and Molecular Mutagenesis 135.
[86] Edith Flaire and others, 'Alternative *in Vitro* Models Used in the Main Safety Tests of Cosmetic Products and New Challenges' [2022] International Journal of Cosmetic Science.
[87] Katherine Gallagher, 'Alternatives to Animal Testing in Cosmetics' (*Treehugger*30 November 2021) <https://www.treehugger.com/alternatives-to-animal-testing-in-cosmetics-5202649>.

experimental data. All scientists should have a fundamental grasp of statistics and experimental design as a requirement before being allowed to conduct animal experiments.[88]

- ❖ Alternative to Refinement

Refinement alternatives are techniques that improve animal welfare while reducing or eliminating pain and suffering. Distress can be brought on by pain, which is the outcome of existing or probable tissue damage from diseases, injuries, or surgeries. An animal under distress exhibits maladaptive behaviour because it is unable to fully adjust to stressors and the stress that follows. Distress is an unpleasant condition. When anaesthetics, analgesics, and tranquillizers are used appropriately, a great deal of potential pain and discomfort can be avoided or reduced. As of right now, there is no easy, standardised method for evaluating animal suffering and pain. Usually, subjective clinical indicators of aberrant conduct and appearance serve as the basis for the examination.[89]

- ❖ Alternative to Replacement

It includes all techniques that allow a certain goal to be accomplished without involving animals in research or other processes. One way to replace an animal might be by relative replacement, which involves killing the animal humanely and using its cells, tissues, or organs for in vitro research, or absolute replacement, which eliminates the need to utilise animals altogether such as Simulated human tissues and cells in continuous cultures or computers.[90] Improved storage, information sharing, and utilisation of past animal experiment data to prevent needless repetition of animal procedures are just a few of the replacement alternative methods and approaches. Other methods include using mathematical and computer models, using physical and chemical techniques and prediction based on molecule properties, using organisms with limited sentience like plants, microbes, and invertebrates, using in vitro methods like tissue slices, subcellular fraction, cell suspension, and perfused organs, and human studies involving the use of human volunteers, post-marketing surveillance, and epidemiology.[91] It is imperative that substitute techniques be grounded on sound scientific principles, and that grandiose claims that lack empirical support be shunned. An animal experiment may only be considered appropriate if it involves the fewest number of animals, produces the least amount of pain and suffering, is essential to achieve a legitimate scientific goal, and cannot be completed in any other manner.[92] Predicting toxicity in particular target organs has been the main focus of efforts to replace animal testing with in vitro alternatives, which has limits for evaluating repeated dosage systemic toxicity.[93] It may be more beneficial to focus on comprehending the biological processes and mechanism of action that result in toxicity :

- ➢ Quantitative structure activity relationship (QSAR):

For predicting genotoxicity and carcinogenicity, Quantitative Structure-Activity Relationship (QSAR) models have been widely used. Chemical carcinogens may be predicted with great precision using the Ames test, which evaluates gene mutation in bacteria. Quantitative QSARs provide more accurate evaluations, especially for sets of comparable substances, whereas most qualitative models provide coarse-grained categorization. Still in their infancy, QSARs for non-genotoxic carcinogenicity are being developed.[94]

- ➢ Read across and grouping of chemicals:

[88] William Moy and Rex Leonard Burch (n 13).
[89] Balls, M., Goldberg and others, 'the Three Rs: The Way Forward: The Report and Recommendations of ECVAM Workshop 11. Alternatives to Laboratory Animals: ATLA, 23(6), 838.'
[90] Michael FW Festing, 'Principles: The Need for Better Experimental Design' (2003) 24 Trends in Pharmacological Sciences 341.
[91] Michael Balls, 'Replacement of Animal Procedures: Alternatives in Research, Education and Testing' (1994) 28 Laboratory Animals 193.
[92] Aysha Akhtar, 'The Flaws and Human Harms of Animal Experimentation' (2015) 24 Cambridge Quarterly of Healthcare Ethics 407 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4594046/>.
[93] Sarah Adler and others, 'Alternative (Non-Animal) Methods for Cosmetics Testing: Current Status and Future Prospects—2010' (2011) 85 Archives of Toxicology 367 <https://link.springer.com/article/10.1007%2Fs00204-011-0693-2>.
[94] Andrey Toropov, Alla Toropova and Emilio Benfenati, 'Additive SMILES-Based Carcinogenicity Models: Probabilistic Principles in the Search for Robust Predictions' (2009) 10 International Journal of Molecular Sciences 3106.

An effective substitute for evaluating each chemical separately is to classify compounds according to their shared physicochemical characteristics. Read-across, trend analysis, and QSARs can be used to fill in data gaps within a category. Analogue read-across is outlined in a step-by-step manner by the OECD guideline, which highlights the significance of comparing toxicokinetics and physicochemical features.[95]

- Threshold of toxic concerns (TTC):

When evaluating cosmetics, the Threshold of Toxicological Concern (hereinafter TTC) technique is frequently used. It sets exposure limits based on structural characteristics and makes the assumption that untested compounds are just as dangerous as the most toxic ones in the group. TTC offers a practical way to set exposure limits in the absence of precise toxicological evidence, notwithstanding its conservatism. TTC is an adaptation of the US FDA's Threshold of Regulation, which emphasises risk assessment above hazard identification alone.[96] Based on a study of carcinogen potencies, the ToR established an exposure limit of 1.5 µg/day for compounds that did not raise concerns about genotoxicity. Regulating constraints prevented this limit from being applied to genotoxic carcinogens.[97]

In toxicological evaluations, the application of computational (in silico), laboratory-based (in vitro), omics technologies, organ-on-a-chip technology, high-throughput screening (hereinafter HTS), and mathematical biology might be complementary.[98] Predicting a chemical's possible effects on live creatures is improved by combining toxicity data with Absorption, Distribution, Metabolism, and Excretion (hereinafter ADME) data.[99] There are several in silico technologies available to forecast the intrinsic toxicity and ADME properties of a substance. As long as they satisfy certain requirements, the European Unions (hereinafter EU's) Registration, Evaluation, Authorisation and Restriction of Chemicals (hereinafter REACH)[100] the law promotes the use of in silico prediction techniques like read-across and QSAR as alternatives to animal testing. Comparably, the Cosmetics Regulation, which went into effect in 2013, forbids animal testing of cosmetic chemicals and products and promotes the use of in silico models for safety assessments.[101] Animal studies should be replaced or reduced wherever feasible, according to the Frank R. Lautenberg Chemical Safety Act in the United States.[102] Cross-sector cooperation and the significance of including toxicokinetic information in read-across are highlighted in a paper by the European Partnership for Alternatives to Animal Testing. This highlights the increasing utilisation of in silico technologies in many businesses, which mirrors the ethical and economic progress made in this domain.[103]

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[95] 'ECHA (2010) Practical Guide on How to Report Categories and Read-Across. Http://Www.www.echa.europa.eu/Doc/Publications/ Practical_guides/Pg_report_readacross_categ.pdf'.

[96] 'US FDA (1995) Department of Health and Human Services. Food Additives; Threshold of Regulation for Substances Used in Food- Contact Articles 21 CFR Parts 5, 25, 170, 171, and 174'.

[97] R Kroes and others, 'Structure-Based Thresholds of Toxicological Concern (TTC): Guidance for Application to Substances Present at Low Levels in the Diet' (2004) 42 Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association 65 <https://pubmed.ncbi.nlm.nih.gov/14630131/>

[98] Catherine Mahony and others, 'New Ideas for Non-Animal Approaches to Predict Repeated-Dose Systemic Toxicity: Report from an EPAA Blue Sky Workshop, Regulatory Toxicology and Pharmacology, Volume 114, 2020, 104668, ISSN 0273-2300, Https://Doi.org/10.1016/J.yrtph.2020.104668. (Https://Www.sciencedirect.com/Science/Article/Pii/S0273230020300945)'.

[99] Judith C Madden and others, 'A Review of in Silico Tools as Alternatives to Animal Testing: Principles, Resources and Applications' (2020) 48 Alternatives to Laboratory Animals 146 <https://journals.sagepub.com/doi/full/10.1177/0261192920965977>.

[100] 'European Commission. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemicals Agency, Amending Directive 1999/45/EC and Repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as Well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Off J Euro Union 2006; L349: 1–849.'

[101] 'European Commission. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. Off J Euro Union 2009; L342: 59–209.'

[102] US Government. Frank R. Lautenberg Chemical Safety for the 21st Century Act, Public Law 114–182, 22 June 2016, https://www.congress.gov/114/plaws/publ182/PLAW-114publ182.pdf.

[103] Charles Laroche and others, 'Finding Synergies for 3Rs – Toxicokinetics and Read-Across: Report from an EPAA Partners' Forum' (2018) 99 Regulatory Toxicology and Pharmacology 5.

CHALLENGES AND ADVANCEMENTS IN ADOPTING ALTERNATIVE METHODS

The expectations on the beauty and cosmetics sector are always changing, and there is ongoing need to innovate in order to meet these demands. Traditional techniques of product creation have been transformed by technological breakthroughs acting as catalysts. This shift shows itself in several ways in the development, testing, promotion, and personalisation of products. Innovations in Artificial Intelligence (hereinafter AI), Augmented reality (hereinafter AR), 3D printing, and nanotechnology have completely changed the development, testing, and marketing of cosmetic products.[104] These technological advancements offer a more ethical and environmentally friendly cosmetics sector while also improving the consumer experience.

Technological advancements:

- Machine learning and Artificial intelligence:

The cosmetics business is undergoing a transformation era to Artificial Intelligence (AI) and Machine Learning (hereinafter ML), which are optimising component efficacy, reducing product development time, and customising goods based on customer preferences.[105] By quickly evaluating large datasets and suggesting optimised formulas, these technologies significantly cut down on the time and effort needed for product development. By using both historical and current data, they may also find novel component combinations that improve the efficacy of products, directing formulators towards creative solutions. By evaluating consumer data, they provide a customised approach to cosmetic formulation, resulting in goods that are matched to specific requirements.[106] These formulas adapt over time to match the changing needs of customers thanks to ongoing feedback loops. Furthermore, by automating monitoring procedures and immediately informing formulators of any problems, these technologies help to ensure regulatory compliance. AI and ML also help the cosmetics sector with sustainability initiatives by optimising formulas to utilise fewer toxic ingredients and produce less waste. With the analysis of supply chain data and certification of conformity to environmental and ethical standards, they also help in the sourcing of ingredients produced responsibly.[107] All things considered, AI and ML are critical to the beauty business since they help to ensure regulatory compliance, personalise formulas, improve product efficacy, and accelerate product development. They also help to promote sustainability.

- 3D Bioprinting:

The development of prototypes for novel formulations and packaging designs is made easier by 3D printing technology. Additionally, it greatly reduces animal testing by helping with the ethical testing of items utilising human tissue models generated from 3D printing.[108]

- Nanotechnology:

By enhancing the composition, application, and efficacy of cosmetics, nanotechnology has completely transformed the cosmetics market. Nanotechnology allows for creative methods of component distribution, formulation, and packaging since it functions at the atomic and molecular size, which is normally 1 to 100 nanometres.[109] Through the creation of nano-sized carriers that can pass through the skin's natural barrier, it improves

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[104] Dr Shilpa Nikam, 'How Technology Is Seeping into the Future of the Beauty Industry?' *The Times of India* (16 February 2023) <https://timesofindia.indiatimes.com/blogs/voices/how-technology-is-seeping-into-the-future-of-the-beauty-industry/>.

[105] 'Advancements in Technology: Steering the Future of Cosmetic Product Development | Cosmetics and Beauty Tech Review' (*sites.psu.edu*) <https://sites.psu.edu/cosmetics/advancements-in-technology-steering-the-future-of-cosmetic-product-development/> accessed 20 March 2024.

[106] Saiyed Umer and others, 'Machine Learning Method for Cosmetic Product Recognition: A Visual Searching Approach' [2020] Multimedia Tools and Applications.

[107] Ameet V Joshi, *Machine Learning and Artificial Intelligence* (Springer International Publishing 2020).

[108] Aditya Bhushan Pant, *Skin 3-D Models and Cosmetics Toxicity* (Springer Nature).

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the delivery of contents by assuring deeper absorption and longer release of active substances, which increases the effectiveness and lifespan of the product. Moreover, reliable and aesthetically pleasing formulations that satisfy consumer preferences may be developed more easily thanks to nanotechnology. Rich colour and coverage are provided while using less material with materials like Nano emulsions and Nano pigments, which enhance texture, look, and performance. Moreover, it stimulates the development of antimicrobial packaging solutions that extend product shelf life and minimise the need for preservatives, in line with the industry's transition to more natural formulations.[110] By improving component distribution, formulation methods, and packaging improvements, nanotechnology has a substantial overall influence on the cosmetics business.

- Augmented Reality:

With the use of augmented reality (AR) technology, one may virtually try things on and link the actual and virtual worlds. Customers will have a better purchasing experience as a result, and useful data will be gathered for preference analysis and product development support. Augmented Reality (AR) combines computer-generated material with real-world settings to let people see things in their own spaces before they buy them.[111] Through improved decision-making and involvement, this interactive experience raises customer happiness and brand loyalty. Additionally, businesses may customise their goods and marketing campaigns by using the data gathered via AR interactions, which offers insights into customer behaviour and preferences. All things considered, augmented reality's adoption by the retail sector revolutionises the way customers purchase and provides companies with insightful data for new product development and marketing tactics.[112]

Challenges:

It might be difficult to develop alternative methods for assessing the quantitative risk of cosmetic components. To characterise risk, three key components of quantitative human health risk assessment must be considered: exposure assessment, dose-response data, and hazard identification. For thorough endpoint coverage, in vivo investigations continue to be essential, even though other methodologies such as in vitro studies can yield some data on dangers. The establishment of starting points for risk categorization, such the No Observed Adverse Effect Level (hereinafter NOAEL), depends heavily on dose-response data obtained from in vivo investigations.[113] Performing risk evaluations is not viable without these data, as it is difficult to evaluate the systemic danger of cosmetic compounds.[114] For an appropriate risk assessment, it is essential to comprehend the mechanisms of action and toxicity. To quantify risk quantitatively, in vitro studies are not sufficient, but they do offer useful insights into cellular systems. To improve the predictability of in vitro systems, physiologically based biokinetic models and the integration of in vitro and human data are essential.[115] But in order to forecast in vivo toxicity—particularly for repeated dose toxicity—these methods still need to be further validated. The accuracy of in vivo research, especially animal models, in predicting toxicity to humans is limited. These restrictions include variations in metabolism between species, insufficient genetic polymorphism coverage, poor assessment of specific toxicity targets, and unclear roles for age and illness characteristics in toxic reactions.[116] Furthermore, there's a chance that insignificant toxicity outcomes may arise

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from the dosage ranges utilised in animal research, which are frequently larger than exposures in humans. Although often used, the process of estimating the hazards to human health by obtaining Margin of Safety (hereinafter MOS) values from animal NOAELs has not been properly verified.[117] It is difficult for in vitro research to replicate the intricately coordinated pathogenic reactions seen in vivo.[118] Although they can identify hazards and rank potencies as well as clarify cellular processes of toxicity, the process of extrapolating dose-response data from in vitro concentrations to whole-body exposure levels is still challenging. In vitro investigations by themselves are insufficient to establish risk categorization in the absence of suitable data on dose-response relationships and threshold values. It is essential to comprehend the mode of action (MOA) of toxicants in order to create alternative testing procedures.[119] A MOA is made up of the critical moments required to produce toxicity and may be evaluated and measured to forecast harmful health consequences. By concentrating on MOA, it could be able to create non-animal methods of toxicity assessment by utilising developments in systems biology, toxicogenomics, and bioinformatics. The goal of projects like the Tox21 programme is to determine how cells react to chemical exposures and to create high-throughput screening instruments that can be used to forecast toxicity in vivo.[120] Research collaborations in the US and Europe are working to create substitute techniques for determining the toxicity of repeated doses. These initiatives concentrate on creating new approaches, refining existing ones, identifying pertinent endpoints, and combining data analytic tools. Despite tremendous advancements, it could take several decades to completely substitute animals in toxicity testing. It is not feasible to substitute animals entirely in acute toxicity testing with an alternate model.[121] Alternative approaches to prevent skin corrosion and irritation have been approved and verified. The Draize rabbit eye test is the only procedure that may be used to test for ocular discomfort. Skin sensitization techniques include improving and reducing the number of animals used. An alternative to in vivo testing might be an in vitro skin absorption test. There isn't a widely approved substitute for the customary in vivo repeated-dose toxicity tests. A variety of in vitro assays can be employed to assess a compound's genotoxic and mutagenic potential. There are two more approaches that might yield knowledge on potential carcinogenic effects.[122]

CONCLUSION

It's clear that views and methods have changed significantly throughout time, from the earliest cases of animal testing to the turning points in the evolution of cosmetic testing. Technological developments, legal pressures, and ethical considerations have all had an impact on these shifts. Advancements in technology have significantly influenced testing methodologies, allowing the creation and verification of substitutes that do not use animals. In vitro methods have become useful instruments that minimise animal suffering while yielding accurate results. The use of computational models and simulations has increased as well because they provide sophisticated ways to evaluate safety and forecast results without using animals in experiments. But even with these developments, there are still obstacles in the way of widely implementing alternative approaches. The primary obstacles still remain regulatory acceptability, standardisation, and validation. The broad adoption of non-animal testing methods is further hampered by industrial practices, institutional and cultural inertia, and other factors.

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