9 Regulatory Requirements for Safety/Toxicity Assessment of Cosmetics/Nanocosmetic Products: Challenges and Opportunities

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Abstract

Cosmetics are regulated globally to maintain their safety and effectiveness. However, different regulatory frameworks adopted by each country adversely affect the competition and economic growth of the cosmetic industry. Further, animal testing for safety and efficacy purposes in cosmetics has sparked controversial debates in the last few decades. Alternative research methodologies have become increasingly popular, particularly after the release of the three R's principles (replacement, reduction, and refinement). Although many alternatives to animal testing have been introduced in the cosmetics industry, studying the safety of cosmetic products and their ingredients is still challenging. In the present chapter, we have attempted to explore the information available on the regulatory frameworks for cosmetics/nanocosmetics in various countries. We have provided a brief overview of the ban on using animals in cosmetic testing and relevant alternative approaches employed in regulatory safety testing. This chapter also covers numerous challenges encountered in substituting animals with alternatives and offers suggestions to overcome the current barriers.

Keywords

Cosmetic safety · Nanocosmetics safety · Cosmetics regulatory requirements · Animal free testing · Alternative to laboratory animals

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9.1 Introduction

Cosmetics are among the most extensively used products worldwide; hence, the scope of regulation of these products is quite broad. The high demand for cosmetics is directly related to their ability to help fulfill men's fundamental desire to look attractive and youthful. Cosmetics and related products have been used for ages to serve various purposes, from increasing appearance to improving confidence (Effiong et al. 2019). Cosmetics are defined as any product or article that is intended to be rubbed, poured, sprinkled, or sprayed on, introduced to, or otherwise applied to the human body or a portion of it, excluding soap, with the goal of cleansing, beautifying, facilitating attractiveness, or altering appearance (US-FDA 2021a). Moisturizers, hair care products, makeup, shaving creams, nail paints, perfumes, toothpaste, mouthwashes, face and body cleansers, and deodorants are a few cosmetic products.

Observable makeup was not regarded as "respectable" in ancient times. Shampoo, lotions, creams, and even makeup were commonly homemade. None were regulated, and some were even deemed risky. For instance, Laird's Bloom of Youth, made around 1860, was used for the skin and complexion but was later revealed to contain harmful amounts of lead. Unfortunately, it is clear from the fact mentioned above that humanity has apparently sacrificed their health and safety throughout history in their pursuit of beauty with various harmful handmade cosmetics.

Additionally, cosmetics were not covered under the original Food and Drug Act, popularly known as "The Pure Food and Drug Act," passed in 1906, although it did include color additives for foods and drugs. Attempts to involve cosmetics in the 1906 Act were unsuccessful because they made up a small portion of the economy, were only utilized by a small population, and were perceived as unnecessary. In the 1920s, changes in commerce started to emerge due to external factors like the use of cosmetics in the film industry and the influx of women into the workforce. These factors promoted the direct sale of cosmetics in retail establishments and beauty parlors. Until the 1930s, there was still concern about regulating foods, medicines, and cosmetics (Katz et al. 2022). Fortunately, the days of ignorance when people used risky, harmful, and even poisonous mixtures to enhance their appearances are over. However, the desire to look beautiful and youthful persists, leading to the implementation of verifiable science and technology under a strict regulatory framework to achieve this goal (Effiong et al. 2019).

Cosmetic regulatory frameworks differ significantly across countries and need to be harmonized, thus posing challenges to the production of cosmetics for sale in the global market. The significant markets adhere to similar regulatory standards, but current discrepancies are substantial enough to affect the cosmetic sector by limiting innovation, lowering the market's growth, and hindering international trade. Several international organizations have been working cooperatively to harmonize the regulatory frameworks for cosmetics in various countries. Examples include the International Cooperation on Cosmetics Regulation (ICCR), the Organization for Economic Cooperation and Development (OECD), and the International Organization for Standardization (ISO) (Ferreira et al. 2022). Furthermore, cosmetics are not

considered a necessary good; they are luxury products. Thus, it is unethical to test cosmetics/cosmetic ingredients on animals to evaluate their safety profiles. Thus, in recent years, there has been increased concern about using animals in cosmetic testing among animal welfare agencies, researchers, and even the general public. Many nations have established laws banning the use of animals in cosmetic product testing to protect animals from unnecessary suffering and harm. These nations are now focusing on developing alternatives to animal testing to assess cosmetics' safety.

This chapter overviews cosmetic and nanocosmetic product regulations worldwide and recent developments in alternative testing approaches. In particular, it discusses the cosmetics regulatory frameworks in different countries, the ban on animal testing, relevant alternatives currently employed in regulatory safety testing, the challenges encountered in substituting animals, and how they can be overcome.

9.2 Nanotechnology in Cosmetics

A nanotechnology is an innovative tool used extensively in the production of cosmetics. The ability of nanotechnology to improve the qualities of cosmetic products has made it a promising addition to the cosmetics sector. For over 30 years, the cosmetic industry has extensively employed nano-based compounds (Pastrana et al. 2018; Carrouel et al. 2020; Revia et al. 2019). Nanotechnology has the potential to alter and enhance properties like absorption, texture, protection for active substances, and the overall effectiveness of cosmetics (Revia et al. 2019). Nanotechnology uses nanoparticles or nanomaterials that are produced artificially or naturally and range in size from 1 to 100 nm (Khezri et al. 2018). Cosmetics made from nanomaterials have distinct advantages over cosmetics made on a micro-scale. The cosmetic industry uses nanoparticles to produce results that persist for a long time and have greater durability. The large surface area of nanomaterials enables the ingredients to be transported through the skin more efficiently (Ahmad et al. 2018). Some of the critical goals in employing nanotechnology in cosmetics include effective penetration of components into the skin for improved product delivery, new color components (such as in lipsticks and nail polishes), transparency (such as in sunscreens), and long-lasting benefits (such as in makeup). The ultimate goal of the cosmetics industry when employing nanomaterials is to achieve long-term stability and deliver the proper amount of ingredients to the desired body areas. The anti-aging lotion Capture™, based on liposomes, was introduced by Christian Dior in 1986. Over the years, nanomaterials have been used in many cosmetic products, and several internationally well-known cosmetic brands have adopted them (Raj et al. 2012). L'Oréal S.A., which invests a significant amount of money in nanotechnology, utilizes up to four nano-ingredients (titanium dioxide (TiO_2), zinc oxide (ZnO), silica (SiO_2), and carbon black) in some of its formulas and ranks sixth in the United States in terms of the number of patents linked to nanotechnology (Rigano and Lionetti 2016). Shiseido employs nano-TiO_2 and nano-ZnO in wet-based formulas (such as emulsions) but avoids using them in aerosols due to

the potential risk of inhalation hazards (Shiseido n.d.). Generally, well-known cosmetic companies worldwide gradually incorporate nanomaterials into their products (Fytianos et al. 2020).

Nevertheless, over the past 10 years, there have been growing concerns regarding the potential effects of cosmetic items incorporating nanomaterials on human health and the environment. The rapid diffusion of cosmetic products containing nanomaterials onto the market has raised alarms about their possible impact on human health and the environment. Concerns about the safety of nanomaterials and their application in consumer products, including cosmetics, have been raised by the World Health Organization (WHO), nongovernmental government agencies, political institutions, and agencies (Pastrana et al. 2018). The Food and Drug Administration (FDA) has established its own guidelines for the use of nanotechnology in industrial products, and the European Commission (EC) has updated the recommendations on the safety evaluation of nanomaterials in cosmetic products (Bernauer et al. 2019). Since animal testing is explicitly forbidden by the EC Cosmetic Regulation No. 1223/2009, future toxicological findings for risk assessments in Europe must not involve animal testing. Instead, safety evaluation must be done employing alternative approaches. According to the 2020 announcement of the European Union Observatory for Nanomaterials (EUON), all manufacturers that produce, utilize, or import nanomaterials will need to be registered under the REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) program. This encourages nanomaterial-based companies to provide consumers with proper product safety information. The use of nanomaterials in consumer products that are not registered under REACH is regarded as illegal. The European Commission (EC) is provided with a priority list of nanomaterials by the Scientific Committee on Consumer Safety (SCCS) for risk assessment of nanomaterials employed in cosmetics (Fytianos et al. 2020).

9.3 Current Regulatory Framework of Cosmetics

The cosmetics sector is a global, dynamic, and expanding industry. Over the last few decades, massive industrial innovation has led to a wide range of new cosmetic products and increased sales. The worldwide cosmetic industry had a value of USD 341.1 billion in 2020 alone, and it is predicted to rise to USD 560.50 billion by 2030, with a compound annual growth rate of 5.1% from 2021 to 2030 (Ferreira et al. 2022). In order to assure the safety and quality of cosmetic products and prevent negative consequences for consumer health, the cosmetic industry must be regulated owing to its highly inventive, dynamic, and complex nature. The ability of the global initiative to sell the same cosmetic product across all markets is substantially hampered by the fact that regulatory frameworks vary significantly between markets and nations and need to be harmonized. The significant markets broadly adhere to similar regulatory standards, but the current discrepancies are substantial enough to affect the cosmetics sector by limiting innovation and lowering the market's potential for growth. These variations may also impact international trade and hamper the

ability of regulatory bodies to ensure that every product complies with the local laws used by individual nations (Ferreira et al. 2022). Therefore, it is crucial to identify solutions that can converge regulatory frameworks for cosmetics, foster innovation, boost market growth, and remove trade obstacles. Several international groups have been working together to attain this goal. One example is the International Cooperation on Cosmetics Regulation (ICCR), formed in 2007, which is a voluntary group of cosmetic regulatory authorities from the United States of America (USA), Brazil, Chinese Taipei, Canada, the European Union (EU), Japan, and the Republic of Korea. This group meets annually to discuss various subjects related to cosmetic safety and regulation (for instance, substitutes for animal testing, nanotechnology, and microbiological restrictions) (US-FDA n.d.). Other organizations that play essential roles in developing global standards for cosmetics and the mutual acceptance of testing method guidelines include the Organization for Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO). However, there is still more that can be done to deepen the current collaboration efforts between the various nations and promote the ongoing dialog. The laws and regulations governing the manufacture and marketing of cosmetic products in the European Union (EU), the USA, Canada, Japan, China, Brazil, Australia, Korea, and India represent some of the leading global markets, which are discussed below.

9.3.1 European Union (EU)

The definition of a cosmetic in the EU includes several additional categories, such as pharmaceuticals, biocides, and medical devices, and is centered on the area of application and possible uses. According to the EU, a cosmetic is defined as "Any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition, or correcting body odors." Before a product is put on the market in the EU, the responsible person (RP), typically the producer or the importer, must guarantee its safety. For this reason, the RP must ensure that a cosmetic product safety report (CPSR) is established and that the cosmetic product passes a safety evaluation based on the relevant data (European Union 2009a). According to Regulation (E.C.) 1223/2009, the safety assessor (SA), who is appointed by the RP and qualified in pharmacy, toxicology, medicine, or a related field, or who has completed a course that is accepted as equivalent by a Member State, conducts the safety assessment. The regulation for the SA enlists just one criterion; it does not include any further prerequisites or a definition. As a result, even though the same laws are enforced throughout the EU, the CPSR may differ because it was prepared by experts with various educational backgrounds, professional experiences, and depth of knowledge. The CPSR is divided into two parts and may be obtained in the product information file (PIF) of the cosmetic—Part A:

cosmetic product safety information, which contains all the information required for the safety assessment of the product; Part B: cosmetic product safety assessment, which is the opinion of the cosmetic safety assessor about the product's safety.

In contrast, the PIF includes the following details: the cosmetic product description, the cosmetic product safety report, a detail of the manufacturing process and a statement of compliance with GMP, proof of the effects claimed for the cosmetic product, and information on any animal test carried out by the producer, his agents, or suppliers in relation to the development or safety analysis of the cosmetic product or its ingredients to meet legislative requirements (European Union 2009a). Whenever product modifications or new information becomes available, the CPSR and PIF must be constantly updated and revised. The RP must also provide some information via the cosmetic products notification portal (CPNP), such as the product category and identity, probable exposure conditions, and the framework formulation. Except for cosmetic goods containing nanomaterials, which are subject to an additional procedure, the notification method is the same for all cosmetic items (Ferreira et al. 2022).

Specific criteria for marketing cosmetic goods containing nanoparticles were set by Regulation (C.E.) No 1223/2009. According to Article 16 of Regulation (CE) No. 1223/2009, manufacturers must notify the EC in advance of their intention to use nanoscale ingredients by sending product-related information to the Cosmetic Products Notification Portal (CPNP) 6 months before releasing the product for sale. An estimate of the amount of nanomaterial in the cosmetic product intended to be marketed annually, its toxicological profile, and safety data of the nanomaterial used in a product, depending on the category of the cosmetic product and its exposure conditions, should all be included in the notification. It must also include information about the nanomaterial identification and its specifications, such as particle size and physical and chemical properties. The Scientific Committee on Consumer Safety (SCCS) published recommendations in 2012 outlining the standards to undertake physicochemical characterization, identify the toxicological profile, and determine a nanomaterial's highly probable exposure conditions (SCCS 2012). In order to ensure that consumers can use cosmetic products containing nanomaterials more safely, the regulation also mandates that manufacturers clearly identify nanomaterials on the label by placing the word "nano" after the INCI name of the ingredient (European Union 2009b). However, there is still debate regarding the effectiveness of such a legal obligation.

9.3.2 United States of America (USA)

For instance, a product may fall under two classifications in the USA simultaneously. For example, an antidandruff shampoo may be both a cosmetic and a medication because it has two purposes: to clean the hair (cosmetic) and to treat dandruff (drug). In these situations, the item in question must adhere to both rules (US-FDA 2022). The Federal Drug and Cosmetic Act (FD&C Act) defines the two major product categories: cosmetics and pharmaceuticals, the latter of which

includes a subcategory of over-the-counter (OTC) medications that can be marketed without a prescription (US-FDA 2018). Cosmetics are defined under the Federal Food, Drug, & Cosmetic Act (FD&C Act) as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance." Products, including skin moisturizers, lipsticks, nail paint, eye and facial makeup formulations, shampoos, permanent waves, hair dyes, toothpaste, and deodorants, are all covered by this definition with any substance manufactured to be used as a cosmetic product component. Both ingredients and finished cosmetics imported into the USA must adhere to the same safety and labeling standards as products manufactured locally in the USA. Except for color additives (other than the coloring substances used in coal-tar hair colors), which must be approved for the specific intended application, the FDA does not need pre-market approval of cosmetics. Therefore, the product's manufacturers or distributors have to guarantee its safety. Cosmetic companies must advertise safe, correctly labeled cosmetics, use no prohibited substances, and abide by limitations on restricted ingredients. Additionally, it is considered best to strictly adhere to safety recommendations and criteria issued by the industry.

The FDA in the USA regulates the use of nanomaterials in cosmetics. The FDA evaluated scientific and regulatory considerations for the safety and efficacy of goods incorporating nanomaterials in the FDA Nanotechnology Task Force Report of 2007 (Fytianos et al. 2020). Guidelines outlining safety concerns for cosmetic items containing nanomaterials were suggested by the Task Force. Based on it, producers ought to take safety measures to ensure the safety of nanomaterial-based cosmetic goods. Nanotechnology and nanomaterials are still not subject to a specific regulatory definition. In 2014, the FDA released guidelines for the industry called "Final Guidance for Industry—Safety of Nanomaterials in Cosmetic Products" (FDA 2014) which evaluates safety concerns and offers guidance to the cosmetic industries (Katz et al. 2015).

9.3.3 Canada

In Canada, a cosmetic product is defined by the Food and Drugs Act as "any substance or mixture of substances, manufactured, sold, or represented for use in cleansing, improving, or altering the complexion, skin, hair, or teeth, and includes deodorants and perfumes." This includes items purchased in bulk by institutions (such as hand soap in schools) and utilized by professional esthetic services, as well as handmade cosmetics offered at craft fairs or products made by home-based companies. In Canada, the manufacturer has to guarantee the cosmetic product's safety. Health Canada must be notified of the sale of any cosmetics in Canada. Within 10 days of the product's initial sale, manufacturers must submit a Cosmetic Notification Form (CNF) for each product. This online notice form provides details such as the manufacturer's address and phone number, the function and type of the cosmetic, and the concentration of ingredients. The producer is mainly responsible

for ensuring that the product complies with all regulatory obligations; thus, the notification does not signify approval for sale or any other kind of guarantee about the product's safety (Canada.ca 2017). The use of nanoparticles in various Canadian cosmetic markets is expanding. According to Health Canada, a nanomaterial is "any substance or product manufactured, and any component material, ingredient, device, or structure if: (1) it is comprised within the nanometric dimensions in at least one external dimension or has an internal dimension or surface structure within the nanoscale, or (2) it is smaller or larger than the nanoscale in all dimensions, but exhibits one or more nanoforms properties or phenomena." A list of hazardous cosmetic compounds, more precisely, a list of cosmetic ingredients that are restricted or forbidden, was created by Health Canada in 2007 (Kumud and Sanju 2018).

9.3.4 Japan

In Japan, cosmetics are described as "articles with mild action on the human body, which are intended to be applied to the human body through rubbing, sprinkling, or other methods, aiming to clean, beautify, increase the attractiveness, alter the appearance, or to keep the skin or hair in good condition" (Japan Ministry of Health 2014). There are six categories of cosmetics: perfume and eau de cologne, makeup, skincare items, hair care products, special-purpose cosmetics, and cosmetic soaps. In order to register a cosmetic product in Japan, the authorities must first obtain cosmetic manufacturing and marketing licenses. Each license has specific demands. However, to preserve their products' integrity, marketing license holders must adhere to the Good Vigilance Practice (GVP) and the Good Quality Practice (GQP) standards. After receiving the necessary licenses, the manufacturers must submit a cosmetic marketing notification to the same prefecture that issued the permit. The product can subsequently be placed on the market once all the previously mentioned standards have been met (Crevedo 2022).

9.3.5 China

According to the definition of cosmetic products in China, these are "daily chemical products intended to be applied on the external part of the human body (such as skin, hair, nails, lips, etc.) by spreading, spraying, or other similar ways for cleansing, protecting, beautifying, or grooming purposes." (Su et al. 2020). The State Administration for Market Regulation (SAMR) and the National Medical Products Administration (NMPA), an independent Drug Administration Bureau governed by SAMR, are the two primary competent bodies in China that oversee cosmetic rules. The NMPA has nine subsidiary departments, one of which is the Cosmetic Safety Supervision Department. Medical Products Administrations (MPAs), located at the provincial level and under NMPA, are in charge of filing domestic, non-special use cosmetics and issuing production licenses to cosmetics firms. The 1989 Regulations concerning the hygiene supervision of cosmetics served as the basis

for China's current regulatory framework. In China, multiple laws must be followed and considered, but the Technical Safety Standard for Cosmetics 2015, which replaced the Hygiene Standard for Cosmetics 2007, is the most significant. Special cosmetics must be registered and approved by the NMPA before manufacture, but regular cosmetics can be put on the market immediately following notice under China's new legislation. From January 1, 2022, prior to registration or notification, the registrant or notifier must either conduct a self-assessment safety review or delegate this duty to a qualified agency. They must also disclose the product safety assessment results at the registration or notification time (Su et al. 2020).

9.3.6 Brazil

Personal care items, including cosmetics and perfumes, are described in Brazil as "Preparations consisting of natural or synthetic substances, for external use on various parts of the human body, including the skin, capillary system, nails, lips, external genital organs, teeth, and mucous membranes of the oral cavity, with the sole or main purpose of cleaning them, perfuming them, altering their appearance, correcting body odours, and or protecting or maintaining them in good condition" (Pomela 2015). The registration processes vary depending on the type of product in Brazil. Pre-market approval processes are required for some of the products classified as grade II cosmetics and mentioned in Annex VIII of Resolution RDC 07/2015. Following the publication date in the Brazilian Official Gazette, these procedures are effective for 5 years and may be renewed for further equal-length periods. Pre-market approval is optional for cosmetic items not listed in Annex VIII of Resolution RDC 07/2015; the Brazilian Health Regulatory Agency (Anvisa) must be notified. The Cosmetic Automation System (SGAS System) is used for the online notification process, which is effective for 5 years from the day the online protocol is finalized and can be extended for further equal-length periods.

Nanomaterials and nanotechnology-specific regulations do not exist in Brazil. A discussion on nanotechnology and security surveillance was encouraged in 2012 by ANVISA (National Agency for Sanitary Vigilance). The Internal Committee of Nanotechnology (CIN) was founded in 2013 to validate the state of our understanding of nanomaterials. They prepared a document outlining the initiatives and regulatory frameworks related to nanotechnologies in other nations and alternative principles and frameworks (Melo et al. 2015).

9.3.7 Australia

Cosmetics, according to the Australian Government, are "substance that is designed to be used on any external part of the human body – or inside the mouth – to change its odors, change its appearance, cleanse it, keep it in good condition, perfume it or protect it." The import, production, marketing, and delivery of cosmetics are tightly controlled and complex in Australia. Before introducing a cosmetic product into the

Australian market, it must undergo various product assessments to determine the necessary approvals and registrations. Trademark and/or patent clearances must also be obtained to reduce the risk of infringing on the intellectual property rights of others. Labeling, packaging, and advertising must also be checked for compliance with the Australian Consumer Law and applicable advertising codes. The Therapeutic Goods Administration (TGA), the Department of Health under the Australian Industrial Chemicals Introduction Scheme (AICIS), and the Australian Competition and Consumer Commission (ACCC) are the three government regulators in charge of monitoring cosmetics regulation in Australia. The National Industry Chemicals Notification and Assessment Scheme (NICNAS) monitors the safety of the ingredients in cosmetics and personal care items in Australia, while the Therapeutic Goods Administration (TGA) regulates sunscreens, which are therefore regarded as drugs. However, neither of these associations makes a distinction between bulk materials and nanoparticles (Raj et al. 2012). In Australia, the NICNAS defines a nanomaterial as "an industrial material intentionally produced, manufactured, or designed to have specific properties or a specific composition and one or more dimensions, typically between 1 and 100 nm." As mandated by the TGA, the Industrial Chemicals Notification and Assessment Act 1989 regulates all chemical ingredients (including natural items) as industrial chemicals (Kumud and Sanju 2018).

9.3.8 Korea

South Korea, which accounts for around 2.8% of the worldwide market, is among the top ten cosmetics markets in the world. "K-Beauty" is rising, and Korea is regarded as the world's center of innovation in the cosmetic industry. South Korean products dazzle with their efficiency, packaging, and sensory appeal, thus inspiring Western brands. In South Korea, cosmetics are rubbed, sprayed, or otherwise applied to the skin or hair to maintain, improve, or enhance the appearance of the skin or hair. Currently valued at around $10 billion, the South Korean cosmetics market is predicted to grow at a CAGR of 4.95% from 2017 to 2030 (Peters and Choi 2020). The South Korean Government issued the comprehensive cosmetics laws known as the Cosmetics Act 3 (Act No. 17250) in 2000 to help improve public health and expand the cosmetics business. The law, which was most recently revised in April 2020, includes provisions for manufacturing, importing, and marketing cosmetics and cosmetic ingredients and specific guidelines for product labeling and promotion (Peters and Choi 2020).

9.3.9 India

The Drugs and Cosmetics Act of 1940, guidelines from 1945, and labeling declarations issued by the Bureau of Indian Standards (BIS) govern cosmetic items in India. The BIS established the cosmetic standards listed in Schedule "s"

of the Drugs and Cosmetics Rules of 1945. In addition, the BIS provided the specifications for skin creams and lipsticks in Indian Standards (IS) 6608:2004 and 9875:1990 (Nanda 2018). Each raw material must pass a heavy metal test in accordance with Indian Standard 6608:2004. If raw materials are screened early, the manufacturer may not need to test the final cosmetic product for heavy metals (CliniExperts 2016). Rule 134 of the Drugs and Cosmetics Rules contains restrictions on the use of cosmetics, including colors, pigments, and dyes besides those listed by the BIS and Schedule Q. Arsenic and lead compounds are no longer allowed to be used as coloring agents in cosmetic products, according to D&C Rule 145. The import of cosmetics containing arsenic or lead is prohibited by Rule 135.

Similarly, the manufacture and import of cosmetics with mercury-containing ingredients are prohibited by Rules 145 D and 135 A (Centre for Science and Environment n.d.). The "Nanotechnology Sectional Committee" group has been established by the Bureau of Indian Standards (BIS) and comprises 33 members from various research institutions and companies. This committee attempts to standardize laws governing nanotechnology (Kumud and Sanju 2018).

9.4 Ban of Cosmetic Testing on Animals

Animals have long been employed in biomedical research as significant experimental subjects due to their physiological resemblance to humans. Animal testing is typically necessary to determine the efficacy and safety of drugs. Animals occasionally experience injury, discomfort, suffering, and even death. Animals are extensively employed in preclinical research for many significant diseases since pharmaceuticals are a necessary commodity. Animal testing has been used for many years to evaluate cosmetic products. Cosmetics are not considered an essential commodity; instead, they are luxury goods. Using in vivo tests for cosmetic items has long raised ethical concerns due to their potential to cause skin irritation, stinging, contact urticaria, allergic sensitization, photoallergy, and phototoxicity. The issue of animal experimentation in cosmetics has received a lot of attention over the years, and consumers are becoming more aware of the issue and imposing higher demands on the sector to ensure the welfare of animals. Fortunately, the cosmetics sector is prioritizing finding alternatives to animal testing, and the number of nations with enforceable bans on animal testing is expanding. Many countries have implemented laws that forbid using animals to test cosmetics in order to prevent unnecessary animal suffering and harm. The present scenario of implementation and bans of animal experimentation in a few nations is discussed herein:

Europe—The European Union was the first to ban animal testing for cosmetics. As of March 2013, the European Union entirely prohibited the sale and import of cosmetics that have undergone animal testing or the use of ingredients that have undergone such testing. The European Union is a significant market for cosmetics businesses worldwide, and this policy has compelled various nations, including China and South America, to seek alternatives for animal testing methods employed in the cosmetic industry (Skincare n.d.; Sreedhar et al. 2020).

USA—Currently, legislation banning the use of animals in cosmetic research has been approved in eight U.S. states: California, Hawaii, Illinois, Maine, Maryland, Nevada, New Jersey, and Virginia (The Human Society of the United States n.d.). Even though the FD&C Act does not entirely ban the use of animals in safety cosmetic studies, and the FDA supports the use of alternative methods for the improvement, reduction, and replacement of animal testing, it is the manufacturers' responsibility to carry out whatever in vivo tests are deemed necessary to maintain the safety of their products in the rest of the country (US-FDA 2021b).

Canada—Animal testing for cosmetics is not prohibited in Canada. Bill S-214 (the Cruelty-Free Cosmetic Act) was introduced in Canada in 2015 to stop using animals for cosmetic research and the sale of cosmetic items produced using these methods. However, this bill has yet to become law, so it is still acceptable to utilize such procedures (Toronto Humane Society 2021).

Japan—Japan is in the process of banning animal experiments for testing cosmetics. Till today, there has been no specific legislative obligation in Japan for all cosmetic goods to be tested on animals, and there are also no laws that forbid such tests. There is no application process for approval, and each cosmetics manufacturer is urged to ensure the quality of their products in accordance with the self-responsibility principle. The manufacturers have been given the authority to conduct their own safety assessments of raw materials and final goods per their requirements.

China—Global trade between regions like the European Union and nations with "cruelty-free" testing standards has long been significantly hampered by China's mandatory animal testing requirement for cosmetics registration, But China has begun to harmonize its laws as many other nations eventually prohibit animal experiments. The need for general cosmetics, whether imported or produced in China, to undergo animal testing was officially abolished in China on May 1, 2021. Nevertheless, a few prerequisites and exceptions may exist. One requirement is to provide the GMP certification from the nation's or region's cosmetic regulatory authority. Since many nations still need to give this form of GMP certification, it is challenging to meet this criterion (RedOrangePeach 2022).

Brazil—Animal testing on cosmetics has already been prohibited in some Brazilian states, including Mato Grosso do Sul, Amazonas, Paraná, Minas Gerais, Pará, Pernambuco, Santa Catarina, Rio de Janeiro, Sao Paulo, and the Federal District. Anvisa guidelines, however, continue to acknowledge the use of animal testing to evaluate the risks associated with cosmetics and their constituents (Humane Society International 2021).

Australia—Animal testing for cosmetic safety is not permitted in Australia after the ban commenced on July 1, 2020. Cosmetics and products tested on animals outside of Australia are also prohibited from being sold in Australia (AG-Department of Health and Aged Care 2019).

Korea—The production of cosmetics involving animal testing was planned to be prohibited by the Korea National Assembly in 2018. South Korea's Ministry of Agriculture, Food, and Rural Affairs has prepared plans to ban the use of animals in cosmetic testing. The Government's Five Year Plan for Animal Welfare forbids

testing of finished cosmetic products on animals, while a ban on testing ingredients is still up for discussion (Cruelty Free International 2016).

India—Animal testing on cosmetics was prohibited in India in 2014 (The Times of India 2014a). The Ministry of Health and Family Welfare has incorporated the new regulation into the already-existing Drugs and Cosmetic Rules, 1945. According to the new law, testing cosmetic products on animals is forbidden. The import of cosmetic products tested on animals is also prohibited in India (The Times of India 2014b).

9.5 Alternative Methods for Animal Testing

For a long time, there has been debate about animal suffering, distress, and death during scientific research. It is argued that as animals are living organisms, they have a right to be free from pain and suffering, and using them in research is considered unethical and ought to be discontinued. Numerous acts and legislative measures have been passed to reduce animal suffering during testing and ensure the ethical use of animals. For instance, the Royal Society for the Prevention of Cruelty to Animals founded the animal rights organization in 1824. Another law was passed in the UK in 1876 to combat animal cruelty. It was introduced in India, France, and the USA in 1960, 1963, and 1966, respectively (Doke and Dhawale 2015). To protect animals from abuse and cruelty, a number of laws and guidelines are currently observed on a global scale. Guidelines for animal housing, breeding, feeding, transportation, and, most importantly, their use in scientific experiments are provided by organizations like ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use), CPCSEA (Committee for the purpose of Control and Supervision on Experiments on Animals), NIH (National Institutes of Health), and OECD (Organization for Economic Cooperation and Development). In addition to the primary ethical issue, other drawbacks of animal experimentation include the need for skilled or trained personnel and time-consuming procedures. Another disadvantage is the exorbitant cost associated with housing and breeding animals and the lengthy protocols of animal research. Several alternatives have been proposed to address some of the issues with animal testing and to prevent unethical practices. The search for the development of new methods and techniques aimed at the reduction, refinement, and replacement of the use of animals in research has become a global trend since the introduction of the 3R's principle (replacement, reduction, and refinement) in 1959 by Russell et al. in their book "The principles of humane experimental technique." The development of alternatives to animals has dramatically increased during the past 30 years. The following section provides a brief summary of several contemporary alternative techniques:

Computational Approaches—The numerous possible biological and toxic effects of a chemical, and potential pharmaceutical candidate are predicted using computational models, which avoid the need for dissecting animals. Examples of such approaches include quantitative structure–activity relationships (QSARs),

grouping and read across, computer-aided drug design (CADD), and physiologically based kinetic (PBK) models. QSARs are computer-based methods for estimating a substance's likelihood of being toxic based on its similarities to other drugs and our understanding of human biology. They have the potential to replace animal experiments with cosmetic testing. QSAR techniques are being used more frequently by companies and governments to avoid using animals in chemical testing. Only the most promising compounds discovered through primary screening are tested in vivo. For instance, in vivo testing is required to determine the receptor-binding site of a drug. A potential receptor-binding site of a drug molecule can be predicted using CADD software. In order to prevent undesirable testing compounds with no biological activity, CADD attempts to identify probable binding sites. These software tools can also specifically customize a novel drug for a given binding site. Finally, animal testing is carried out to get conclusive data. The computer database does a remarkable job of predicting possible properties of drug candidates, like carcinogenicity and mutagenicity. The most modern QSAR software provides more accurate results indicating a molecule's ability to cause cancer.

The procedures' speed and relatively low cost make computer models superior to traditional animal models (Doke and Dhawale 2015). In silico predictions are also a great strategy when combined with partially concluded data, such as the in vitro mutagenicity test. While the in vitro micronucleus assay is necessary to examine genotoxicity, a QSAR prediction can assist in better understanding a substance's ability to cause DNA damage before the tests are carried out. According to the ICH M7 guideline, such methods are widely approved for the regulatory assessment of pharmaceutical impurities (Fioravanzo et al. 2012). Organs simulated on a chip and vast chemical databases are now available to researchers to determine whether a cosmetic is likely to adversely affect humans. The COSMOS project has developed sophisticated computer models that can predict where a chemical will end up in the body after coming into contact with human tissue. A database of more than 5000 ingredients used in cosmetics and their effects is also being generated as part of the COSMO project (European Commission 2015).

In vitro test systems—An important substitute for animal testing is in vitro cell and tissue cultures, which involve the growth of cells outside the body in a laboratory setting. After being removed from the animal, the cells and tissues from the liver, kidneys, brain, skin, and other organs can be maintained outside the body for a few days to several months or even a few years in an appropriate growth medium. Animal and human cells are isolated and grown as a monolayer on the surface of culture plates or flasks during in vitro culture. It is also possible to use cellular components like membrane fragments and enzymes. There are many different uses for various cultures, including cell, callus, tissue, and organ culture. The advantages of in vitro methods are their simplicity, efficiency, time-saving, and low cost. To assess the toxicity and efficacy of potential therapeutic compounds and chemicals, several in vitro approaches are routinely used (Clift and Doak 2021). These in vitro tests determine the effectiveness and toxicity of almost all cosmetics, pharmaceuticals, and chemicals. Researchers at Wyss Institute, Harvard University, have developed "organs-on-chips technology" that mimics the microenvironment

and physiological processes of human organs like the lung, liver, brain, and skin. Compared to animal research, they are more accurate in simulating human physiology and can substitute animals that endure painful, lengthy tests to determine whether cosmetics are toxic or likely to irritate the skin, eyes, or other body tissues. MatTek's cornea-like 3D tissue structures made from human cells can be employed instead of rubbing or dripping cosmetic products into the eyes of rabbits (Lee et al. 2017).

In vitro dermal absorption tests, capable of predicting probable dermal absorption in humans, are the gold standard method for studying skin pharmacokinetics. Through in vitro skin absorption tests, several formulation types, including hair dyes, shampoos, foundations, moisturizers, cleansers, soaps, sunscreen, suspensions, foams, patches, and aqueous formulations, can be tested. Detailed instructions for conducting in vitro skin absorption tests can be found in the OECD Guidelines 2004, 2011, and 2019 (OECD 2004a, b, 2011). A first set of "basic criteria" for the in vitro evaluation of skin absorption of cosmetic ingredients was adopted by the SCCNFP (Scientific Committee on Cosmetics and Nonfood Products) in 1999 and amended in 2003 (SCCNFP/0750/03). In 2010, the SCCS (Scientific Committee on Consumer Safety) revised this opinion and released it as (SCCS/1358/10) (Barthe et al. 2021).

For performing appropriately in vitro skin absorption tests for cosmetic chemicals, the OECD 428 guideline and the SCCS "Basic Criteria" (SCCS/1358/10) are viewed as necessary. When conducting an in vitro dermal absorption test, a skin sample is placed in a Franz-type diffusion cell between two chambers (a donor chamber and a receptor chamber), with the stratum corneum facing the donor compartment and the dermis touching the receptor compartment. Most of the time, patients undergoing plastic surgery provide human skin samples. The most convenient skin to work with is abdominal skin, owing to its large surface area. When skin viability and metabolic activity are not being explored, carefully managed frozen human skin is adequate for investigating the passive penetration of cosmetic compounds (Barbero and Frasch 2016). However, fresh skin samples are required for studies requiring the presence of live epidermal tissue, such as analyses of drug transporters (Clerbaux et al. 2019) *and skin metabolism* (Alriquet et al. 2015).

Living human keratinocytes have been cultured to produce a multi-layered, highly differentiated epidermis for the RHE skin model. The model contains a functional skin barrier with an in vivo-like lipid profile and well-structured basal cells. Eye irritation testing is conducted using a commercially available 3D model based on a reconstructed human cornea-like epithelium (RhCE) (OECD Test Method 492) (OECD 2019). Living human cells were used to develop the multi-layered, differentiated corneal epithelium that makes up the RhCE corneal model. The endpoint used in both RhE and RhCE test procedures is the reduction of MTT (3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide) by cells into a blue formazan salt, which is quantitatively evaluated after extraction from tissues. Interleukin-1 (IL-1) production measurement is a second endpoint that can be utilized to enhance sensitivity. If the viability of the test item is greater than 50% (RhE) or 60% (RhCE) (no label or UN GHS no category), it is classified as a

non-irritant. If the viability of the test item is less than or equal to 50% in the RhE model, it is classified as an irritant (Barthe et al. 2021). No prediction can be made if the viability of the RhCE model is less than or equal to 60%; additional testing may be necessary. The hen's egg test on chorioallantoic membrane (HET-CAM) has been utilized for eye irritation and toxicity testing. These tests have described irritation levels ranging from barely irritating to severely irritating compounds (Prinsen et al. 2017). In vitro systems like the KeratinoSens™ assay, which uses immortalized human keratinocytes (HaCaT) lineage transfected with a selected plasmid, have been validated to evaluate the sensitization potential of chemical compounds (Natsch et al. 2015). EpiDerm™, EpiSkin™, and SkinEthic™ are OECD-validated models that reasonably resemble human skin (16–18).

The exposure of the skin to solar irradiation and photoreactive xenobiotic compounds, including cosmetics, may cause unusual skin problems. Phototoxicity is an acute light-induced reaction that occurs when photoreactive agents in cosmetics are activated by sunlight and converted into toxic products in skin cells. The primary focus is on non-animal test methods, such as in vitro and chemico (cell-free test tube methods), which determine the phototoxicity of cosmetics to minimize animal suffering and agony. Standard fibroblast cells derived from Swiss mouse embryonic tissue cells (3 T3) are used in the in vitro 3 T3 NRU ultraviolet experiment (95% correlation with in vivo assay) to assess the 50% mean inhibitory concentration (IC50), with and without exposure to solar radiation (Nabarretti et al. 2022). *In chemico,* methods have been employed to detect the formation of reactive oxygen species or DNA strand break activity in cosmetics with a potential for phototoxicity. Other in vitro test systems include the erythrocyte photohemolysis test and the phototoxicity test employing a human 3-dimensional (3D) epidermis model (Kim et al. 2015).

There are a few drawbacks associated with these isolated systems as well. Typically, they cannot provide all of an organism's physiological responses. When removed from the organism, the components frequently degenerate and lose the capacity to carry out their unique functions. Another disadvantage is that the impact of the exposure route, which has a significant effect on the test results, cannot be evaluated with these approaches.

Alternative Organisms—The use of higher model vertebrates for experimentation, such as guinea pigs, rats, dogs, and monkeys, have been greatly restricted by ethical concerns. The use of alternative organisms such as plants, single-celled organisms, invertebrates, and other non-animal organisms has been suggested that can be used in cosmetic testing in place of experimental animals. All of these can react to certain noxious stimuli, and some may experience pain. Nevertheless, many analysts suggest that they do not sense pain or suffering in the same manner as animals do, especially when there is no brain or neural tissue present.

Microorganisms—The use of bacteria and fungi to evaluate various genotoxic effects has received increasing attention in recent years. These organisms have the significant benefit of being much simpler and quicker to culture than most animal or human cells. Their genetic makeup is more straightforward than that of animals and humans. Furthermore, a broad understanding of their physiology and functions

facilitates their use, particularly in toxicological research that leads to the development of new techniques. Genetic material alterations are relatively simple to identify and characterize. The use of fungi in mutagenicity testing has been demonstrated to be very beneficial, and they appear to be more sensitive than bacteria.

Brewing yeast, or Saccharomyces cerevisiae, is the most well-known and significant model organism due to its quick growth, ease in replica plating and mutant isolation, dispersed cells, precisely defined genetic system, and highly adaptable DNA transformation system. S. cerevisiae contributes to our understanding of the fundamental cellular biology in neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's diseases by analyzing endogenous or heterologous proteins whose aggregation is the root cause of these ailments (Pereira et al. 2012). Slime molds, algae, and protozoa have also been shown to be beneficial. Protozoa typically have specialized functions that resemble humans, although they are generally relatively primitive. The cilia in the human bronchial tube, for example, and those of protozoa both react to smoke or phenols. Smoke toxicity tests have utilized a variety of protozoans. Protozoans are currently being considered for use in screening tests for carcinogenesis, mutagenesis, and reproductive toxicity (Doke and Dhawale 2015). These microorganisms can effectively be used for toxicity testing cosmetic products and their ingredients.

Invertebrates—Animals employed in laboratories are frequently replaced with invertebrate species. Invertebrates have been used to study various diseases, including Parkinson's disease, endocrine and cognitive disorders, muscle dystrophy, wound healing, cell aging, programmed cell death, retrovirus biology, diabetes, and toxicological testing (Castillo and de la Guardia 2017). There are several restrictions on the use of invertebrates in treating human diseases since they lack the adaptive immune system and have undeveloped organ systems. However, many invertebrates can be studied in a single experiment in a short time with fewer ethical issues because of their numerous advantages, like their short life cycle, small size, and superficial anatomy. In comparison with animals, their maintenance costs are also lower. For instance, a shelter that can only house a few mice could house thousands of flies. One of the most extensively researched invertebrate species is the fruit fly, *Drosophila melanogaster* (Allocca et al. 2018). Its genome has been extensively studied, making it possible to investigate the molecular processes that underlie human diseases. Its entire genome, which contains more than 14,000 genes on four chromosomes, has been sequenced and annotated. The majority of *Drosophila melanogaster's* genome is carried by just three genes. It is believed that the fly has functional homologs of over 75% of the genes linked to human diseases. Numerous organs in the fly, including the heart, lungs, intestines, kidneys, and reproductive system, perform similar activities to those of mammals. Various molecular and genetic methods are currently available for determining the mutagenicity, teratogenicity, and reproductive toxicity of *Drosophila melanogaster.* Many drugs that affect the central nervous system produce similar responses in flies as in humans. Fruit flies were an exceptional and sensitive model for studying human genetics and diseases because of their many similarities in development and behavioral activities. The *Drosophila melanogaster* (Meigen) somatic mutation and

recombination test, also known as the "wing spot test," was used to assess the genotoxicity of 10 essential oil constituents used as flavoring agents or cosmetic ingredients as part of a screening project aimed at determining their mutagenic activity (Mademtzoglou et al. 2011).

Caenorhabditis elegans (C. elegans), another invertebrate model, has been widely used in toxicity testing. It is a tiny nematode that may be maintained using in vitro methods at a low cost. It has been frequently demonstrated that toxicity ranking screens in *C. elegans* are just as predictive of LD50 rankings in rats and mice. Furthermore, numerous cases of conservation of toxicant modes of action between *C. elegans* and mammals have been identified. These strong correlations support the use of *C. elegans* assays in early safety testing of cosmetics and as a part of tiered or integrated toxicity evaluation techniques. Still, they do not suggest that nematode data may substitute for mammalian data in assessing health hazards and toxicological assessments of cosmetics. Cosmetic safety testing studies employing *C. elegans* would provide findings from an entire organism with intact and metabolically functioning digestive, reproductive, endocrine, sensory, and neuromuscular systems, in contrast to toxicity testing utilizing in vitro cell cultures. The Complex Object Parametric Analyzer and Sorter™ (COPAS) automates the examination of several endpoints on hundreds of *C. elegans* per minute using microfluidics and laser-based technologies. Studies evaluating six or seven water-soluble compounds have revealed that the COPAS ranking for these endpoints in *C. elegans* coincides with the mouse LD50 ranking for the same compounds. The COPAS has also been used to evaluate larval growth and reproductive production (Hunt and The 2017). COPAS-based quantification of hundreds of compounds from the U.S. EPA's ToxCast™ phase I and phase II libraries on *C. elegans* larval growth correctly predicts developmental toxicity in rabbits or rats with a balanced accuracy of 45–53%, which is somehow less than the concordance for developmental toxicity between rats and rabbits, which was 58% (Boyd et al. 2016).

Lower Vertebrates—A small freshwater fish known as zebrafish (Danio rerio) has been used as a cost-efficient alternative to filling the void between fully synthetic techniques and mammalian model systems. Research on embryonic zebrafish provides an excellent middle ground for testing cosmetic products and their ingredients by enabling scientists to access the benefits of working with mammals more responsibly and ethically while still helping high-throughput cosmetics testing. During early development, the optical clarity of zebrafish enables easy screening, direct observation of gene expression, developmental stages, and phenotypic traits, and efficient evaluation of cosmetics toxicity test endpoints. Its laboratory maintenance on cell culture plates and petri dishes is favored by its small size, short life cycle, and high fecundity (Lachowicz et al. 2021). Organ-focused data could be obtained using zebrafish embryos. This information can only be provided by organoids or other non-animal models such as in vitro, tissue-based, or ex vivo. This offers a comparative benefit compared to in vitro cellular experiments. The transparency of zebrafish embryos enables noninvasive tests and permits easy monitoring, which could be particularly advantageous for developmental toxicity tests for cosmetics and their ingredients. The in vivo visualization of tissue/cells in

acute toxicity studies for cosmetics is a significant advantage of zebrafish. These in vivo readouts are far more accurate and informative because the cells or tissues used in in vitro tests are just a part of a living organism.

Human Volunteers—Prior to large-scale human trials, a technique known as "microdosing" might offer crucial information about the safety of cosmetics. Volunteers are exposed to a very low, one-time dose of the test compound, and the effects are monitored using advanced technologies. Microdosing can substitute specific animal tests for cosmetic safety testing and help identify cosmetics that would not work on humans, preventing their testing on animals. High levels of safety must always be maintained. Human interests should always receive priority over scientific and societal interests. As a result, the investigator should stop conducting the study as soon as it is recognized that the risks exceed the expected benefits. An ethical committee should be consulted for compatibility tests for cosmetic items that could harm volunteers, provided that the committee complies with all applicable rules and regulations in the country where the study is being conducted. Human volunteers should be fully informed of the study's objectives, procedures, and potential discomfort (Nobile 2016).

Prior to participating in the study, free and informed written consent is required from all volunteers. Modern brain imaging and recording methods using human volunteers, such as functional magnetic resonance imaging (fMRI), could replace antiquated cosmetic testing studies using animals with brain damage. Researchers may now safely study the human brain at the level of a single neuron (using intracranial electroencephalography), and they can even use transcranial magnetic stimulation to temporarily or irreversibly induce brain diseases.

Human–Patient Simulators—Computerized human–patient simulators, which can breathe, bleed, convulse, talk, and even die, could be used to study the biological response to the application of cosmetics and their ingredients. These simulators are much more effective at teaching students about physiology and pharmacology than simple exercises involving the dissection of animals. The most advanced simulators simulate diseases and injuries and provide appropriate biological effects for pharmacological therapies. Human simulators, virtual reality platforms, and computer simulators have largely replaced animal laboratories in medical colleges across the USA, Canada, and India. Systems like TraumaMan, which simulate breathing, and bleeding human torso with realistic skin and tissue layers, ribs, and internal organs, are frequently used to teach emergency surgical techniques for more advanced medical training. These systems are more effective at imparting lifesaving skills than programs that require students to cut into live pigs, goats, or dogs (Liventsev et al. 2021).

9.6 Roadblocks to the Implementation of Animal Alternatives

Scientific Constraints—Current scientific methods for testing theories present a significant obstacle to substituting animals for cosmetic safety testing. The standard procedure mainly entails testing a chemical in models with increasing complexity

while developing trust in the hypothesis as it overcomes each obstacle. The most frequent justification for using animals is the apparent requirement to test a cosmetic in a "complex, entire being" before being sufficiently confident that testing on people can be done safely (Taylor 2019). This is based on the notion that testing cosmetics on a sophisticated and complex creature will be able to identify all potential, unforeseen ways in which a cosmetic could be damaging (or ineffective), thus bypassing the damage to human volunteers. In vitro-based approaches are not considered sufficient because they are perceived as inadequate due to their apparent lack of complexity. The potential quest to record every possible interaction of cosmetics within complex animals may raise the issue of cosmetics being tested on the wrong species. Researchers who support alternative techniques find this extremely irrational, and there is a significant gulf between the two parties regarding the importance of complexity versus relevancy. The complexity vs. relevance dispute may be resolved using the adverse outcome pathway (AOP) framework. The AOP is a systematical procedure that uses the available details about a toxicological response and explains the mechanistic interconnections between an initial molecular event, several intermediate critical events, and the adverse outcome. The AOP framework offers practical recommendations to encourage the development of alternative cosmetic testing procedures (Halappanavar et al. 2020). Another solution to increase complexity and relevance is to use "lab-on-a-chip" techniques and more advanced in vitro techniques like "3D tissue constructs" and "mini-brains" (Caruso 2017).

Traditional Barriers—Despite significant advancements in reducing and improving animal testing, the scientific and regulatory communities frequently still view animal testing as the "gold standard" to which all alternative tests must comply. Additionally, researchers that use animals in their studies will attest to the challenges associated with publishing research that employs a strategy that is distinct from the standard approach and obtaining funding for developing/using innovative alternatives. Researchers often complain behind closed doors about journal editors even requesting that their proposal or research be tested in an animal model before it is published (Cronin 2017). It is challenging for new ideas to get acceptance by the current scientific community. Research groups dissatisfied with this situation are frequently unwilling to speak up because it might adversely impact their research funding or university tenure.

Absence of Strict Laws—Under EU rules, in vitro alternatives to animal testing may be used in place of in vivo testing. Validated in vitro tests can take the place of animal studies as long as the test results are of equivalent quality and value for assessing safety. This leads to the conclusion that there are no mandatory legal requirements for alternatives to animal testing in EU cosmetics law. The language is cautious; instead of using the words "preferred" or "obliged," it is "permitted to employ" alternative approaches. Furthermore, Directive 2001/83/EC of the European Parliament and the Council of the EU often communicate mixed information. On one side, it supports the 3R's principles, but on the other, it explicitly mentions animal testing, even specifying in one instance the type of animal that should be utilized (e.g., rodent or non-rodent). This raises concerns for researchers

regarding the potential replacement of animal testing entirely by in vitro tests and other recent technologies. It is currently more appropriate to replace the traditional animal test with integrated testing strategies (ITS), which incorporate both in vivo and in vitro tests (Vermeire et al. 2013).

Lack of Funding—Despite extensive efforts and notable advancements in this area, raising funds to develop alternatives to animal testing is still quite challenging. For instance, the EC and the cosmetic sector each committed €25 million to develop substitutes for using animals to assess long-term toxicity following bans on animal testing for cosmetics in 2009 (Taylor 2019). In addition, the EC has invested €180 million in replacement approaches under the most recent significant scientific funding stream, Framework Project 7 (2007–2013) (EC 2021). The total budget for Framework Project 7 was €45.3 billion, and the commission only allocated 0.4% of its research budget to alternatives to animal testing. Thus, investment in alternative development is extremely low when compared to overall science funding. National funding levels are considerably lower than central funding, possibly indicating a general lack of interest in improving scientific processes due to ethical concerns. The rate of progress in developing alternatives to animal testing of cosmetics is expected to be slow until funding levels substantially increase and are proportional to the magnitude of the problem.

Bureaucratic Barriers—The adoption of alternatives is often delayed due to bureaucratic obstacles, especially when it comes to regulatory acceptance. An effort to synchronize testing requirements globally often results in bureaucratic delays. For instance, the reconstituted skin model's initial validation was confirmed by ECVAM for detecting corrosive compounds in 1998 (ECVAM Scientific Advisory Committee (ESAC) 1998), but the OECD did not approve it until 2004 (OECD, TG 431). The OECD did not adopt the skin irritation model (OECD TG 439) till 2010, despite the fact that its initial version had been validated in 2007 (ESAC 2007). Using unusual protocols, political pressure caused the EU to accept skin procedures before the OECD for corrosion in 2000 (European Parliament and Commission of the European Communities 2000) and irritation in 2009 (Commission of the European Communities 2009). The EU never seemed to adopt this method, even though other systems have experienced comparable delays. For instance, the direct peptide reactivity assay (DPRA) for skin sensitization was not made public as OECD TG 44C until 2015, despite being authorized in 2012 (European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHC) 2012). It took more than 2 years following its OECD publication for it to be published in the EU Test Methods Regulation (Commission of the European Communities 2017). The timing of the cycle for revising test guidelines is one factor causing delays at the OECD and the EU. The process at the OECD is annual; a whole year is wasted if the deadline for submitting techniques is missed. Given enough political will, this process can be expedited by increasing the frequency of meetings. Additionally, the majority of EU members in Europe are also OECD members, thus negating the need for a second round of negotiations to revise the Test Methods Regulation.

Lack of International Harmony—The most "cautious" regulatory body establishes the permissible degree of "risk" because cosmetic items are typically

produced for a global market. Manufacturers frequently continue to use animal-based models, notwithstanding the availability of alternatives or the encouragement of alternatives by particular regulators to comply with the applicable regulations in most countries and reduce the risk of a delay or rejection. This obstacle will be overcome only by harmonizing regulatory requirements internationally (Vonk et al. 2015). Harmonization is desirable since it ensures that all participating nations will accept the results of a single (animal) test undertaken in a laboratory in one country for the regulatory submission of cosmetics, thus saving time and resources. This is termed "mutual acceptance of data (MAD)." Over the past 20 years, significant efforts have been made to promote global harmony in the chemical and pharmaceutical sectors. Alternatives to animal testing must also go through the same harmonizing procedures as traditional approaches. Despite several initiatives to strengthen harmonization, different national interpretations may result in additional requirements for regulatory acceptance between nations. Other regulators may sometimes share the European desire to promote the 3R's strategies more widely. Although alternatives are now acceptable in Europe, due to a lack of international harmonization of categorization and labeling standards, rabbit skin irritation tests are still performed in Europe for non-EU regulators (Taylor 2019).

Validation of Alternative Approaches—Before alternative approaches can replace animal experiments in the market authorization process, they must undergo stringent validation procedures (Kooijman 2013). Validation of alternative approaches is relatively easy but time-consuming and demands significant financial investments from manufacturers and/or the scientific community. Its scientific output is also not highly appreciated by the scientific community and only yields small scientific credits. Additionally, recovering the expenses of the validation studies is impossible due to the lack of a market for alternative methods, which is partially attributable to the unwillingness of regulatory bodies to accept data obtained through alternative testing procedures (Kooijman 2013). The additional animal testing required to obtain adequate data for the particular context in which the alternative approach will be employed occasionally makes the validation of alternatives an effort that is not worth undertaking. Manufacturers, scientists, and regulators frequently continue to use animal-based models despite the availability of alternatives to reduce the possibility of rejection or delay (Vonk et al. 2015).

9.7 Overcoming Roadblocks to Implement Alternatives

- **It is generally recognized that only one alternative method involving** a single in vitro test or in silico prediction method **could completely replace in vivo animal tests.** Hence, Integrated Approaches to Testing and Assessment (IATA) must be used to evaluate cosmetic products' safety profiles based on AOP data. AOPs are the main component of the toxicological knowledge framework that provides a current understanding of the relationship between a molecular initiating event and an adverse outcome. Several AOPs are now being developed for various complicated toxicity endpoints in the OECD AOP initiative. The

AOPs are expected to aid in developing numerous precise in vitro test procedures and innovative integrated approaches for efficiently assessing the safety of several cosmetics and their ingredients.

- Currently, it is the researcher's responsibility rather than the regulators to demonstrate that there is no alternative available to replace animal testing in their proposed projects. The regulator evaluating a project that offers to utilize animals is not often an expert in the field. When an alternative method that can prevent animal experimentation or partially replace it is available, regulatory agencies should simply take responsibility for enforcing the law. Currently, some animal protection organizations consider it the responsibility of regulators and hold them accountable. Regulators who are genuinely committed to questioning the need for animal testing, such as those with vast knowledge of alternatives or animal protection, must only be involved in conducting ethical evaluations of research projects involving animals. Furthermore, a strict attitude adopted by regulators under a tough directive from their governments would be beneficial (Taylor 2019).
- The backbone of developing or promoting alternatives to using animals in cosmetic research and product safety testing is funding. National and international regulatory agencies must allocate substantial funds to provide essential seed money to researchers or companies interested in developing novel alternative procedures. Numerous financial incentives must also be given to companies and research laboratories to encourage the use of alternatives to animal testing in cosmetics.
- International efforts to support the work of animal welfare organizations and a general shift in public attitude resulted in a number of animal testing bans being imposed globally, along with the development of new alternatives. However, many nations continue to employ animal-based models to adhere to the prevalent laws in most countries and minimize the possibility of the global market rejecting or delaying the release of cosmetic items. The main impediments to completely eliminating animal-based tests in cosmetic testing were a need for mutual acceptance and international harmonization. An internationally harmonized testing of cosmetics and their ingredients could help companies and their products be more competitive worldwide and eliminate unnecessarily repeated testing, thus saving time and resources. Several organizations with global recognition have been promoting universal values and harmonizing animal welfare in research and safety testing. However, effective global harmonization still needs to be improved, and there is a need to develop international standards and guidelines to promote alternative approaches in the worldwide market. Several global organizations like People for the Ethical Treatment of Animals (PETA), the Food and Agricultural Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), WSPA (World Society for the Protection of Animals), Animal Welfare Institute (AWI), Coalition for Consumer Information on Cosmetics (CCIC), and European Coalition to End Animal Experiments (ECEAE) must form alternative animal councils that promote the use of animal alternatives in cosmetic testing globally to ensure and advance animal welfare.

- The validation of alternative methods is the major challenge for cosmetic toxicity and safety testing. It may be worthwhile to develop more and more substantial incentives to encourage the government, business, and academia to participate in the validation process after creating an alternative test approach. This can be done, for instance, by designating a portion of public funds for research programs to only be used for the validation of in vitro and other alternative tests that are intended to replace animal testing.
- There is an urgent need to form country-specific federal agencies (for instance, the Indian Centre for Validation of Alternative Methods (InCVAM) in India) to institutionalize alternative testing methods for fostering cooperative relationships among domestic and foreign organizations for reviewing and validating proposed alternatives. This body will be intended to keenly respond to the current global trends by introducing and promoting alternative test methods developed by various organizations in the country. This body must provide policy support for developing and accepting alternative test methods that replace animal testing. It should also provide education and training regarding alternative test methods. These national agencies must also join the International Cooperation on Alternative Test Methods (ICATM), which includes other members like the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the European Union Reference Laboratory (EURL), the European Centre for the Validation of Alternative Methods (ECVAM), the Japanese Center for the Validation of Alternative Methods (JaCVAM), the Korean Centre for the Validation of Alternative Methods (KoCVAM), and Health Canada.

9.8 Conclusion

The fast-growing, highly competitive, and science-driven cosmetics sector contributes significantly to the social and economic well-being of national and regional economies worldwide (Singh et al. 2018). Due to the numerous potential applications of nanoparticles and their enhanced characteristics, there is an increasing rush to incorporate them into cosmetic preparations, and the cosmetic market is already overrun with "nano-enhanced" formulations. Nanocosmetics may offer many advantages, but one must recognize the risks associated with some nanomaterials. The nanomaterial risk assessment must be done item by item, employing relevant data. Furthermore, several regulatory agencies worldwide, each with their own set of laws and regulations, control cosmetic/nanocosmetic products. The international trade of cosmetics on the global market is substantially affected by numerous legislative measures undertaken by various nations. For decades, the cosmetic industry has been working to achieve international regulatory harmonization in cosmetic development and safety assessment for promoting global trade and animal welfare. To ensure global coverage, regulatory agencies must collaborate internationally in exchanging information about cosmetic ingredients, safety evaluation profiles, and their effects on human health. Harmonizing regulatory regulations has numerous advantages, including maintaining a favorable marketing

environment, fostering productivity and competition, and minimizing unnecessary clinical testing duplication.

The welfare of animals is a subject that is as crucial as human welfare. In recent years, there has been a minor but considerable shift away from whole-animal testing toward in vitro and non-animal approaches, possibly as a result of advancements in biological techniques, ethical grounds, and in reaction to political and economic pressures. Numerous alternatives to using animals have been proposed; these alternatives must be effectively implemented. Several in vitro and computational models have been developed for the safety assessment of cosmetics and their ingredients, and some of these models have also been included in the test guidelines. Although most alternatives have not yet been fully validated, they have the potential to replace animal testing in the screening of cosmetics shortly. Alternative models have significant drawbacks, such as the inability to assess systemic toxicity and pharmacokinetic profiles and the difficulty of establishing complete physiological organ–organ interactions. As a result, the cosmetic must be tested in several contexts. In this regard, the OECD standards explicitly recommend using integrated technologies based on the AOP framework to produce more accurate results, prevent under- or overestimation of a particular cosmetic's toxicity, and improve understanding of the underlying mechanisms (Nabarretti et al. 2022).

Alternatives implementation in cosmetic testing is hindered by several barriers, including scientific constraints; traditional barriers; a lack of funding, strict laws, global harmonization, rigid regulations; bureaucratic barriers; and validation of alternatives. The obstacles to alternative implementation can be overcome by using integrated strategies based on AOP data, offering adequate funding and financial incentives for developing, validating, and using alternatives, and fostering international harmonization. Thus, research concerning the implementation of animal alternatives for the safety assessment of cosmetics and their ingredients is still a growing field that needs global cooperation between regulators, research institutes, universities, and industry. The efficient implementation of alternatives to animal testing in the cosmetic sector calls for extensive efforts to address numerous unmet needs for achieving policy changes, regulatory approval, and investment in innovation.

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