


Review

Cosmetic Formulations from Natural Sources: Safety Considerations and Legislative Frameworks in the European Union

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Abstract: Consumer preferences, safety, and sustainability aspects of conventional cosmetic ingredients have contributed to an increase in the demand for natural cosmetic ingredients and products. Naturally derived active cosmetic agents and excipients may come into contact with various naturally occurring and synthetic contaminants throughout the supply chain, and substantiating their safety is essential. This review examines the safety and legislative requirements applicable to natural cosmetic ingredients in the European Union (EU). Cosmetic safety requirements include technical data based on the ingredient profile, presence of hazards and the risks associated with the intended conditions of use. The hazard analysis includes screening for microbial contaminants such as aerobic mesophilic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*; chemical contaminants such as lead, cadmium, arsenic, and mercury; and naturally occurring toxins, such as allergens. The toxicological assessment considers both local effects (such as skin sensitisation, eye/skin irritation, and photo-induced effects) and systemic effects (including acute dermal toxicity, sub-acute and sub-chronic toxicity, mutagenicity and carcinogenicity, reproductive toxicity, and toxicokinetics). The EU legislative requirements prohibit the use of animal-based tests for the toxicological evaluation of cosmetic ingredients, paving the way for alternatives termed as New Approach Methodologies (NAMs). The validation of NAMs is critical for their wider usage, and despite advancements, few have been validated, particularly for systemic toxicity testing. The use of NAMs in evaluating the safety of complex natural cosmetic ingredients is further examined.

Keywords: new approach methodologies; hazard analysis; EU regulatory framework; safety assessments; natural cosmetic ingredients



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1. Introduction

Natural cosmetic ingredients and products have received increasing attention from both consumers and the cosmetics industry. This surge in interest is driven by consumer demand for cosmetics that are sustainable, environmentally friendly, organic, safe, and effective. Furthermore, with evolving regulatory landscapes in key cosmetic markets such as the European Union (EU), there is an increasing pressure on the global cosmetics industry to maintain an animal-free cosmetics supply chain.

Ongoing research is focused on deriving natural cosmetic compounds to meet consumer demand, with an emphasis on demonstrating their efficacy [1,2]. Natural sources of cosmetics (e.g., polyphenol-rich extracts, polysaccharides, and organic acids) offer numerous benefits to skin health and appearance, including anti-aging, moisturising, anti-hyperpigmentation, and skin-conditioning properties.

Research on the safety and toxicology of natural cosmetic ingredients compared to synthetic or manufactured counterparts, which have undergone extensive safety assessments,

is still emerging. There is a general misconception that natural extracts used in cosmetics are inherently safe due to their natural origin [3]. However, their safety must be demonstrated. As the cosmetic industry derives new biological or natural extracts, regulatory authorities require their safety and toxicological assessments to safeguard consumer health.

Natural products are intrinsically complex with respect to their composition, which poses a challenge for their safety assessments. Unlike single compounds, definitive chemical mixtures, and purified fractions of extracts, crude extracts often contain thousands of molecules that are difficult to fully quantify. Additionally, factors such as horticultural practices, seasonality, cultivar, and geography of cultivation contribute to compositional variability [4].

The EU Cosmetic Regulation (1223/2009) is central to the regulation of cosmetic products and ingredients in the EU [5]. The goal of the legislation is to ensure the safety of cosmetics under their intended usage. Although there is no specific legislation governing natural cosmetic ingredients, all ingredients, whether natural or manufactured, are subject to existing EU legislations. Consequently, all cosmetics must undergo safety assessments, and a cosmetic product safety report must be submitted before market entry. The safety assessments of cosmetics generally rely on the toxicological profiles of the ingredients [6], which further provide insights into the safe concentrations for formulations while achieving the desired efficacy.

Safety assessments for natural cosmetic ingredients follow a risk assessment approach involving hazard identification, hazard characterisation, exposure assessment, and risk characterisation. This entails assessing the presence of chemical (e.g., heavy metals such as mercury, lead, and cadmium as well as pesticides specific to the value chain of the raw material), microbial (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, aerobic mesophilic bacteria, etc.), or physical components that pose risks to human health, as well as evaluating the likelihood of local (e.g., skin sensitisation and irritation/corrosion) and systemic toxicological (e.g., mutagenicity/genotoxicity, carcinogenicity, and reproductive toxicology) effects.

The cosmetics industry has made considerable progress in transitioning to non-animal testing methods facilitated by policy-driven measures such as the 3Rs (replacement, reduction, and refinement) of animal testing and the ban on animal testing for cosmetic ingredients and products in the EU [7]. Consequently, alternative approaches such as New Approach Methodologies (Table 1) have emerged as substitutes for animal testing, with ongoing efforts to validate these methods [8,9]. While the majority of validated NAMs primarily address local toxicity effects, further research and policy efforts are needed to advance validation in other areas [10].

Table 1. Examples of regulatory-approved NAMs relevant for cosmetic ingredient toxicological evaluation according to the TSAR * website.

Alternative Test	Toxicological Endpoints	Validated Method
Human cell activation test (h-CLAT)	Skin sensitisation	OECD TG 442E
Amino acid derivative reactivity assay (ADRA)	Skin sensitisation	OECD 442C
Direct peptide reactivity assay	Skin sensitisation	OECD 442C
LuSens	Skin sensitisation	OECD TG 442D
KeratinoSens	Skin sensitisation	OECD TG 442D
IL-8 luciferase assay for skin sensitisation	Skin sensitisation	OECD TG 442E
epiCS skin irritation test	Skin irritation	OECD TG 439
LabCyte EPI-MODEL24 skin irritation test	Skin irritation	OECD TG 439
Isolated chicken eye	Serious eye damage/Eye irritation	OECD TG 438

Table 1. Cont.

Alternative Test	Toxicological Endpoints	Validated Method
Vitrigel-eye irritancy test	Serious eye damage/Eye irritation	OECD TG 494
LabCyteCORNEAMODEL24 eye irritation test	Serious eye damage/Eye irritation	OECD TG 492
Ocular irritation	Serious eye damage/Eye irritation	OECD TG 496
SkinEthic HCE eye irritation test	Serious eye damage/Eye irritation	OECD TG 492
EpiOcular human cell construct EIT	Serious eye damage/Eye irritation	OECD TG 492
NRU phototoxicity assay	Skin irritation	OECD TG 432
Reactive oxygen species (ROS) assay for photoreactivity	Skin irritation, Skin sensitisation, Genotoxicity/Mutagenicity	OECD TG 495
Neutral red uptake for starting doses for acute oral toxicity	Acute toxicity, Basal cytotoxicity	OECD Document 129
Ames test	Mutagenicity	OECD TG 437
In vitro mammalian cell micronucleus test	Genotoxicity/Mutagenicity	OECD TG 487
In vitro BALB/c 3T3 cell transformation assay	Carcinogenicity	OECD Guide 231

* The Tracking System for Alternative methods towards Regulatory acceptance (TSAR) is an initiative of the EURL ECVAM to document and track the progression of alternative and non-animal methods from test submission through validation and regulatory acceptance.

This review discusses the safety and toxicological requirements in relation to natural cosmetic ingredients within the EU regulatory framework. Additionally, it examines the adoption of New Approach Methodologies and highlights the progress and gaps in their validation. The need for ongoing research and validation efforts, while aligning safety assessments of natural cosmetic ingredients with regulatory requirements, is essential to ensure consumer health protection.

2. Safety Considerations

2.1. Hazard Assessment

Cosmetic products are susceptible to various kinds of contamination along the supply chain and require hazard analyses to ensure consumer safety. Hazard assessments remain an important requirement for both natural and synthetic cosmetic ingredients and products. The main hazards of concern with potential risks to human health are chemical, physical, and microbiological contaminants and naturally occurring allergens. Chemical contaminants in cosmetics have been a subject of concern over the years [11]. Heavy metals such as mercury and lead have been associated with pigments used in cosmetics [12]. Pesticide residues in natural cosmetic ingredients may pose health risks to consumers when present above the maximum allowable limits [13]. Microbial contaminants need to be analysed in cosmetic products to ensure they are within the safety limits.

2.1.1. Microbial Contaminants Relevant for Natural Cosmetic Ingredients

Cosmetics that are water-based can provide thriving environments for microbial growth. Ingredients with mild or neutral pH levels and storage at room temperature can further encourage microbial proliferation. Preservatives are commonly added to maintain the safety and quality throughout the product's shelf life. However, minimising microbial contamination in natural cosmetic ingredients and ensuring hygienic manufacturing practices can reduce the reliance on preservatives [14]. Microbial contaminants can compromise product safety, quality, and shelf life, leading to undesirable changes and potential health risks for consumers. Therefore, understanding the microbial load of natural cosmetic ingredients can inform preservative use and ensure formulations with reduced microbial risks.

Annex I of the EU Cosmetic Regulation demands the microbiological assessment of both cosmetic raw materials and finished products [5]. The common microbial contaminants that need to be assessed include aerobic mesophilic bacteria, yeasts and moulds, *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans* [6]. The existing microbial standards for cosmetic products were established by the International Organisation for Standardisation (ISO 17516:2014) and adopted by the Scientific Committee on Consumer Safety (SCCS) [6,15]. According to the standard, *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans* should not be detected in 1 g or 1 mL of the cosmetic product. The limits placed on aerobic mesophilic bacteria, yeasts, and moulds differ according to the area of application and the target population. For example, products applied to the eye area or mucous membranes and those produced for children and immunocompromised individuals should not exceed a total plate count of 100 CFU/g or mL.

Reports of microbial safety have focused predominantly on finished cosmetic products compared to their raw materials and utilise traditional culture-based methods. Furthermore, the majority of the internationally approved or validated methods rely on culture-based enumeration techniques (e.g., the ISO 21149:2017) [16]. The drawbacks of the traditional colony count method include the inability to determine viable but non-culturable microorganisms and the substantial use of disposable culture media [17]. Alternative methods that seek to address these challenges are reported in the literature, with few internationally recognised standardised protocols [18]. There is a need for the standardisation of non-culture methods.

The commonly isolated bacterial contaminants in commercial cosmetic products include *P. aeruginosa*, *Serratia* spp., *S. aureus*, *E. coli*, *Streptococcus pneumoniae*, *S. epidermis*, *Bacillus subtilis*, and *Citrobacter* spp., whereas fungal isolates such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Candida* spp. have been reported [19,20]. Almukainzi et al. [21] employed the colony count method to assess the microbial quality of a wide range of personal care products, including shampoos, toothpaste, lotion, baby oil, and hand cream. The findings confirmed that the microbial quality of cosmetics is product-dependent; baby oil, baby powder, and make-up powder exceeded the safety limits set by the SCCS. Similar to existing trends in the scientific literature, the study identified *S. aureus* and *Bacillus* spp. as the main isolated microorganisms.

The microbial stability of cosmetics during their shelf life ensures consumers are exposed to the minimum risk. Studies have shown that cosmetic products can be contaminated during processing and during their usage [22,23]. Consequently, the EU Cosmetic Regulation requires the performance of a microbial challenge test with standard protocols published in the ISO 11930:2019 [24]. The European Pharmacopoeia (Ph. Eur.) has set the recommended acceptance criteria for the microbial challenge test. According to one criterion, a cosmetic product inoculated with bacteria at 10^5 to 10^6 CFU/mL should attain a log 2 reduction on the 2nd day and a log 3 reduction on the 7th and 14th days, without any increase in microbial growth on the 28th day [25]. In a study by Alshehrei [26], foaming gel and body face cream failed to achieve a log 2 reduction against *S. aureus* after day two of the challenge testing. The microbial quality of natural cosmetic ingredients can therefore influence the overall product safety and associated consumer health risks. Natural cosmetic ingredients with demonstrated antimicrobial activity can be explored as bio-preservatives against the commonly isolated microbial contaminants in cosmetic products.

2.1.2. Chemical Contaminants Associated with Natural Cosmetic Ingredients

Chemical hazards that can be found in natural cosmetic ingredients include heavy metals, pesticides, and other persistent organic pollutants. In contrast to manufactured cosmetics, where metallic compounds are utilised in applications such as pigments and UV filters, natural cosmetics may be contaminated via different routes [27]. The manufacturing plant used in the extraction, preparation, or concentration of the ingredient may be a source of heavy metal contamination. Therefore, the potential of heavy metals leaching into cosmetic ingredients needs to be addressed and regulated. In addition, botanicals sourced

from heavily polluted soils or environments with industrial activity may present a risk of heavy metal contamination. Metalloids (e.g., arsenic) and heavy metals of concern in cosmetics include nickel, chromium, lead, cadmium, and mercury, and their concentrations in some natural products have been documented in the scientific literature.

Mercury contamination has been associated with skin lightening products. Mercuric compounds are used as common ingredients in skin-brightening preparations due to mercury's anti-tyrosinase activity. Podgórska et al. [28] found detectable levels of mercury contamination in a pool of 268 natural and conventional personal care products, which ranged between 0.348 to 37.768 µg/kg. The study found no significant differences ($p < 0.05$) between mercury concentrations in natural and synthetic products; however, mercury levels were significantly higher ($p < 0.05$) in facial care products than in body care products. Ho et al. [29] investigated the levels of mercury in differently priced facial skin-lightening preparations and found varying concentrations, from non-detectable to 1.13 ppm. One cosmetic product exceeded the permissible limits set by the US Food and Drug Administration (US FDA) for cosmetics (1 ppm). Furthermore, a study by Abbas et al. [30] found a correlation ($r^2 = 0.72$) between the mercury levels in participants' scalp hair and the facial skin-lightening creams they used.

Other studies have assessed heavy metal levels in a common natural cosmetic dye called henna (*Lawsonia inermis* plant) employed both for domestic uses and industrial formulations. The findings have been variable and geography-specific. In a study conducted in Turkey, the levels of lead in henna samples were found to range from 0.6 to 0.93 µg/g, while cadmium was detected at levels less than 0.01 µg/g [31]. Additionally, nickel was found to be present in concentrations ranging between 0.49 to 1.06 µg/g. In another study by Rezaeian et al. [32] in Iran, the lead and arsenic content in henna powders were found to range between 9.56–16.94 ppm and 0.25–1.12 ppm, respectively. The authors acknowledged that over 50% and 70% of the samples assessed exceeded the maximum limits permitted by the World Health Organisation (WHO) for lead and arsenic, respectively. Yahya et al. [33] analysed henna samples originating from India and Turkey and found that the lead and cadmium in the samples ranged from 1.3–6.5 ppm and 0.11–0.54 ppm, respectively. The distribution of heavy metals in raw materials can vary depending on factors such as geographical location, soil health indicators (such as pH and soil organic matter), and agricultural practices [34,35].

Some essential oils (EOs) commonly used as natural cosmetic ingredients have been found to contain varying levels of heavy metals. In a study which highlighted variations in the heavy metal concentrations of commercial EOs (derived from *Thymus vulgaris* L., *Lavandula angustifolia* Mill., *Mentha piperita* L., *Pinus sylvestris* L., and *Juniperus communis* L.) sourced from different manufacturers, mercury and lead were found to be the main contaminants, with mercury as high as 0.612 ppm [36]. Other heavy metals, for example, nickel and chromium, were higher in certain thyme EOs (nickel, 1.44 ppm; chromium, 2.55 ppm) compared to some mint EOs (nickel, 1.00 ppm; chromium, 2.94 ppm). Kereeditse et al. [37] determined comparatively lower levels of heavy metals (lead, 0.02 ppm; arsenic, 0.003 ppm; and nickel, 0.063 ppm) in hydro-distilled EOs derived from vetiver grass, a common fragrance oil for cosmetic applications, cultivated in polluted soils.

The European Pharmacopoeia (Ph. Eur.) indicates that the risks associated with EOs can be influenced by the nature of the contaminant, source of the plant material, and the extraction process [38]. It further acknowledges that cold-pressed essential oils potentially present higher risks than those that are distilled. The Ph. Eur. has set the limits for the following heavy metals in essential oils: lead, 5 ppm; cadmium, 1 ppm; and mercury, 0.1 ppm [38]. According to Brkljača et al. [39], olive oil extracted through mechanical pressing exhibited higher lead content than oil derived through centrifugation. Luka and Akun, [40] additionally noted that contaminated extraction equipment could be a source of heavy metal contamination.

Chemical contaminants can penetrate through the stratum corneum and result in dermal absorption and systemic exposures. Heavy metals penetrate through the human

dermal layer via different pathways based on their physiochemical properties, such as lipophilicity, molecular weight, affinity, form (organic or inorganic), type of compound (e.g., salt), and oxidative state [41]. For example, lipophilic heavy metals, such as organic forms of lead and mercury, metallic mercury, and mercury salts (HgCl_2), can permeate cells both transcellularly through the lipid plasma membrane and intercellularly via the lipid bilayer [11,41]. Such heavy metals are absorbed into the bloodstream and accumulate in organs such as the kidneys, resulting in organ toxicity [12]. Alternatively, some heavy metals (e.g., mercury) can permeate through the stratum corneum via a transfollicular absorption route facilitated by a compromised epidermal layer [30]. In this route, heavy metals penetrate the intercellular spaces between corneocytes (dead keratinocytes) [11]. Other heavy metals (e.g., nickel and the inorganic form of lead) generally exhibit poor percutaneous permeation. They accumulate in the stratum corneum due to their affinity for sulfhydryl groups and can form metalloprotein complexes, which interrupt normal physiological processes [11]. Nickel and cobalt, in particular, may covalently modify skin proteins and trigger contact allergic reactions. The daily or frequent use of contaminated cosmetics can therefore lead to increased exposure and accumulation in tissues.

While diet has been regarded as the major source of lead and arsenic exposure, lip care cosmetics can be another route of lead ingestion [42]. Lead absorption occurs in the human gastrointestinal tract and further accumulates in bone tissues and other soft tissues such as the liver and kidneys [43]. Adults exposed to elevated levels of lead are at risk of health complications such as renal and neurological damage, anaemia, and cardiovascular disorders. Lead toxicity in children has been linked to behavioural abnormalities and impaired cognitive development [44]. Arsenic has the tendency to bioaccumulate in the skin, resulting in dermatological anomalies including hyperpigmentation, skin lesions, and skin cancers [45]. The chronic exposure to high levels of arsenic could result in the development of neurological dysfunctions, cardiovascular ailments, lung cancer, and diabetes [46]. Chronic mercury exposure can result in its accumulation in the skin and its appendages, such as nails and hair [30]. Other adverse health effects associated with mercury poisoning include skin disorders such as flushing, desquamation, rashes, and dermatitis [11].

Other cosmetic agents such as tattoos and permanent make-up (PMU) have come under regulatory scrutiny due to the identification of compounds that can elicit local cutaneous reactions and systemic toxicity upon exposure. The common ingredients found in tattoos include metallic salts and organic compounds such as mercury sulphide, cadmium yellow, lead chromate, and cobalt blue [47]. Studies have highlighted the potential health risks associated with these ingredients found in tattoos and PMU inks [48,49]. The EU has therefore implemented ongoing policy measures to restrict consumer exposure to potentially carcinogenic and reproductive toxic chemicals found in these cosmetic preparations [50]. Natural and safer alternatives to the existing PMU inks and tattoos need to be further addressed.

Pesticides are another class of chemical contaminants that could be present in natural cosmetic ingredients depending on the raw material. Long-term exposure to pesticide residues can pose health risks to consumers, such as blood cancer and reproductive disorders [51]. Consequently, consumers are increasingly interested in organic sources and third-party labels such as COSMOS Organic and Natrue, which certify organic cosmetic products. However, it appears that organic sources are rarely completely devoid of environmental pollutants. Therefore, cosmetic ingredients may require pesticide testing based on the assumptions in their risk assessments. According to the Ph. Eur. "Monographs on essential oils", pesticide levels in distilled essential oils have rarely exceeded permissible limits and therefore may require testing on a case-by-case basis [38].

The significant use of oils derived from oleaginous fruits and oilseeds as natural cosmetic ingredients necessitates the control of potential pesticide residues. For instance, in the EU, the European Food Safety Authority (EFSA) requires the analysis of insecticides such as dimethoate, deltamethrin, and imidacloprid in olive oil [52]. Reviews by

Tascone et al. [13] and Nikolic et al. [53] discussed pesticide residues in natural cosmetic ingredients (extracts, absolutes, and essential oils) and attributed the sources of contamination to raw materials and environmental conditions (soil, rainfall, and processing equipment). A synthesis of over 225,000 datasets of pesticides in essential oils revealed that cold-pressed oils have a higher probability of pesticide contamination than distilled oils [54]. The challenge associated with pesticide analyses of natural extracts is the co-elution of constituents with similar physicochemical properties as the pesticide of interest [13]. Other environmental pollutants that are of concern are polycyclic aromatic carbons (PAHs). PAHs have mostly been associated with petroleum-derived cosmetic ingredients and products [55,56].

2.1.3. Allergens in Natural Cosmetic Ingredients

The presence of allergens in cosmetics can lead to adverse skin reactions in sensitive individuals. The mechanism of skin allergies is discussed further in subsequent sections.

Due to the considerable use of nut oils in cosmetic formulations, allergenic nut oils (e.g., almond, peanut, and hazelnut) can become an issue of concern. To address this, cosmetic products are labelled with the text “contains (named nut) oil”, e.g., “contains almond oil,” to inform consumers. Furthermore, the possibility of removing allergenic proteins from relevant oils has been explored, especially in peanut oil, with potential considerations for other allergenic oils [57,58].

Furthermore, lavender, tea tree, and peppermint essential oils are potential sources of allergenic substances [59]. Annex III of the EU Cosmetic Regulation includes a list of allergenic substances commonly found in botanical extracts and establishes usage restrictions (further discussed in subsequent sections). Examples of such sources used in cosmetic formulations include tree moss extract, coumarin, citronella, linalool, eugenol, citral, and D-limonene [5].

A key constraint associated with the testing of allergic fragrances is that they are mostly undetectable by existing commercial fragrance markers [60,61]. Patch testing is therefore recommended to mitigate such limitations.

Patch testing is a classical approach for determining the skin sensitivity potential of cosmetic substances and is essential for assessing the allergenic potential of natural cosmetic ingredients. This is important due to the diverse chemical compositions of natural ingredients and the variability in individual skin sensitivities [4,59]. Furthermore, patch testing aids in identifying potential allergens present in natural cosmetic extracts and the evaluation of allergic cutaneous reactions, such as contact dermatitis.

2.2. Toxicological Aspects

2.2.1. The Matrix Complexity of Natural Cosmetic Ingredients

Natural cosmetic ingredients such as botanical extracts, essential oils, biopolymers, and seed oils can be compositionally complex [62–64]. According to de Groot and Schmidt [65], essential oils can contain about 100 to 500 compounds. The matrix composition of natural extracts can vary from batch to batch and harvest season. In addition, the solubility of lipophilic matrices, stability of compounds in solvents, accessibility of molecules to test models, and the interference of pigmented matrices in spectrophotometric readings are some key considerations. These factors present challenges for the safety testing of natural cosmetic ingredients. Generally, the main constituents and their physicochemical properties (such as lipophilicity, molecular weight, chemical structure, etc.) are employed in the prediction of their toxicokinetic properties. Currently, no minimum quantification level is established for naturally complex extracts, in contrast to other substances such as smoke flavourings, in which at least a 50% characterisation of the matrix is required by Regulation (EC) No 627/2006 [66].

2.2.2. Recommended Toxicological Testing for Natural Cosmetics

Cosmetic ingredients may elicit local toxicity effects on a defined area of application (e.g., eye or skin) and are often determined through endpoints such as skin/eye irritation,

skin sensitisation, and photo-induced effects. When a considerable level of dermal absorption is demonstrated, the absorption, distribution, metabolism, and excretion (ADME) properties and systemic toxicology effects, such as reproductive toxicology and repeated dose toxicity, need to be investigated [6]. The utilisation of NAMs (in vitro, in silico, and in chemico techniques) in the assessment of local and systemic toxicological effects of naturally complex cosmetic matrices is an area of ongoing research.

1. Skin sensitisation

Skin sensitisation is a toxicological endpoint that evaluates dermatological reactions caused by substances known as haptens. Haptens form covalent bonds and modify skin proteins, which are recognised as foreign substances to the body, and an adaptive immune response is elicited. The visible dermal reaction typically occurs between 12 to 72 h after contact with the hapten and results from a delayed T-cell-mediated immune response [67]. The symptoms of the immunological response may include itching, urticaria, and redness. Individuals exposed to a sufficient dose of the hapten may develop a skin condition known as allergic contact dermatitis (ACD) [68]. Cross-reactivity allergic reactions have also been identified as a possible cause of ACD.

Certain natural sources are associated with skin sensitisation effects, and plant-induced ACD is known to be the most common form of T-cell-mediated reactions [69]. EO and aromatic extracts have been a subject of concern due to reports of their skin sensitisation potential [59]. According to Buonomo and Warshaw [70], about 80 essential oils, including tea tree oil, thyme oil, neem oil, and eucalyptus oil, have been associated with contact allergy. Skin sensitisation is therefore an important dermal toxicological endpoint in the safety evaluation of natural cosmetic ingredients.

In times past, conventional skin sensitisation testing was achieved with guinea pigs and mice. These assays included the guinea pig maximisation test (Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 406), the Buehler occluded patch test (OECD TG 406), and the Local Lymph Node Assay (LLNA, OECD TG 429) [71]. The LLNA emerged as an alternative to the guinea pig maximisation test (GPMT) due to its improved sensitivity and reduced pain and trauma to animal test candidates [72]. The GPMT assesses dermal sensitisation through induction by injection, followed by topical application, and then a challenge with the same substance at a lower dose, whereas the LLNA measures lymphocyte proliferation by draining the lymph nodes after dermal exposure [73,74]. While the LLNA may no longer be applicable for regulatory purposes due to the EU ban on animal testing, data generated from past LLNA assays have served as modelling datasets for predicting the toxicity of unknown compounds in modern in silico tools [68,75].

Recent policy shifts and research efforts have made progress on the utilisation of NAMs for determining skin sensitisation potential (Table 1). A major advantage of NAMs is the generation of insights into the various toxicological processes that result in an adverse outcome. The adverse outcome pathway (AOP) framework for skin sensitisation demonstrates the sequential mechanistic processes that lead to an adverse skin reaction [76]. Four key initiating events and the respective NAMs for their testing have been proposed (Figure 1) [76]. Puginier et al. [77] utilised the AOP approach to evaluate the skin sensitisation potential of 16 botanical extracts (including myrrh and beet extracts) used as cosmetic ingredients (Table 2).

The potential of in silico tools for evaluating skin sensitisation effects of cosmetics has been reported in literature. Chilton et al. [68] recently utilised an in silico tool (Derek Nexus) to predict three dermal sensitivity thresholds (non-reactive, reactive, and high potency) by including additional LLNA-generated data in their model. However, as highlighted by Ta et al. [78] in their review, existing in silico tools are limited by their capability of binary classification of chemicals (as sensitisers or non-sensitisers) which does not take into account mild and moderate sensitisers.

Micro-physiological test systems for the conduct of skin sensitisation are other potential viable alternatives [10]. A microfluidic model was developed to assay the skin

sensitisation potential of capsaicin, a common naturally occurring cosmetic ingredient [79]. It is noteworthy that the standardisation and acceptance of NAMs are paramount for their inclusion in regulatory risk assessments. Considerable research efforts and policy instruments together with the work of the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) have led to the standardisation of several NAMs relevant for skin sensitisation testing (Table 1).

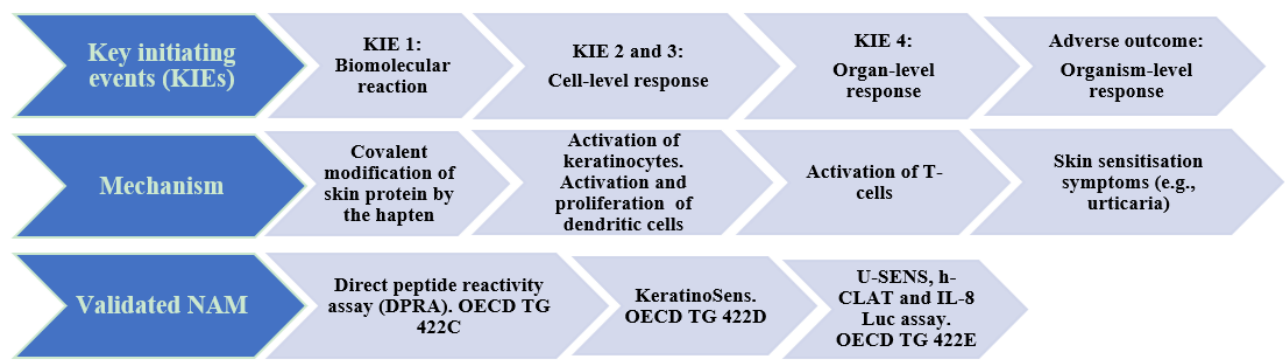


Figure 1. The adverse outcome pathway (AOP) of skin sensitisation according to the Organisation for Economic Co-operation and Development [76].

Table 2. Examples of toxicological studies on natural cosmetic ingredients incorporating New Approach Methodologies (NAMs).

Natural Cosmetic Ingredient	Endpoint	New Approach Methodology	Experimental Conditions	Findings	References
Selected botanical extracts	Repeated dose toxicity	Threshold of toxicological concern (TTC)	Meta-analysis was used to derive the TTC for botanical extracts.	Authors proposed a new TTC of 663 µg/day for botanical extracts used in cosmetics.	[80]
16 botanical extracts	Skin sensitisation	Sen-Is h-CLAT ¹ , Keratino-Sens	Extracts underwent testing in the Sen-Is assay; negative results were confirmed using the h-CLAT followed by the KeratinoSens assay.	Three botanical extracts (<i>Orbignya phalerata</i> , <i>Arctium lappa</i> , and Apiaceae herb extracts) showed sensitisation potential.	[77]
<i>Galenia Africana</i> extract	Dermal irritation	SkinEthic Episkin irritation assay	The cell viability of Episkin RHE ² was assessed after 3-h exposure to 1% and 20% doses of extracts.	<i>Galenia Africana</i> extract is a non-irritant.	[81]
Aqueous extracts of <i>C. micranthum</i> and <i>A. occidentale</i> hexane extracts of <i>M. oleifera</i> and <i>A. digitata</i> seeds	Repeated dose toxicity	In vitro reconstructed human pigmented epidermis (RHPE) model for four days	The cell viability of Episkin RHPE was assessed after a 4-day daily administration of variable doses of the three extracts.	The extracts were non-toxic to cells, except for <i>A. occidentale</i> and <i>A. digitata</i> at certain doses.	[82]
Twenty natural extracts	Photoreactivity and phototoxicity	Reactive oxygen species (ROS) assay, micellar ROS assay, and the 3T3 neutral red uptake phototoxicity test	The absorbance of extracts, irradiated for 1 h, was measured to detect ROS formation. The viability of 3T3 cells was assessed after incubation in darkness or irradiation following a 1-h exposure to variable extract doses.	Three extracts (St. John's wort powder, tagetes oil, and cumin seed oil) exhibited the highest phototoxicity.	[83]
Water-in-oil-in-water emulsion with 7.5% rosemary extract, 24.18% flaxseed mucilage, and 44.03% oatmeal suspension	Irritation, phototoxicity	In vitro skin irritation and phototoxicity assay with EpiDerm skin model	The viability of the EpiDerm skin tissue was measured after treatment with sample for 3, 5, and 18 h. The viability of the EpiDerm skin tissue was assessed after incubation in darkness or irradiation following an 18-h exposure to variable extract doses.	Cytotoxicity was observed for tissues treated for 18 h. Sample showed no phototoxicity, irrespective of the concentration.	[84]

¹ h-CLAT: human cell activation test. ² RHE: reconstructed human epidermis.

2. Dermal and eye irritation/corrosion

Dermal irritation tests evaluate the potential of a test substance to cause reversible damage to the skin within four hours of contact. The mechanism of skin irritation involves a non-immune mediated inflammatory response characterised by visible effects such as erythema and oedema. The assessment of dermal irritation involves the administration of a single or repeated dose of the test substance, and the extent of irritation (no reaction, slight, moderate, or severe) is determined [85]. In contrast, skin corrosion assesses the irreversible skin damage of a substance, with symptoms such as ulcers, bleeding, and tissue necrosis [86].

Traditional approaches to dermal irritation testing have relied on animal models, particularly the use of albino rabbits (OECD TG 404, acute dermal irritation/corrosion test) [86]. However, in response to the need for alternatives to animal testing, various in vitro assays have been developed, which primarily employ the reconstructed human epidermis (RHE) model. The RHE is a three-dimensional (3D) in vitro tissue model derived from human keratinocytes embedded in a polycarbonate membrane cultured in a chemically defined medium. The 3D RHE model closely mimics the human epidermis and can address the lack of cell-to-cell interactions in 2D cell culture systems [87]. Despite this, Nabarretti et al. [10] argued that the current RHE models lack components such as sebaceous glands and filaments, sensory nerves, and lymphatic vessels, which need consideration in human-relevant toxicological predictions.

Alternative tests such as the EpiSkin skin irritation test, EpiDerm skin irritation test (SIT), and the epiCS SIT have been validated by the European Centre for the Validation of Alternative Methods (ECVAM), with others documented in Table 1 [88]. The RHE model was employed to assess the dermal irritation potential of *Galenia Africana*, a natural product known for its traditional usage in treating skin diseases and used as a common ingredient in lotions and shampoos [81] (Table 2).

The in vivo eye irritation test (OECD TG 405, acute eye irritation/corrosion test) employs the albino rat model due to its large eyes and economic advantages [89]. However, the rabbit eye is considered insufficient in modelling the inflammatory and recovery mechanisms of the human eye, and Prinsen et al. [71] recommended the isolated chicken eye (ICE) test as a replacement. While a viable alternative, the ICE model still involves the use of animals.

Non-animal alternatives, such as the human cornea epithelium (HCE) 3D model, have emerged for detecting eye irritation potential [71]. The SkinEthic HCE time-to-toxicity test method (OECD TG 492) has been recommended as a conditional replacement for the in vivo eye irritation test, which is a major achievement in accelerating regulatory adoption of NAMs [6,90]. Other in vitro assays developed for mucosal membrane and eye irritation include the EpiOral assay (mouth and lips) [91], the HET-CAM, and the EpiOcular eye irritation test [90].

In line with the 3Rs of animal testing, the OECD recommends that in vivo tests only occur after the utilisation of existing in vitro tests in accordance with the Integrated Approaches to Testing and Assessment (IATA) framework for serious eye damage and eye irritation [92].

3. Photo-induced effects

The OECD TG 432 standardises the in vitro test for phototoxicity with the 3T3 mouse fibroblasts in the neutral red uptake (NRU) assay. In the test, monolayer 3T3 cells are exposed to variable doses of the test substance in the presence and absence of light. The cells' ability to uptake the neutral red dye is measured as an indicator of cell viability. Few studies have documented the phototoxicity of natural cosmetic substances. The 3T3 NRU assay has been reported to potentially over-estimate the phototoxicity of test substances, leading to false positives when compared with in vivo data. Aguiar et al. [93] adopted an alternative to the 3T3 NRU assay by employing the human keratinocyte cell line (HaCaT) to conduct an in vitro study to assess the phototoxic effects of five natural phenolic antioxidant

compounds (*p*-coumaric acid, ferulic acid, caffeic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), and rutin), commonly used in cosmetics. The authors found that the doses of the compounds tested (up to 1000 µM) did not induce phototoxicity in the human cell line. Other photo-safety assessments of natural extracts are summarised in Table 2 [83,84]. According to Maddaleno and Vinardell [94], there is a lack of validated in vitro and in silico methods specifically for the assessment of photoallergenicity. This presents both a challenge in the use of non-validated NAMs and the opportunity to progress the validation and regulatory approval of alternatives relevant for the photoallergic determination of cosmetic ingredients.

4. Dermal absorption

Dermal absorption determines the fate of the cosmetic substance after application and has remained a relevant parameter for skin care products with active agents. A dermal toxicokinetic study is deemed relevant when a considerable cutaneous permeation is confirmed [95]. The transdermal absorption is a contributing factor for any potential systemic toxicological risk and further tests (e.g., reproductive toxicity, acute toxicity, and carcinogenicity) may be required when sufficient dermal absorption is demonstrated [6]. A low dermal absorption is predicted when a molecule's physicochemical properties align with those outlined in Table 3. For instance, natural antioxidant molecules have been shown to exhibit low dermal absorption properties and strategies to improve their cutaneous permeation, such as nano-encapsulation have been discussed [96,97]. In addition, the use of excipients such as jojoba oil was demonstrated in an in vitro study to potentiate the dermal absorption of retinol [98].

Table 3. Physicochemical characteristics of dermally absorbable molecules according to the Scientific Committee on Consumer Safety [6,95].

Physicochemical Property	Value
Molecular weight	>500 Da
Degree of ionisation	High
Octanol water partition coefficient, Log P _{ow}	≤ −1 or ≥ 4
Topological surface area	>120 Å ²
Melting point	>200 °C

Furthermore, Nishida et al. [83] indicated that the overall physicochemical properties (based on those of the quantifiable main constituents) of natural extracts are often subjected to inaccurate estimation due to their complex matrix properties. This often affects the accurate toxicokinetic and dermal absorption predictions of natural extracts. Kojic acid is an example of a naturally derived compound from fungal species (*Aspergillus oryzae*), and its well-researched skin permeation studies have shown it to be dermally absorbed in both in vivo and in vitro models [99,100]. The European Commission therefore recommends up to 1% kojic acid in the formulation of skin care products [101]. Further insights into the dermatotoxicokinetic properties of natural components in cosmetics can support policies on their safe usage.

Various standardised protocols for dermal absorption developed by the OECD are in vivo based, such as the TG 417 (in vivo toxicokinetics) and TG 427 (in vivo skin absorption). An alternative to these animal tests is the OECD TG 428 (in vitro assessment of dermal absorption) based on the Franz diffusion cell. The advantage of this assay is the ability to mimic the flow of blood beneath human skin, and it can be conducted in both static and dynamic setups. Furthermore, small volumes of cosmetic test substances are used in the assay with minimal manipulation of the skin model. Currently, the Franz diffusion cell is one of the commonly employed NAMs for dermatological ADME studies. However, Filaire et al. [75] noted an inherent limitation in the use of Franz cells; molecules are metabolised by the skin tissue during the study. The authors suggested the use of a micro-physiological equivalent to the Franz diffusion cell to address this limitation. Ac-

cordingly, Pulsoni et al. [102] developed a novel micro-fluidic alternative to the Franz diffusion cell and observed a comparable transdermal absorption rate using caffeine as a test molecule. Another commonly employed in vitro model for skin permeation studies is the skin Parallel Artificial Membrane Permeation Assay (PAMPA), which has been in existence for decades but has yet to attain validation by ECVAM.

In silico models have been suggested for the prediction of the dermal permeability of cosmetic ingredients and are mainly based on the quantitative structure–activity relationship (QSAR) approaches. Recently developed QSAR systems include the Vermeer Cosmolife and the SkinPerm calculator, which are relevant for predicting the skin permeation properties of substances [78].

5. Acute toxicity

Acute toxicity tests for cosmetic ingredients evaluate their effects based on the routes of exposure (dermal, oral, and inhalation). Acute oral and dermal toxicological studies assess the adverse effects of a substance after single or multiple doses of administration to the skin within 24 h of exposure, while acute inhalation toxicity determines the toxic effect within four hours of exposure. Acute toxicity studies yield the median lethal dose (LD50), which is the dose sufficient to cause a fatal outcome in 50% of the administered group. The existing validated acute toxicity assays which rely on animal models include acute oral toxicity (OECD TG 401, replaced by three methods: TG 420, 423, and 425), acute dermal toxicity (OECD TG 402), and acute inhalation toxicity (OECD TG 403).

Amongst the three acute toxicological endpoints, only oral acute toxicity has a validated in vitro test. The test is based on the 3T3 NRU assay. Due to the ban on the use of animals for the testing of cosmetic ingredients in the EU, viable and validated alternatives for acute toxicity are needed. In the current context of limited alternatives, in vivo acute toxicity assays are only performed when skin absorption is evidenced and when oral and inhalation toxicity are not expected [103]. In addition, the SCCS notes that acute toxicity data may not be required if a weight of evidence based on alternative tests (including in vitro and in silico approaches) can justify the safety of the ingredients [6]. These are strategies to ensure reduction in the unnecessary use of animal models.

Various in vitro basal cytotoxicity tests are prominent in the literature with limited validation, including the Alamar blue, Trypan blue, MTT assay, lactate dehydrogenase (LDH) assays, and incorporate mammalian cell lines (e.g., 3T3 mouse fibroblast cells). Barker-Treasure et al. [104] argued that the 3T3 NRU assay may not sufficiently reflect human toxicity mechanisms. In that regard, the authors proposed an animal-free alternative which uses human dermal fibroblasts as test models and human-derived serum in the cell culture growth media. However, this novel approach has not attained regulatory approval for inclusion in regulatory science in the context of cosmetic ingredients. Riebeling et al. [103] underscored that the complex nature of acute dermal system toxicity mechanisms places a challenge on the development and regulatory acceptance of alternatives. Borba et al. [105] recently developed in silico models (STopTox, <https://stoptox.mml.unc.edu/> (accessed on 18 March 2024)) to predict acute (dermal, oral, and inhalation) toxicity based on experimental results documented in the REACH database. Currently, there are limited studies on the in silico prediction of the acute toxicity of natural cosmetic compounds.

6. Repeated dose toxicity studies

Repeated dose toxicity studies assess the toxicological effects of a daily repeated dose of a test substance on an animal model from 21 days to 2 years. These studies are based on sub-acute, sub-chronic, chronic, and combined chronic/carcinogenicity approaches and generate data on the dose–effect relationships and organ and systemic toxicological effects of a test substance. The measures derived from these studies are the No Observed Adverse Effect Level (NOAEL), Lowest Observed Adverse Effect Level (LOAEL), and the threshold of safety exposure. These tests are conducted with animal subjects such as rabbits, guinea pigs, and rats. The SCCS notes that there are currently no alternative models developed for repeated dose toxicity studies. Therefore, efforts are made to en-

sure the judicious use of the existing in vivo tests with justification. As an alternative to conventional repeated dose toxicity studies, Kawamoto et al. [80] derived a TTC value of 663 µg/day based on 213 experimental data of botanical extracts used in cosmetic formulations. Zeitoun et al. [82] performed in vitro repeated dose studies to investigate the cytotoxicity of three natural extracts with potential use as skin-lightening agents in a reconstructed human pigmented epidermis (RHPE) model for four days. The study administered daily doses of up to 2 g/L each of aqueous extracts of *C. micranthum* and *A. occidentale* and up to 36 µL of hexane extracts of *M. oleifera* and *A. digitata* seeds. The authors noted that the extracts were non-toxic to cells except at doses of 0.6 and 1 g/L of *A. occidentale* extracts and 24 µL and 36 µL *A. digitata* seed oil, respectively. According to Filaire et al. [75], microfluidic models have potential as promising alternatives for repeated dose toxicity studies.

7. Mutagenicity/genotoxicity

The mutagenicity endpoint detects direct-acting mutagens, while genotoxicity detects changes in genetic material that may or may not result from mutations. Regulatory requirements for cosmetic substances include assays demonstrating genotoxic mechanisms such as gene-level mutations, chromosome breakage, and alterations in chromosomal numbers [6]. In the case of complex matrices, the SCCS recommends an initial in vitro genotoxicity testing of the whole matrix, and if required, the threshold of toxicological concern (TTC) can be employed as a follow-up approach considering both the whole botanical extract and its individual constituents. The commonly employed in vitro genotoxicity assays include the Ames assay, the micronucleus test, the comet assay, and the DNA adducts formation test.

The hen's egg test for micronucleus induction (HET-MN) has been introduced as an alternative for genotoxicity testing with applicable relevance to natural cosmetic ingredients. The hen's egg model can metabolise test substances, making it comparable to in vivo biological systems [10]. Although the HET-MN has yet to attain regulatory approval, various validation studies have been published [106], and its classification as an animal or non-animal approach depends on the jurisdiction [107]. Ongoing research is exploring the application of the principles of the in vitro micronucleus test and the in vivo comet assay to RHE skin model for genotoxicity testing [102].

In silico models have been demonstrated to provide relevant insights on the mutagenesis/genotoxicity and carcinogenesis of cosmetic substances [6]. Raitano et al. [108] demonstrated the use of the quantitative structure–activity relationship (QSAR) approach to predict the mutagenicity of about 18,000 chemicals found in natural plant extracts and cosmetic ingredients. Various databases, with in vitro study data such as OASIS genotoxicity and ISSSTY bacterial mutagenicity and in vivo data such as ECVAM genotoxicity and ISSMIC micronucleus are currently integrated into the OECD QSAR toolbox (<https://qsartoolbox.org/resources/databases/>) (accessed on 20 March 2024).

8. Carcinogenicity

The two-year animal-based assay for the in vivo carcinogenicity assessment of cosmetic ingredients is prohibited in the context of the current EU Cosmetic Regulation. There are ongoing developments in the validation of alternatives that can cover a wide range of carcinogenic mechanisms for detecting both DNA-reactive (genotoxic carcinogens) and non-DNA-reactive (non-genotoxic carcinogens) substances. The current validated in vitro tests detecting genotoxic carcinogens (e.g., Ames assay) are employed as a pre-screening strategy for carcinogenicity studies. The cell transformation assay (CTA) has emerged as an alternative in vitro test to determine non-genotoxic carcinogenic mechanisms by identifying substances that initiate and promote tumour development (Table 1). However, due to the inability of the CTA to fully model the complexity of in vivo carcinogenesis mechanisms, the OECD recommends its use as a complimentary assay in conjunction with validated tests [109].

The in vivo carcinogenicity assay is recommended to be carried out only when the existing battery of in vitro assays for genotoxicity show positive results or generate incon-

clusive data [6]. Such long-term studies are carried out when there is chronic exposure to a substance. The probability of chronic exposure to cosmetics is prominent due to their daily or regular consumer usage, and there is the need for alternative tests that can model chronic toxicity mechanism. Transcriptomic-based assays have been identified as a promising alternative for the *in vivo* carcinogenic assessment of substances. Further studies are necessary for their development and validation.

9. Reproductive toxicity

The reproductive toxicity endpoint assesses the potential of a substance to cause adverse health effects on mammalian reproduction, including all the cycles of reproduction. Due to the complexity of the mammalian reproductive cycle, it has remained a challenge to delineate an alternative assay that fully mimics the entire cycle. Therefore, a battery of alternative assays has been developed. These include the Whole Embryo Culture (WEC) test, the MicroMass (MM) test, and the Embryonic Stem Cell Test (EST). However, these tests are restricted to embryotoxicity assessments and do not fully cover all reproductive toxicity mechanisms. In addition, the WEC test is considered animal-based and therefore restricted for prioritised testing. [6]. Research projects such as the ReProTest have developed various *in vitro* alternatives for reproductive toxicology [110]. However, these have yet to be standardised and fully applied in regulatory decision making. Animal-based tests have remained the gold standard for reproductive toxicity testing, and efforts are needed for the development and validation of alternatives.

3. The European Union Legislative Framework

The legislative framework for cosmetic ingredients has undergone several developments in the past decades in the EU. The three main aspects of the EU Cosmetic Regulation (EC) No. 1333/2009 relevant for natural cosmetic ingredients include the requirements for safety testing, the ban on animal testing, and the restrictions on natural and synthetic cosmetic ingredients [5]. Aspects that apply to finished cosmetic products that are relevant for the end user, such as labelling and product presentation, are not a key focus in this section.

3.1. Requirements for Safety Reporting

Article 11 of the EU Cosmetic Regulation establishes the components of the safety reporting of cosmetic products taking into account that of the raw materials [5]. Therefore, a careful selection of the cosmetic ingredients to ensure their safety at the final concentration in the finished product is recommended [6]. Furthermore, the Annex 1 of the legislation outlines the specific requirements for safety reporting, including qualitative and quantitative ingredient descriptions, physicochemical characteristics, microbial safety, purity, anticipated use scenarios, exposure considerations, toxicological profiles, adverse effects, human studies, and usage instructions or restrictions. The legislation additionally requires that cosmetic product safety reports must be retained for a minimum of 10 years following product market entry.

3.2. Approaches for Safety Testing: Ban on Animal Tests for Cosmetic Ingredients

The use of animal-based toxicological testing was banned in 2004 and in 2009 for cosmetic products and ingredients, respectively. Exceptions were granted for skin sensitisation tests and other higher-tier endpoints that evaluate systemic toxicity, such as reproductive, carcinogenicity, and toxicokinetics; an extension until March 2013 was provided. This facilitated the emergence of alternative tests for toxicity testing of cosmetics and their validation and standardisation.

Over the past decade, considerable advancements in the development of alternatives and their regulatory acceptance have been observed for skin sensitisation in comparison to NAMs relevant for toxicokinetics, reproductive toxicity, and repeated dose toxicity studies. It is projected that the scientific community can reach the complete replacement of *in vivo* tests for skin sensitisation and toxicokinetics in the next decade [88]. It is worth

acknowledging that a myriad of in vitro assays have been developed with restrictions on research purposes due to the lack of validated and standardised protocols. A consideration of their regulatory approval is key in the selection of tests for commercially focused research.

3.3. Requirements for Cosmetic Ingredients with Restrictions and Permissible Ingredients

Prohibitions and restrictions on compounds present in natural cosmetic ingredients are put in place to protect people from adverse health effects. Awareness of these restrictions is important during the development of extraction techniques for natural ingredients and the formulation of cosmetics [111]. The intentional usage of heavy metals in cosmetics is prohibited in the EU. Furthermore, the presence of naturally occurring allergens in cosmetics are restricted. Examples of restricted bioactive allergenic compounds include D-limonene, coumarin, and eugenol [5]. According to the legislation, these compounds should not exceed concentrations of 0.001% for leave-in products and 0.01% for rinse-off products. Furthermore, allergic compounds that are not technically feasible to be removed should be included in the label of the cosmetic product. These allergens may be co-extracted, and achieving their complete removal from natural extracts presents a research challenge. In addition, the removal of biologically active compounds (e.g., antibacterial compounds) which are allergenic could present formulation challenges for the industry.

Natural ingredients that are employed as preservatives, colouring agents, and UV filters should be included in the permissible list provided by the legislation [5]. In the instance where they are not included in the approved list, the ingredient must undergo safety and toxicological assessment by the SCCS. A safety dossier must therefore be submitted to obtain approval for use as a cosmetic substance.

4. Future Perspectives

Considering the increased demand for natural cosmetic ingredients, safety assessments must accompany their efficacy studies. Further research and policy attention are needed to standardise non-culture methods for detecting microbial contaminants in cosmetics. In addition, techniques that address matrix effects during the detection of chemical contaminants and allergens need further development. The complex nature of natural cosmetic ingredients presents inherent challenges for their safety assessment, necessitating key considerations, particularly in the use of New Approach Methodologies (NAMs).

The ban on animal testing for cosmetic ingredients and products requires accelerated progress in the development of alternatives for relevant endpoints. Alternatives for photo-induced tests have been validated, yet challenges regarding the potential over-estimation and the limited tests for photo-allergenicity need to be addressed. NAMs targeting systemic toxicity tests are comparatively limited and few are validated. There remains the need to accelerate the development of alternatives for evaluating the reproductive toxicology, toxicokinetic, repeated dose toxicity, and carcinogenicity of natural cosmetic ingredients. The next generation risk assessment concept needs to be discussed and practically demonstrated in the context of natural cosmetic ingredients.

A regulatory framework specific to natural cosmetic ingredients and products is necessary. Furthermore, health-conscious consumers may prioritise the environmental and social impact, as well as the sustainability of natural cosmetic sources, indicating a need for regulation in this area.

5. Conclusions

The demonstration of the safety of natural cosmetic ingredients is crucial to establishing their risk and benefit windows. The safety assessment of these ingredients must progress in tandem with efficacy studies to ensure their safe utilisation. The use of New Approach Methodologies (NAMs) offers a more cost-effective, rapid, human-relevant, and humane approach to the safety evaluation of naturally derived cosmetic materials. NAMs can facilitate prioritisation and early decision making regarding the safety of novel natural cosmetics sources and promote innovation in the cosmetics industry. However, further

research and collaboration are needed to progress the validation of NAMs and ensure their widespread adoption while contributing to the development of safe and sustainable cosmetic products. Furthermore, the pace of innovations in natural cosmetic ingredients needs to align with the legislative requirements to ensure consumer health protection.

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References

- Goyal, N.; Jerold, F. Biocosmetics : Technological Advances and Future Outlook. *Environ. Sci. Pollut. Res.* **2023**, *30*, 25148–25169. [[CrossRef](#)] [[PubMed](#)]
- Meléndez-Martínez, A.J.; Mandić, A.I.; Bantis, F.; Böhm, V.; Borge, G.I.A.; Brnčić, M.; Bysted, A.; Cano, M.P.; Dias, M.G.; Elgersma, A.; et al. A Comprehensive Review on Carotenoids in Foods and Feeds: Status Quo, Applications, Patents, and Research Needs. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 1999–2049. [[CrossRef](#)] [[PubMed](#)]
- Silvério, L.A.L.; Coco, J.C.; de Macedo, L.M.; dos Santos, É.M.; Sueiro, A.C.; Ataíde, J.A.; Tavares, G.D.; Paiva-Santos, A.C.; Mazzola, P.G. Natural Product-Based Excipients for Topical Green Formulations. *Sustain. Chem. Pharm.* **2023**, *33*, 101111. [[CrossRef](#)]
- Shipkowski, K.A.; Betz, J.M.; Birnbaum, L.S.; Bucher, J.R.; Coates, P.M.; Hopp, D.C.; MacKay, D.; Oketch-Rabah, H.; Walker, N.J.; Welch, C.; et al. Naturally Complex: Perspectives and Challenges Associated with Botanical Dietary Supplement Safety Assessment. *Food Chem. Toxicol.* **2018**, *118*, 963–971. [[CrossRef](#)] [[PubMed](#)]
- EC 1223/2009; The European Parliament and the Council of the European Union Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. European Union: Brussels, Belgium, 2009; Volume 10, pp. 1–376.
- Scientific Committee on Consumer Safety (SCCS). *The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation 12 Th Revision*; European Commission: Luxembourg, 2022; pp. 1–203.
- Westmoreland, C.; Bender, H.J.; Doe, J.E.; Jacobs, M.N.; Kass, G.E.N.; Madia, F.; Mahony, C.; Manou, I.; Maxwell, G.; Prieto, P.; et al. Use of New Approach Methodologies (NAMs) in Regulatory Decisions for Chemical Safety: Report from an EPAA Deep Dive Workshop. *Regul. Toxicol. Pharmacol.* **2022**, *135*, 105261. [[CrossRef](#)]
- Cattaneo, I.; Astuto, M.C.; Binaglia, M.; Devos, Y.; Dorne, J.L.C.M.; Fernandez Agudo, A.; Fernandez Dumont, A.; Garcia-Vello, P.; Kass, G.E.N.; Lanzoni, A.; et al. Top of Form Implementing New Approach Methodologies (NAMs) in Food Safety Assessments: Strategic Objectives and Actions Taken by the European Food Safety Authority. *Trends Food Sci. Technol.* **2023**, *133*, 277–290. [[CrossRef](#)]
- Manful, M.E.; Ahmed, L.; Barry-Ryan, C. New Approach Methodologies (NAMs) for Safety Testing of Complex Food Matrices: A Review of Status, Considerations, and Regulatory Adoption. *Trends Food Sci. Technol.* **2023**, *142*, 104191. [[CrossRef](#)]
- Nabarretti, B.H.; Rigon, R.B.; Burga-Sánchez, J.; Leonardi, G.R. A Review of Alternative Methods to the Use of Animals in Safety Evaluation of Cosmetics. *Einstein* **2022**, *20*, eRB5578. [[CrossRef](#)]
- Ullah, H.; Aslam, S.; Mustafa, G.; Waseem, A.; de Freitas Marques, M.B.; Gul, Z.; Usman Alvi, M.; Anwar, S.; Sabir, M.; Hamid, A.; et al. Potential Toxicity of Heavy Metals in Cosmetics: Fake or Fact: A Review. *Int. J. Environ. Anal. Chem.* **2023**, 1–32. [[CrossRef](#)]
- Arshad, H.; Mehmood, M.Z.; Shah, M.H.; Abbasi, A.M. Evaluation of Heavy Metals in Cosmetic Products and Their Health Risk Assessment. *Saudi Pharm. J.* **2020**, *28*, 779–790. [[CrossRef](#)]
- Tascone, O.; Roy, C.; Filippi, J.J.; Meierhenrich, U.J. Use, Analysis, and Regulation of Pesticides in Natural Extracts, Essential Oils, Concretes, and Absolutes. *Anal. Bioanal. Chem.* **2014**, *406*, 971–980. [[CrossRef](#)] [[PubMed](#)]
- Halla, N.; Fernandes, I.P.; Heleno, S.A.; Costa, P.; Boucherit-Otmani, Z.; Boucherit, K.; Rodrigues, A.E.; Ferreira, I.C.F.R.; Barreiro, M.F. Cosmetics Preservation: A Review on Present Strategies. *Molecules* **2018**, *23*, 1571. [[CrossRef](#)] [[PubMed](#)]
- ISO 17516:2014; Cosmetics—Microbiology—Microbiological Limits. International Organisation for Standardisation: Geneva, Switzerland, 2014.
- ISO 21149:2017; Cosmetics—Microbiology—Enumeration and Detection of Aerobic Mesophilic Bacteria. International Organisation for Standardisation: Geneva, Switzerland, 2017.
- Pazos-Rojas, L.A.; Cuellar-Sánchez, A.; Romero-Cerón, A.L.; Rivera-Urbalejo, A.; Van Dillewijn, P.; Luna-Vital, D.A.; Muñoz-Rojas, J.; Morales-García, Y.E.; Bustillos-Cristales, M.d.R. The Viable but Non-Culturable (VBNC) State, a Poorly Explored Aspect of Beneficial Bacteria. *Microorganisms* **2024**, *12*, 39. [[CrossRef](#)] [[PubMed](#)]
- Foddai, A.C.G.; Grant, I.R. Methods for Detection of Viable Foodborne Pathogens: Current State-of-Art and Future Prospects. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4281–4288. [[CrossRef](#)] [[PubMed](#)]
- Budecka, A.; Kunicka-Styczyńska, A. Biotechnology and Food Sciences Microbiological Contaminants in Cosmetics—Isolation and Characterization. *Biotechnol. Food Sci.* **2014**, *78*, 15–23.

20. Alshehrei, F.M. Isolation and Identification of Microorganisms Associated with High-Quality and Low-Quality Cosmetics from Different Brands in Mecca Region—Saudi Arabia. *Saudi J. Biol. Sci.* **2023**, *30*, 103852. [CrossRef] [PubMed]
21. Almukainzi, M.; Alotaibi, L.; Abdulwahab, A.; Albukhary, N.; El Mahdy, A.M. Quality and Safety Investigation of Commonly Used Topical Cosmetic Preparations. *Sci. Rep.* **2022**, *12*, 18299. [CrossRef] [PubMed]
22. Bashir, A.; Lambert, P. Microbiological Study of Used Cosmetic Products: Highlighting Possible Impact on Consumer Health. *J. Appl. Microbiol.* **2020**, *128*, 598–605. [CrossRef] [PubMed]
23. Kim, H.W.; Seok, Y.S.; Cho, T.J.; Rhee, M.S. Risk Factors Influencing Contamination of Customised Cosmetics Made on-the-Spot: Evidence from the National Pilot Project for Public Health. *Sci. Rep.* **2020**, *10*, 1561. [CrossRef]
24. ISO 11930:2019; Cosmetics—Microbiology—Evaluation of the Antimicrobial Protection of a Cosmetic Product. International Organisation for Standardisation: Geneva, Switzerland, 2019.
25. European Pharmacopoeia Efficacy of Antimicrobial Preservation. In *European Directorate for the Quality of Medicines and HealthCare*; EQDM: Strasbourg, France, 2008; p. 529.
26. Alshehrei, F.M. Microbiological Quality Assessment of Skin and Body Care Cosmetics by Using Challenge Test. *Saudi J. Biol. Sci.* **2024**, *31*, 103965. [CrossRef]
27. Barbaud, A.; Lafforgue, C. Risks Associated with Cosmetic Ingredients. *Ann. Dermatol. Venereol.* **2021**, *148*, 77–93. [CrossRef]
28. Podgórska, A.; Puścion-Jakubik, A.; Grodzka, A.; Naliwajko, S.K.; Markiewicz-żukowska, R.; Socha, K. Natural and Conventional Cosmetics—Mercury Exposure Assessment. *Molecules* **2021**, *26*, 4088. [CrossRef] [PubMed]
29. Ho, Y.B.; Abdullah, N.H.; Hamsan, H.; Tan, E.S.S. Mercury Contamination in Facial Skin Lightening Creams and Its Health Risks to User. *Regul. Toxicol. Pharmacol.* **2017**, *88*, 72–76. [CrossRef]
30. Abbas, H.H.; Sakakibara, M.; Sera, K.; Nurgahayu; Andayanie, E. Mercury Exposure and Health Problems of The. *Cosmetics* **2020**, *7*, 58. [CrossRef]
31. Ozbek, N.; Akman, S. Determination of Lead, Cadmium and Nickel in Hennas and Other Hair Dyes Sold in Turkey. *Regul. Toxicol. Pharmacol.* **2016**, *79*, 49–53. [CrossRef]
32. Rezaeian, M.; Mohammadi, M.; Ahmadiania, H.; Mohammadi, H.; Ghaffarian-Bahraman, A. Lead and Arsenic Contamination in Henna Samples Marketed in Iran. *Environ. Monit. Assess.* **2023**, *195*, 913. [CrossRef] [PubMed]
33. Yahya, M.; Kesekler, S.; Durukan, İ.; Arpa, Ç. Determination of Prohibited Lead and Cadmium Traces in Hair Dyes and Henna Samples Using Ultrasound Assisted-Deep Eutectic Solvent-Based Liquid Phase Microextraction Followed by Microsampling-Flame Atomic Absorption Spectrometry. *Anal. Methods* **2021**, *13*, 1058–1068. [CrossRef]
34. Kabaran, S.; Güleç, A.; Besler, H.T. Is There Any Potential Health Risk of Heavy Metals through Dietary Intake of Olive Oil That Produced in Morphou, Cyprus. *Prog. Nutr.* **2020**, *22*, 2020018. [CrossRef]
35. Prabagar, S.; Dharmadasa, R.M.; Lintha, A.; Thuraisingam, S.; Prabagar, J. Accumulation of Heavy Metals in Grape Fruit, Leaves, Soil and Water: A Study of Influential Factors and Evaluating Ecological Risks in Jaffna, Sri Lanka. *Environ. Sustain. Indic.* **2021**, *12*, 100147. [CrossRef]
36. Iordache, A.M.; Nechita, C.; Voica, C.; Roba, C.; Botoran, O.R.; Ionete, R.E. Assessing the Health Risk and the Metal Content of Thirty-Four Plant Essential Oils Using the ICP-MS Technique. *Nutrients* **2022**, *14*, 2363. [CrossRef]
37. Kereeditse, T.T.; Pheko-Ofitlhile, T.; Ultra, V.U.; Dinake, P. Effects of Heavy Metals on the Yield of Essential Oil from Vetiver Grass Cultivated in Mine Tailings Amended with EDTA and Arbuscular Mycorrhizal Fungi. *Nat. Prod. Commun.* **2023**, *18*, 1–12. [CrossRef]
38. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM). Essential Oils: Revised Monograph and New General Chapter in the Ph. Eur. Available online: <https://www.edqm.eu/en/-/essential-oils-revised-monograph-and-new-general-chapter-in-the-ph-eur>. (accessed on 16 March 2024).
39. Brkljača, M.; Giljanović, J.; Prkić, A. Determination of Metals in Olive Oil by Electrothermal Atomic Absorption Spectrometry: Validation and Uncertainty Measurements. *Anal. Lett.* **2013**, *46*, 2912–2926. [CrossRef]
40. Luka, M.F.; Akun, E. Investigation of Trace Metals in Different Varieties of Olive Oils from Northern Cyprus and Their Variation in Accumulation Using ICP-MS and Multivariate Techniques. *Environ. Earth Sci.* **2019**, *78*, 578. [CrossRef]
41. Sartorelli, P.; Montomoli, L.; Sisinni, A.G. Percutaneous Penetration of Metals and Their Effects on Skin. *Prev. Res.* **2012**, *2*, 158–164. [CrossRef]
42. Shaaban, H.; Issa, S.Y.; Ahmad, R.; Mostafa, A.; Refai, S.; Alkharraa, N.; Albaqshi, B.T.; Hussien, D.; Alqarni, A.M. Investigation on the Elemental Profiles of Lip Cosmetic Products: Concentrations, Distribution and Assessment of Potential Carcinogenic and Non-Carcinogenic Human Health Risk for Consumer Safety. *Saudi Pharm. J.* **2022**, *30*, 779–792. [CrossRef] [PubMed]
43. Halmo, L.; Nappe, T.M. Lead Toxicity. In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2023; Volume 43.
44. Ruebner, R.L.; Hooper, S.R.; Parrish, C.; Furth, S.L.; Fadrowski, J.J. Environmental Lead Exposure Is Associated with Neurocognitive Dysfunction in Children with Chronic Kidney Disease. *Pediatr. Nephrol.* **2019**, *34*, 2371–2379. [CrossRef] [PubMed]
45. Rajiv, S.V.; George, M.; Nandakumar, G. Dermatological Manifestations of Arsenic Exposure. *J. Ski. Sex. Transm. Dis.* **2023**, *5*, 14–21. [CrossRef]
46. Hong, Y.-S.; Song, K.-H.; Chung, J.-Y. Health Effects of Chronic Arsenic Exposure. *J. Prev. Med. Public Health* **2014**, *47*, 245–252. [CrossRef]
47. Chalarca-Cañas, D.; Caviedes-Cleves, M.A.; Correa-Londoño, L.A.; Ospina-Gómez, J.P.; Velásquez-Lopera, M.M. Tattoos: Risks and Complications, Clinical and Histopathological Approach. *An. Bras. Dermatol.* **2024**; in press. [CrossRef]

48. Laux, P.; Tralau, T.; Tentschert, J.; Blume, A.; Dahouk, S.A.; Bäuml, W.; Bernstein, E.; Bocca, B.; Alimonti, A.; Colebrook, H.; et al. A Medical-Toxicological View of Tattooing. *Lancet* **2016**, *387*, 395–402. [\[CrossRef\]](#)
49. Islam, P.S.; Chang, C.; Selmi, C.; Generali, E.; Huntley, A.; Teuber, S.S.; Gershwin, M.E. Medical Complications of Tattoos: A Comprehensive Review. *Clin. Rev. Allergy Immunol.* **2016**, *50*, 273–286. [\[CrossRef\]](#)
50. Bäuml, W.; Hauri, U.; Liszewski, W.; McCombie, G.; Schreiver, I.; Schubert, S. Alignment with Toxicological and Medical Reality Is Urgently Needed: A Plea after More Than One Year of European Regulation on Tattoo and Permanent Make-up Inks. *J. Eur. Acad. Dermatol. Venereol.* **2024**, 1–2. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Tudi, M.; Li, H.; Li, H.; Wang, L.; Lyu, J.; Yang, L.; Tong, S.; Yu, Q.J.; Ruan, H.D.; Atabila, A.; et al. Exposure Routes and Health Risks Associated with Pesticide Application. *Toxics* **2022**, *10*, 335. [\[CrossRef\]](#) [\[PubMed\]](#)
52. European Food Safety Authority. The 2019 European Union Report on Pesticide Residues in Food. *EFSA J.* **2021**, *19*, e06491. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Nikolic, N.; Höferl, M.; Buchbauer, G. Pesticides in Essential Oils and Selected Fragrance Extracts. Some Examples. A Review. *Flavour Fragr. J.* **2018**, *33*, 373–384. [\[CrossRef\]](#)
54. Knödler, M.; Häfner, E.; Klier, B.; Albert, H.; Binder, G.; Schenk, A.; Steinhoff, B. Evaluating a Comprehensive Database on Pesticide Residues in Essential Oils: An Update. *J. Appl. Res. Med. Aromat. Plants* **2021**, *20*, 100283. [\[CrossRef\]](#)
55. Wang, S.W.; Hsu, K.H.; Huang, S.C.; Tseng, S.H.; Wang, D.Y.; Cheng, H.F. Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Cosmetic Products by Gas Chromatography-Tandem Mass Spectrometry. *J. Food Drug Anal.* **2019**, *27*, 815–824. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Roda, G.; Arnoldi, S.; Casagni, E.; Dei Cas, M.; Silva, L.; Carini, M. Determination of Polycyclic Aromatic Hydrocarbons in Lipstick by Gas-Chromatography Coupled to Mass Spectrometry: A Case History. *J. Pharm. Biomed. Anal.* **2019**, *165*, 386–392. [\[CrossRef\]](#)
57. Crevel, R.W.R.; Kerkhoff, M.A.T.; Koning, M.M.G. Allergenicity of Refined Vegetable Oils. *Food Chem. Toxicol.* **2000**, *38*, 385–393. [\[CrossRef\]](#)
58. Yunginger, J.W.; Calobrisi, S.D. Investigation of the Allergenicity of a Refined Peanut Oil-Containing Topical Dermatologic Agent in Persons Who Are Sensitive to Peanuts. *Cutis* **2001**, *68*, 153–155.
59. Sindle, A.; Martin, K. Art of Prevention: Essential Oils—Natural Products Not Necessarily Safe. *Int. J. Women's Dermatol.* **2021**, *7*, 304–308. [\[CrossRef\]](#)
60. Amornruk, N.; Siranart, N.; Sittiwattanawong, P.; Kueanjinda, P.; Loplumert, S.; Wongpiyabovorn, J. The Immediate Patch Test Reaction to Fragrance in Patients with Allergic Contact Dermatitis to Fragrance: A Prospective Study. *J. Am. Acad. Dermatol.* **2022**, *87*, 1042–1048. [\[CrossRef\]](#) [\[PubMed\]](#)
61. De Groot, A.C. Fragrances: Contact Allergy and Other Adverse Effects. *Dermatitis* **2020**, *31*, 13–35. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Sharmeen, J.B.; Mahomoodally, F.M.; Zengin, G.; Maggi, F. Essential Oils as Natural Sources of Fragrance Compounds for Cosmetics and Cosmeceuticals. *Molecules* **2021**, *26*, 666. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Rybczyńska-Tkaczyk, K.; Grenda, A.; Jakubczyk, A.; Kiersnowska, K.; Bik-Małodzińska, M. Natural Compounds with Antimicrobial Properties in Cosmetics. *Pathogens* **2023**, *12*, 320. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Gupta, S.; Sharma, S.; Kumar Nadda, A.; Saad Bala Husain, M.; Gupta, A. Biopolymers from Waste Biomass and Its Applications in the Cosmetic Industry: A Review. *Mater. Today Proc.* **2022**, *68*, 873–879. [\[CrossRef\]](#)
65. de Groot, A.C.; Schmidt, E. Essential Oils, Part III: Chemical Composition. *Dermatitis* **2016**, *27*, 161–169. [\[CrossRef\]](#) [\[PubMed\]](#)
66. EC 2065/2003; European Commission Commission Regulation (EC) No 627/2006 of 21 April 2006 Implementing Regulation (EC) No 2065/2003 of the European Parliament and of the Council as Regards Quality Criteria for Validated Analytical Methods for Sampling, Identification and Characterisation. European Union: Brussels, Belgium, 2006; Volume 74, pp. 3–6.
67. Basketter, D.; Darlenski, R.; Fluhr, J.W. Skin Irritation and Sensitisation: Mechanisms and New Approaches for Risk Assessment—2. Skin Sensitisation. *Ski. Pharmacol. Physiol.* **2008**, *21*, 191–202. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Chilton, M.L.; Api, A.M.; Foster, R.S.; Gerberick, G.F.; Lavelle, M.; Macmillan, D.S.; Na, M.; O'Brien, D.; O'Leary-Steele, C.; Patel, M.; et al. Updating the Dermal Sensitisation Thresholds Using an Expanded Dataset and an in Silico Expert System. *Regul. Toxicol. Pharmacol.* **2022**, *133*, 105200. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Sheehan, M.P. Plant Associated Irritant and Allergic Contact Dermatitis (Phytocontact dermatitis). *Dermatol. Clin.* **2020**, *38*, 389–398. [\[CrossRef\]](#)
70. Buonomo, M.; Warshaw, E. Contact Dermatitis to Essential Oils. *Curr. Dermatol. Rep.* **2021**, *10*, 148–172. [\[CrossRef\]](#)
71. Prinsen, M.K.; Hendriksen, C.F.M.; Krul, C.A.M.; Woutersen, R.A. The Isolated Chicken Eye Test to Replace the Draize Test in Rabbits. *Regul. Toxicol. Pharmacol.* **2017**, *85*, 132–149. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Gerberick, F.G.; Ryan, C.A.; Dearman, R.J.; Kimber, I. Local Lymph Node Assay (LLNA) for Detection of Sensitisation Capacity of Chemicals. *Methods* **2007**, *41*, 54–60. [\[CrossRef\]](#) [\[PubMed\]](#)
73. OECD. *Guideline Testing Chemicals 442A: Skin Sensitisation: Local Lymph Node Assay*; OECD: Paris, France, 2010.
74. OECD. *Test Guideline No. 406: Skin Sensitisation: Guinea Pig Maximisation Test and Buehler Test*; OECD: Paris, France, 2022; pp. 1–49.
75. Filaire, E.; Nachat-Kappes, R.; Laporte, C.; Harmand, M.F.; Simon, M.; Poinot, C. Alternative in Vitro Models Used in the Main Safety Tests of Cosmetic Products and New Challenges. *Int. J. Cosmet. Sci.* **2022**, *44*, 604–613. [\[CrossRef\]](#) [\[PubMed\]](#)
76. OECD. *Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins Part 1: Scientific Evidence Series on Testing and Assess*; OECD: Paris, France, 2014.

77. Puginier, M.; Roso, A.; Groux, H.; Gerbeix, C.; Cottrez, F. Strategy to Avoid Skin Sensitisation: Application to Botanical Cosmetic Ingredients. *Cosmetics* **2022**, *9*, 40. [\[CrossRef\]](#)
78. Ta, G.H.; Weng, C.-F.; Leong, M.K. In Silico Prediction of Skin Sensitisation: Quo Vadis? *Front. Pharmacol.* **2021**, *12*, 655771. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Lee, J.S.; Kim, J.; Cui, B.; Kim, S.K.; Cho, S.A.; An, S.; Cho, S.W. Hybrid Skin Chips for Toxicological Evaluation of Chemical Drugs and Cosmetic Compounds. *Lab Chip* **2022**, *22*, 343–353. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Kawamoto, T.; Fuchs, A.; Fautz, R.; Morita, O. Threshold of Toxicological Concern (TTC) for Botanical Extracts (Botanical-TTC) Derived from a Meta-Analysis of Repeated-Dose Toxicity Studies. *Toxicol. Lett.* **2019**, *316*, 1–9. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Ng'uni, T.; Klaasen, J.A.; Fielding, B.C. Acute Toxicity Studies of the South African Medicinal Plant *Galenia Africana*. *Toxicol. Rep.* **2018**, *5*, 813–818. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Zeitoun, H.; Michael-Jubeli, R.; El Khoury, R.; Baillet-Guffroy, A.; Tfayli, A.; Salameh, D.; Lteif, R. Skin Lightening Effect of Natural Extracts Coming from Senegal Botanical Biodiversity. *Int. J. Dermatol.* **2020**, *59*, 178–183. [\[CrossRef\]](#)
83. Nishida, H.; Hirota, M.; Seto, Y.; Suzuki, G.; Kato, M.; Kitagaki, M.; Sugiyama, M.; Kouzuki, H.; Onoue, S. Non-Animal Photosafety Screening for Complex Cosmetic Ingredients with Photochemical and Photobiochemical Assessment Tools. *Regul. Toxicol. Pharmacol.* **2015**, *72*, 578–585. [\[CrossRef\]](#)
84. Zlabiene, U.; Baranauskaite, J.; Kopustinskiene, D.M.; Bernatoniene, J. In Vitro and Clinical Safety Assessment of the Multiple W/O/W Emulsion Based on the Active Ingredients from *Rosmarinus officinalis* L., *Avena sativa* L. and *Linum usitatissimum* L. *Pharmaceutics* **2021**, *13*, 732. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Parasuraman, S.; Balamurugan, S.; Vanishya, R. Overview of Safety Assessment and Toxicological Screening of Dermal Formulations. *SBV J. Basic Clin. Appl. Health Sci.* **2020**, *3*, 96–103. [\[CrossRef\]](#)
86. OECD. No. 404 Acute Dermal Irritation/Corrosion: OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects; OECD: Paris, France, 2015; pp. 1–8.
87. Sandner, G.; König, A.; Wallner, M.; Weghuber, J. Alternative Model Organisms for Toxicological Fingerprinting of Relevant Parameters in Food and Nutrition. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 5965–5982. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Almeida, A.; Sarmiento, B.; Rodrigues, F. Insights on in Vitro Models for Safety and Toxicity Assessment of Cosmetic Ingredients. *Int. J. Pharm.* **2017**, *519*, 178–185. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Teixeira, L.; Dubielzig, R.R. Special Senses. In *Fundamentals of Toxicologic Pathology*; Schafer, K.A., Bolon, B., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 673–747. ISBN 9780128098424.
90. OECD. Test Guideline No. 492 Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage; OECD Guidelines for the Testing of Chemicals; OECD: Paris, France, 2023.
91. Aizawa, S.; Yoshida, H.; Umeshita, K.; Watanabe, S.; Takahashi, Y.; Sakane, S.; Sakaguchi, H.; Kataoka, S. Development of an Oral Mucosal Irritation Test Using a Three-Dimensional Human Buccal Oral Mucosal Model. *Toxicol. Vitro.* **2023**, *87*, 105519. [\[CrossRef\]](#) [\[PubMed\]](#)
92. OECD. Guidance Document No 263 on Integrated Approaches To Testing and Assessment (Iata) for Serious Eye Damage and Eye Irritation. Series on Testing and Assessment; Organisation for Economic Co-operation and Development; OECD: Paris, France, 2023; pp. 1–110.
93. Aguiar, B.; Carmo, H.; Garrido, J.; Sousa Lobo, J.M.; Almeida, I.F. In Vitro Evaluation of the Photoreactivity and Phototoxicity of Natural Polyphenol Antioxidants. *Molecules* **2022**, *27*, 189. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Maddaleno, A.S.; Vinardell, M.P.; Mitjans, M. Innovative Strategies for Photoallergy Assessment: Breaking Free from Animal Models in Cosmetic Ingredient Development. *Cosmetics* **2024**, *11*, 47. [\[CrossRef\]](#)
95. Ates, G.; Steinmetz, F.P.; Doktorova, T.Y.; Madden, J.C.; Rogiers, V. Linking Existing in Vitro Dermal Absorption Data to Physicochemical Properties: Contribution to the Design of a Weight-of-Evidence Approach for the Safety Evaluation of Cosmetic Ingredients with Low Dermal Bioavailability. *Regul. Toxicol. Pharmacol.* **2016**, *76*, 74–78. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Santos, A.C.; Rodrigues, D.; Sequeira, J.A.D.; Pereira, I.; Simões, A.; Costa, D.; Peixoto, D.; Costa, G.; Veiga, F. Nanotechnological Breakthroughs in the Development of Topical Phytocompounds-Based Formulations. *Int. J. Pharm.* **2019**, *572*, 118787. [\[CrossRef\]](#)
97. Ferreira, L.; Pires, P.C.; Fonseca, M.; Costa, G.; Giram, P.S.; Mazzola, P.G.; Bell, V.; Mascarenhas-Melo, F.; Veiga, F.; Paiva-Santos, A.C. Nanomaterials in Cosmetics: An Outlook for European Regulatory Requirements and a Step Forward in Sustainability. *Cosmetics* **2023**, *10*, 53. [\[CrossRef\]](#)
98. Gruber, J.V.; Terpak, N.; Massard, S.; Schwartz, A.; Bojanowski, K. Passive Enhancement of Retinol Skin Penetration by Jojoba Oil Measured Using the Skin Parallel Artificial Membrane Permeation Assay (Skin-PAMPA): A Pilot Study. *Clin. Cosmet. Investig. Dermatol.* **2023**, *16*, 317–324. [\[CrossRef\]](#) [\[PubMed\]](#)
99. Burnett, C.L.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Final Report of the Safety Assessment of Kojic Acid as Used in Cosmetics. *Int. J. Toxicol.* **2010**, *29*, 244S–273S. [\[CrossRef\]](#) [\[PubMed\]](#)
100. Nohynek, G.J.; Kirkland, D.; Marzin, D.; Toutain, H.; Leclerc-Ribaud, C.; Jinnai, H. An Assessment of the Genotoxicity and Human Health Risk of Topical Use of Kojic Acid [5-Hydroxy-2-(Hydroxymethyl)-4H-Pyran-4-One]. *Food Chem. Toxicol.* **2004**, *42*, 93–105. [\[CrossRef\]](#) [\[PubMed\]](#)

101. EU 2024/996; The European Commission Commission Regulation (EU) 2024/996 of 3 April 2024 Amending Regulation (EC) No 1223/2009 of the European Parliament and of the Council as Regards the Use of Vitamin A, Alpha-Arbutin and Arbutin and Certain Substances with Potential Endocrine Disrupting P. European Union: Brussels, Belgium, 2024; Volume 996, pp. 1–8.
102. Pulsoni, I.; Lubda, M.; Aiello, M.; Fedi, A.; Marzagalli, M.; von Hagen, J.; Scaglione, S. Comparison between Franz Diffusion Cell and a Novel Micro-Physiological System for In Vitro Penetration Assay Using Different Skin Models. *SLAS Technol.* **2022**, *27*, 161–171. [[CrossRef](#)] [[PubMed](#)]
103. Riebeling, C.; Luch, A.; Tralau, T. Skin Toxicology and 3Rs—Current Challenges for Public Health Protection. *Exp. Dermatol.* **2018**, *27*, 526–536. [[CrossRef](#)] [[PubMed](#)]
104. Barker-Treasure, C.; Coll, K.; Belot, N.; Longmore, C.; Bygrave, K.; Avey, S.; Clothier, R. Non-Animal Replacements for Acute Toxicity Testing. *ATLA Altern. Lab. Anim.* **2015**, *43*, 199–203. [[CrossRef](#)] [[PubMed](#)]
105. Borba, J.V.B.; Alves, V.M.; Braga, R.C.; Korn, D.R.; Overdahl, K.; Silva, A.C.; Hall, S.U.S.; Overdahl, E.; Kleinstreuer, N.; Strickland, J.; et al. STopTox: An in Silico Alternative to Animal Testing for Acute Systemic and Topical Toxicity. *Environ. Health Perspect.* **2022**, *130*, 27012. [[CrossRef](#)] [[PubMed](#)]
106. Reisinger, K.; Fieblinger, D.; Heppenheimer, A.; Kreutz, J.; Liebsch, M.; Luch, A.; Maul, K.; Poth, A.; Strauch, P.; Dony, E.; et al. The Hen’s Egg Test for Micronucleus Induction (HET-MN): Validation Data Set. *Mutagenesis* **2022**, *37*, 61–75. [[CrossRef](#)]
107. ICCVAM. ICCVAM-Recommended Test Method Protocol: Hen’s Egg Test—Chorioallantoic Membrane (HET-CAM) Test Method. In *ICCVAM Test Method Evaluation Report: Current Validation Status of In Vitro Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products*; National Institutes of Health: Durham, NC, USA, 2010; Volume 13, ISBN 1661-6596.
108. Raitano, G.; Roncaglioni, A.; Manganaro, A.; Honma, M.; Sousselier, L.; Do, Q.T.; Paya, E.; Benfenati, E. Integrating in Silico Models for the Prediction of Mutagenicity (Ames Test) of Botanical Ingredients of Cosmetics. *Comput. Toxicol.* **2019**, *12*, 100108. [[CrossRef](#)]
109. OECD. *Guidance Document on the in Vitro Bhas 42 Cell Transformation Assay*; OECD: Paris, France, 2017; pp. 1–35.
110. Schenk, B.; Weimer, M.; Bremer, S.; van der Burg, B.; Cortvrindt, R.; Freyberger, A.; Lazzari, G.; Pellizzer, C.; Piersma, A.; Schäfer, W.R.; et al. The ReProTect Feasibility Study, a Novel Comprehensive in Vitro Approach to Detect Reproductive Toxicants. *Reprod. Toxicol.* **2010**, *30*, 200–218. [[CrossRef](#)]
111. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM) *Guidance on Essential Oils in Cosmetic Products*; Council of Europe: London, UK, 2016.

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