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Safety evaluation for human and aquatic environment of physical and chemical UV-filters used in
cosmetics
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Abstract

Sunscreens play a fundamental role in the prevention of ultraviolet-induced skin damage and skin cancer, yet growing attention has been directed toward the environmental fate of UV filters released into aquatic ecosystems during product use. Among these, titanium dioxide (TiO₂) is widely employed as an inorganic UV filter in both micro-sized and nano-sized forms, particularly in modern broad-spectrum sunscreen formulations. Despite its extensive use, the environmental behaviour and ecotoxicological implications of TiO₂ remain insufficiently understood, especially when assessed in the context of complete formulations rather than as isolated raw materials.

The aim of this study was to evaluate the environmental release and ecotoxicological effects of TiO₂-based sunscreen systems using a formulation-aware approach designed to better approximate realistic wash-off scenarios. Both micro-TiO₂ and nano-TiO₂ were investigated as isolated active ingredients and as components of finished sunscreen formulations. Leachate-based exposure methods were applied to simulate product release into aquatic media, and ecotoxicological responses were assessed using a multi-species test battery representative of different trophic levels and biological functions. Physicochemical characterisation and titanium quantification were integrated with biological testing in order to support interpretation of the observed effects.

The results indicate that the environmental behaviour of TiO₂-based sunscreen systems is strongly influenced by formulation context, particle characteristics, and exposure medium. Differences between micro- and nano-sized materials were not determined exclusively by nominal particle size, but also by aggregation behaviour, matrix interactions, and environmental conditions. Moreover, the ecotoxicological profile of formulation-derived leachates differed from that of isolated TiO₂ active ingredients, underscoring the limitations of substance-only testing for the environmental assessment of sunscreen products.

Overall, this study demonstrates that realistic ecotoxicological evaluation of TiO₂-based sunscreens requires a combined consideration of particle physicochemistry, formulation architecture, and biologically relevant exposure conditions. These findings contribute to the development of more robust environmental risk assessment strategies and support the design of sunscreen products that better reconcile effective human photoprotection with reduced ecological impact.

Keywords: sunscreen; titanium dioxide; TiO₂; nanoparticles; inorganic UV filters; ecotoxicology; environmental risk; leachates; formulation science; aquatic toxicity.

1.Introduction

1.1 Historical and Regulatory Background of Photoprotection

1.1.1 Introductory Section - Regulatory, Historical, and Conceptual Foundations of Photoprotection.

The field of photoprotection occupies a remarkably distinctive position within the broader landscape of cosmetic science and regulation. Unlike most categories of cosmetic products—moisturizers, cleansers, decorative cosmetics, or hair care formulas—sunscreens lie at the intersection of public health, dermatology, toxicology, materials science, environmental science, and European regulatory law. This intersection is not superficial but structurally constitutive: photoprotection has evolved precisely because scientific understanding of ultraviolet (UV) radiation, human skin physiology, and long term health risks required an increasingly sophisticated regulatory architecture capable of incorporating emerging knowledge into consumer protection frameworks [11,12]. During much of the twentieth century, sun exposure was celebrated culturally, marketed commercially, and trivialized medically. Tanned skin was widely seen as a sign of vitality, leisure, and modernity—a social phenomenon reinforced by fashion, cinema, and post war tourism. Yet beneath this cultural valorization, medical evidence regarding UV induced damage was accumulating gradually. The transformation from a cosmetic gesture (applying sun oils to enhance tanning) to a scientifically grounded public health practice (photoprotection) was slow, uneven, and marked by conflicting industrial and medical interests. Today, however, regulatory authorities, dermatologists, and public health organisations uniformly recognize that UV radiation is a proven carcinogen, responsible for a substantial proportion of preventable skin cancers worldwide [2,7]. This consensus, developed over decades of epidemiological and photobiological research, has reshaped the scientific and legal status of sunscreens. They are still classified formally as cosmetics under European law—specifically under Regulation (EC) No 1223/2009—but their functional relevance far exceeds that of typical cosmetic products. Sunscreens play a pivotal role in the primary prevention of UV induced conditions such as erythema, photoageing, photoimmunosuppression, and certain non melanoma skin cancers [1,3,14]. The European Commission explicitly acknowledges this preventive potential in the Commission Recommendation 2006/647/EC, which provides detailed criteria for sunscreen efficacy, labelling, and consumer guidance [11]. Understanding why sunscreens occupy this borderline conceptual space—cosmetics by legal definition, yet quasi medical in purpose—requires a reconstruction of the historical and scientific dynamics that shaped the modern regulatory framework. Unlike categories of cosmetics whose regulatory evolution followed relatively linear pathways, sunscreen regulation emerged as a reactive and adaptive process, responding to:

- new scientific discoveries about UV radiation;
- shifting public health priorities;
- industry driven innovation in UV filter chemistry;
- methodological refinements in efficacy testing;
- environmental and ethical concerns about materials and testing methods;

- cross jurisdictional divergences (notably between EU and FDA frameworks);
- increasing expectations for evidence based claims.

A comprehensive historical account must therefore trace the parallel evolution of scientific knowledge, consumer behaviour, industrial practices, and regulatory norms. It must consider how the inadequacies of early sunscreen products created public health challenges that later demanded harmonized intervention; how dermatology and photobiology matured into quantitative, mechanistic sciences; how UV filter technology progressed from simple aromatic compounds to sophisticated organic and inorganic systems; and how regulatory institutions—most notably the European Commission, the Scientific Committee on Consumer Safety (SCCS), and the Central Laboratory for Photoprotection Methodology in various international collaborations—came to shape the current standards of efficacy and safety [11–13]. At the heart of this evolution lies a shift in epistemology: from phenomenological observation (“this product seems to reduce sunburn”) to quantitative, reproducible measurement (SPF, PPD, critical wavelength, broad spectrum criteria) [1,11]. The introduction of standardized test methods, international collaborations, and formalised annexes of permitted UV filters within European legislation marked the consolidation of photoprotection as a scientific discipline. The milestone moment in this consolidation was the 2006 Commission Recommendation, which—although not binding—became highly influential in harmonising the European sunscreen market, defining technical performance requirements, preventing misleading claims, and shaping consumer education [11].

Finally, any contemporary analysis must recognise that sunscreens are no longer evaluated solely in terms of human safety and efficacy. They must also be assessed for their environmental impact, including potential toxicity to coral reefs and aquatic ecosystems, as documented in studies such as Downs et al. (2016) [17]. Additionally, the increasing use of nanomaterials in UV filters (TiO₂ and ZnO) has stimulated new toxicological paradigms, prompting regulatory scrutiny over their physicochemical properties, dermal penetration, and photoreactivity [13,14,27]. In sum, the story of sunscreen regulation is a story of convergence: a slow but decisive movement toward an integrated system where science, law, and public health mutually reinforce each other. To fully appreciate the current regulatory landscape—and the place of sunscreens within it—one must examine the historical trajectories that led to the recognition of UV radiation as a major health hazard and the classification of sunscreens as essential protective tools within a strictly cosmetic regulatory framework [11,12].

The following sections provide a detailed monographic exploration of this evolution, from the earliest sunscreen formulations of the 1930s to the sophisticated, regulated products of the 21st century.

1.1.2 The Historical Evolution of Sunscreen Regulation

Understanding the present regulatory status of sunscreens requires a careful reconstruction of the historical forces that shaped their development. Photoprotection did not emerge in a vacuum: it is the outcome of converging trajectories in medical science, consumer culture, industrial chemistry, and regulatory jurisprudence. These trajectories evolved at different speeds and often with conflicting priorities. The history of sunscreen regulation is therefore not a straightforward narrative of linear progress; rather, it is a patchwork of scientific breakthroughs, cultural shifts, industrial pressures, and regulatory responses.

In this expanded historical account, we examine how early sunscreen products were conceived, how scientific understanding of UV radiation gradually deepened, how regulators responded—or failed to respond—to emerging evidence, and how institutional structures ultimately converged in the modern European framework.

1.1.3 The early era of photoprotection (1930s–1950s): a pre scientific field

The first products marketed as sunscreens did not arise from a well developed understanding of photobiology. Instead, they emerged from a general recognition—largely phenomenological rather than mechanistic—that certain substances could reduce the incidence of sunburn. The dominant paradigm was that of empirical tanning aids rather than scientifically validated protection. Products of this period often contained oily or reddish substances intended to deepen the appearance of a tan, rather than prevent UV-induced damage.

One of the earliest attempts at a functional sunscreen is attributed to the chemist Franz Greiter, who in 1938 is reported to have formulated a rudimentary protective cream after suffering severe sunburn while mountaineering. This anecdote, often cited in cosmetic histories, illustrates the broader context: early sunscreens were born out of practical need but lacked any systematic scientific foundation. At the same time, the mid 20th century witnessed a cultural transformation in the Western perception of sun exposure. Whereas pale skin had historically symbolized aristocracy, leisure, and avoidance of manual labor, the interwar and post war periods saw tanning redefined as a sign of health, cosmopolitanism, and wealth. This shift was reinforced by the rise of seaside tourism, the popularization of swimwear, and the association of sunbathing with modern, active lifestyles. However, this cultural enthusiasm often obscured the biological risks of UV exposure. Medical literature of the time mentions erythema as a common acute reaction but rarely frames it as a precursor to long term damage. Dermatology remained primarily descriptive, and the links between cumulative UV exposure and skin carcinogenesis were not yet fully established. As a result, early sunscreen products operated largely outside the purview of medical scrutiny and regulatory intervention.

The scientific awakening (1950s–1970s): early UV filters and the seeds of regulation.

By the mid 20th century, certain aromatic compounds had been identified as effective UVB absorbers. Among these, para aminobenzoic acid (PABA) and its esters were the most widely used. PABA-containing formulations proliferated from the 1950s onward, becoming the prototype for what we would now call “active UV filters.” Yet these early filters protected almost exclusively against UVB wavelengths responsible for sunburn, while offering little to no protection against UVA radiation, which we now know to be deeply implicated in photoaging, photoimmunosuppression, and photocarcinogenesis [3,8].

This scientific limitation had regulatory consequences. Because the products achieved visible, immediate results—reducing erythema—they were perceived as effective, despite leaving users vulnerable to long term UVA-mediated biological damage. The absence of long-term clinical endpoints, such as photoaging or cumulative immunological effects, meant that regulators had little basis for intervention.

In the 1960s and early 1970s, dermatologists began to publish more systematic observations on the effects of sunlight on the skin. Photodermatology as a sub-specialty gradually took shape, supported

by advancements in UV measurement technologies and in laboratory methods for analyzing photochemical processes in skin [24]. However, this emerging scientific foundation had not yet crystallized into regulatory frameworks. Sunscreens remained largely unregulated consumer products, marketed with considerable variability in quality, safety, and composition. This period also witnessed the first concerns about photosensitivity reactions, particularly associated with PABA and some cinnamate derivatives [5,34]. Such reactions were sporadically reported in dermatological journals but did not lead to systematic regulatory oversight. The absence of harmonized standards across European states meant that products varied widely in their composition and claimed efficacy, often relying on unverified or exaggerated marketing statements. The seeds of future regulation were nonetheless planted during this era. As dermatologists grew increasingly aware of the risks posed by UV radiation, the foundations were laid for a conceptual shift: from sunscreens as optional cosmetic aids to sunscreens as public health instruments.

The emergence of regulatory consciousness (1970s): the prelude to European harmonization.

The 1970s marked a transitional phase in which scientific insights into UV radiation intensified, and regulators began acknowledging the need for harmonization. Several developments converged: Scientific clarity about UVB vs. UVA. Research increasingly demonstrated that UVB radiation, though historically considered the primary cause of erythema, was not the sole agent of photodamage. UVA wavelengths were shown to penetrate more deeply, damaging dermal structures and contributing to long term carcinogenic processes [8,10]. The rapid growth of the cosmetics industry during the post-war period, combined with increasing cross-border trade, generated pressure for harmonized standards across the European Economic Community (EEC).

1.1.4 Public concern about consumer safety.

Several high profile cases related to cosmetic safety—not necessarily involving sunscreens—prompted European policymakers to re-examine cosmetic regulation in general. Divergent national approaches. Each EEC member state maintained its own cosmetic regulations, leading to barriers in the free movement of goods. It was in this context that Directive 76/768/EEC emerged—a landmark piece of European legislation that would shape the trajectory of sunscreen regulation for decades. Initially, this Directive did not address sunscreens specifically, but it established two fundamental principles:

- cosmetic products must be safe under reasonably foreseeable conditions of use;
- claims must not mislead consumers regarding the product's characteristics.

These provisions would later form the legal backbone for the interpretation of sunscreen labelling, efficacy standards, and claim restrictions in the European Union. The Directive also mandated the creation of annexes listing substances permitted, restricted, or prohibited in cosmetic products. Although these annexes did not yet include a positive list of UV filters, they set the structural precedent for what would eventually become Annex VI of the modern Regulation (EC) No 1223/2009 [6,12].

The stage was thus set for the next phase in the evolution of sunscreen regulation—one in which scientific advances in photobiology would accelerate dramatically and regulatory frameworks would begin to reflect the transformative role of sunscreens in public health.

The Consolidation of European Cosmetic Law: Directive 76/768/EEC and its Long Shadow.

Directive 76/768/EEC, adopted on 27 July 1976, occupies a foundational place in the history of European cosmetic regulation. Although sunscreens were not explicitly singled out upon its adoption, the Directive established the conceptual and legal architecture that would shape photoprotection for decades to come. It also laid the groundwork for the later development of more specialised norms and technical criteria, including those set forth in the 2006 Commission Recommendation [6,11]. The Directive functioned on two levels: first as a public health instrument, ensuring that cosmetic products circulating within the European Economic Community (EEC) did not pose risks to consumer safety; and second as a market integration mechanism, harmonising national laws in order to facilitate the free movement of cosmetic goods. Its importance for sunscreen regulation lies in the structural principles it introduced, particularly:

- the requirement that cosmetic products be safe under normal or reasonably foreseeable conditions of use;
- the prohibition of misleading claims—an essential provision that would later be used to constrain inappropriate sunscreen labelling;
- the establishment of annexes listing ingredients subject to restrictions or bans.

These annexes became the backbone of the EU's ingredient based regulatory approach, later evolving into the detailed, harmonised system of positive lists (including the list of approved UV filters) found in modern EU law. Importantly, the Directive required manufacturers to hold information on product composition, safety assessment, and manufacturing conditions available for competent authorities. This requirement was an early precursor of today's Product Information File (PIF) under Regulation (EC) No 1223/2009 [6,12]. Although modest in scope, the Directive's data retention obligations reflected a shift toward evidence based cosmetic oversight, a shift that would later become central in sunscreen regulation as scientific knowledge expanded. At the time of its adoption, however, the Directive did not foresee the complexities of photoprotection. Sunscreens remained conceptually aligned with cosmetics; they were not yet recognized as tools with significant public health implications. What the Directive did accomplish was to create the conceptual apertures through which later harmonisation efforts could flow. As knowledge accumulated about UV radiation and skin cancer risk, these apertures widened, eventually giving rise to more technically detailed and scientifically grounded regulatory measures.

1.1.5 The rise of photobiology and its regulatory implications (1980s–1990s)

If the 1970s laid the legal foundation, it was the 1980s and 1990s that constituted the scientific renaissance of photoprotection. Dermatology underwent a profound transformation during this

period, shifting from a largely observational discipline to one increasingly informed by molecular biology, photochemistry, and epidemiology. This transformation had immediate relevance for sunscreens.

Several domains advanced simultaneously:

1. Insights into UV-induced DNA damage

Scientists identified cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts as primary forms of UV-induced DNA lesions [7]. These discoveries illustrated, with molecular precision, how UV radiation initiates carcinogenic pathways, providing mechanistic evidence for the public health importance of sunscreen use.

2. Understanding the distinct biological roles of UVB and UVA

By the mid-1980s, researchers had demonstrated that UVB photons, though comprising a small fraction of solar UV reaching the earth's surface, are highly energetic, directly damaging DNA and producing erythema. UVA radiation, though less energetic, penetrates deeper into the skin, generating reactive oxygen species and contributing to long-term dermal degradation [8,10]. This dichotomy undermined earlier sunscreen models that protected almost exclusively against UVB.

3. Epidemiological breakthroughs

Studies began confirming an association between cumulative UV exposure and increased incidence of non melanoma skin cancer, and certain patterns of intermittent intense exposure (especially in fair skinned populations) with melanoma risk. The pivotal meta-analysis by Gandini et al. (2005) later synthesized decades of these findings into a coherent epidemiological narrative [2].

4. Advances in sunscreen chemistry

The development of new UV filters—such as benzophenones, cinnamates, salicylates, and later dibenzoylmethanes—expanded the protective spectrum of sunscreen products. Yet many of these molecules demonstrated limitations: photoinstability, skin penetration concerns, or incomplete UVA coverage [5,34].

5. Emergence of modern efficacy metrics

Perhaps most crucial for regulation was the emergence of quantitative methods to measure sunscreen efficacy. In the late 1970s and early 1980s, the Sun Protection Factor (SPF) began to be standardized as a measure of protection against UVB-induced erythema [1,12]. Subsequently, the PPD (Persistent Pigment Darkening) method was introduced in Japan as a measure of UVA protection.

These advances collectively shifted sunscreens from phenomenological consumer products to quantifiable, scientifically assessable tools of photoprotection. Regulators could now build norms on empirical data—not anecdote or marketing claims.

1.1.6 International and institutional dynamics: COLIPA and the harmonisation of testing methods

Parallel to the scientific maturation of photobiology, institutional structures evolved to support standardized evaluation of sunscreen products. The European trade association COLIPA (Comité de Liaison des Industries de la Parfumerie), now known as Cosmetics Europe, played a central role in developing harmonized test methods for both SPF and UVA metrics. In the 1990s, COLIPA worked extensively with international counterparts—particularly from Japan, the United States, and South Africa—to standardise:

- in vivo SPF testing parameters;
- UVA protection evaluation methods (PPD, UVA PF)
- the critical wavelength method, which quantifies how uniformly a sunscreen absorbs UV across wavelengths from 290 to 400 nm.

This international cooperation was not merely technical; it represented a form of proto regulatory diplomacy. Regulators in many jurisdictions lacked the scientific expertise or institutional capacity to develop detailed photoprotection standards independently. Industry, especially COLIPA and Japanese research institutions, provided pivotal scientific infrastructure that would later feed directly into official EU guidelines.

As these collaborative efforts progressed, European regulators—and above all the European Commission—became increasingly aware of the growing need for harmonised, transparent efficacy standards. Without such standards, discrepancies between products, inconsistent labelling, and misleading claims proliferated. This set the stage for the decisive regulatory intervention of 2006.

The scientific and regulatory trajectory toward the 2006 Commission Recommendation The early 2000s were characterised by a surge in public-health campaigns on UV risks, fuelled by rising incidence rates of skin cancers across Europe, particularly in Northern and Western populations with fair skin phenotypes. At the same time, industry continued to launch new UV filters with broader spectra and improved photostability, which further exposed inconsistencies in labelling practices and claims.

This convergence of public-health pressure, scientific advances, and industry innovation generated a regulatory expectation that the European Commission could no longer ignore. Policymakers recognised that sunscreens, although legally cosmetics, played a crucial societal role. The absence of harmonised technical criteria was increasingly untenable.

By the mid-2000s, dermatologists, national authorities, and consumer organisations were urging the Commission to provide authoritative guidance on:

- how sunscreen efficacy should be measured;
- how protection should be communicated to consumers;
- what claims should be prohibited as misleading;
- how to align UVA and UVB protection criteria;
- how to reconcile the proliferation of SPF numbers with consumer comprehension;

- how to address inconsistent or deceptive marketing terminology.

The result of these pressures was the Commission Recommendation 2006/647/EC, which we will examine in depth as follows. This Recommendation became one of the most influential documents in the history of sunscreen regulation, despite its non binding nature. It crystallised the scientific and regulatory advances of the previous three decades into a coherent set of principles and criteria that would transform the European sunscreen market.

1.1.7 The 2006 Commission Recommendation in Depth

The Commission Recommendation 2006/647/EC, adopted on 22 September 2006, represents a pivotal moment in the evolution of European sunscreen regulation. More than any prior legal or scientific initiative, this Recommendation served to consolidate decades of dermatological research, photobiological understanding, and institutional deliberation into a coherent framework that redefined what sunscreen efficacy means in the European context [11]. Although formally a Recommendation—and therefore not legally binding under EU law—it rapidly acquired quasi normative authority. It shaped regulatory expectations, guided market practices, influenced national supervisory bodies, and provided a blueprint for subsequent harmonisation at both scientific and commercial levels. In practice, the Recommendation became the de facto European standard for sunscreen efficacy and labelling, even before the adoption of Regulation (EC) No 1223/2009 [11,12].

To appreciate fully the significance of this document, we must examine its historical context, structural content, scientific reasoning, and regulatory implications. The Recommendation is not merely a set of technical guidelines; it is a conceptual milestone that reshaped the understanding of what sunscreens should be, how they should be assessed, and how consumers should be guided in their use.

Historical and scientific context: why the Recommendation was necessary.

By the early 2000s, the sunscreen market presented several systemic problems:

1. Lack of harmonisation

European sunscreens displayed wide variations in labelling, particularly regarding UVA protection. Products could claim broad spectrum efficacy even if they absorbed negligibly beyond 340 nm [4,11]. Different national agencies used different metrics, and many products boasted numerical indicators that were neither standardised nor reproducible.

2. Misleading claims

Many products marketed themselves with terms like “total protection,” “sunblock,” or “all-day protection.” Such claims were fundamentally incompatible with photobiological realities: no topical formulation can block 100% of UV radiation, and no sunscreen remains fully effective without reapplication. The Commission deemed these claims not only scientifically inaccurate but also dangerous to consumers.

3. Consumer confusion over SPF numbers

By 2005, products advertised a vast range of SPF values, often exceeding SPF 60. Yet the incremental protection gained between values above SPF 30 is small when expressed in terms of percentage UVB absorption. Consumers could be misled into thinking that SPF 60 offered twice the protection of SPF 30—an interpretation the Commission viewed as deeply problematic [1,11].

4. Growing recognition of UVA risks

While SPF measures UVB induced erythema, it does not capture UVA protection. Scientific evidence had increasingly shown that UVA contributes significantly to long term photodamage, photoaging, and carcinogenesis [8,10]. The absence of harmonised UVA standards allowed products with meagre UVA absorption to present themselves as “high protection.”

5. Increasing public health importance of sunscreens

Skin cancer rates were rising sharply, particularly in fair skinned populations. Sunscreens were increasingly recognised as essential tools in broad public health strategies, not mere cosmetic adjuncts. Regulators saw a responsibility to ensure that products marketed as protective actually delivered meaningful protection.

The Commission thus identified an urgent need for clarification, harmonisation, and guidance. The Recommendation was crafted not as a symbolic exhortation but as a detailed and pragmatic response to persistent regulatory gaps.

1.1.8 Core principles of the 2006 Recommendation

The Recommendation articulates several foundational principles that underpin modern photoprotection in Europe. These principles continue to influence regulatory and scientific discourse.

1. Sunscreens must protect against both UVB and UVA

The Commission recognizes that sunscreens must not privilege UVB protection at the expense of UVA, stating unequivocally that products should protect “not less” against UVA than UVB [11]. This principle is operationalised through specific quantitative criteria, including the UVA/UVB ratio, the PPD minimum (one-third of the SPF), and the critical wavelength threshold.

2. No sunscreen may claim “total protection”

The Commission explicitly prohibits claims that suggest 100% UV blockade. This includes formulations such as:

- “sunblock”
- “total protection”
- “complete screen”
- “total defence”

The prohibition stems not only from scientific impossibility but from a public health

imperative: consumers who believe they are fully protected may engage in prolonged sun exposure, increasing long term harm [3,11].

3. Emphasis on correct use and reapplication

The Recommendation stresses that efficacy measurements assume use at 2 mg/cm², a dose rarely achieved by consumers. It therefore obliges manufacturers to include instructions emphasising:

- pre exposure application
- frequent reapplication
- reapplication after sweating, swimming, or towelling
- warnings that inadequate application reduces protection significantly (often by more than 50%) [1,11]

4. Harmonised categories of protection

Instead of an unbounded proliferation of SPF values, four harmonised categories are introduced:

- Low protection
- Medium protection
- High protection
- Very high protection

Each category corresponds to a specific range of SPF values and UVA minimum criteria.

This system enhances consumer comprehension and prevents deceptive marketing based on minimal numerical differences.

5. Preference for in vitro methods

While acknowledging existing in vivo methods, the Commission expresses a clear preference for validated in vitro methodologies, largely on ethical grounds (reducing human testing) but also recognising their reproducibility and consistency. This foreshadows the later ethical imperatives embedded in the animal-testing bans under Regulation 1223/2009 [11,12].

1.1.9 Technical criteria introduced by the Recommendation

To operationalise these principles, the Recommendation provides detailed scientific standards. It is here that the document transforms from a policy statement into a quasi technical protocol.

1. Minimum SPF

A product labelled as sunscreen must provide at least SPF 6, as measured by the International SPF Test Method (2006). This constitutes the baseline for UVB protection.

2. Minimum UVA protection

UVA protection must meet two simultaneous criteria:

UVA-PF $\geq$ 1/3 of SPF, measured via the PPD method modified by AFSSAPS.

Critical wavelength $\geq$ 370 nm, ensuring broad absorption across the UVA spectrum.

This dual requirement is more rigorous than many non EU standards, including the current U.S. FDA monograph [11,43].

3. Labelling clarity and visibility

The category of protection (e.g., “high protection”) must be as prominent as the numerical SPF value. This requirement reflects the Commission’s view that UV protection categories should become intuitive consumer information, similar to energy labels or food nutrition categories.

1.1.10 Interpretative significance of the Recommendation

Although not legally binding, the Recommendation attained a form of “soft law” status, widely cited in enforcement actions, marketing review, and regulatory guidance across EU Member States. Its influence stems from several sources:

1. Scientific credibility

The Recommendation was grounded in robust photobiological evidence, including mechanistic, epidemiological, and methodological research. It integrated insights from multiple scientific fields, giving it legitimacy among dermatologists and toxicologists.

2. Institutional momentum

The Recommendation arrived at a moment when European regulatory institutions were transitioning from directive based to regulation based frameworks. It provided a scientific template later absorbed by Regulation 1223/2009 [11,12].

3. Industry alignment

The cosmetic industry, through COLIPA, had already invested in harmonised testing methods consistent with the Recommendation. Thus, industry compliance was facilitated by pre existing infrastructure.

4. Consumer demand for clarity

Public-health campaigns increasingly stressed the importance of photoprotection. Consumers were seeking clarity amid confusing SPF claims, and the Recommendation met this demand.

1.1.11 Critical evaluation: strengths and limitations

The Recommendation remains influential, but it is not without critiques. Scholars and public-health experts have noted both strengths and limitations.

Strengths :

- Establishes a clear scientific basis for UV protection.
- Reduces misleading claims, particularly around “total protection.”
- Harmonises disparate national approaches.

- Recognises UVA as equally important as UVB.
- Encourages ethical and reproducible test methods.
- Enhances consumer comprehension and decision making.

Limitations :

- Lacks legal enforceability (though widely adopted in practice).
- Relies heavily on in vivo SPF tests still raising ethical concerns.
- Offers limited guidance on photostability, despite its importance.
- Does not fully address environmental impacts of UV filters.
- Does not tackle endocrine disruption concerns, which emerged more prominently post 2010.

Despite these limitations, the Recommendation remains a foundational document in European photoprotection, shaping both the normative expectations and the technical practices of the entire sunscreen industry.

The Recommendation thus stands as a rare example of regulatory guidance where science, public health, and industry converged to produce a coherent and widely adopted framework.

1.1.12 Regulation (EC) No 1223/2009 and the Shaping of the Modern Photoprotection Framework

The adoption of Regulation (EC) No 1223/2009, which replaced Directive 76/768/EEC, constitutes one of the most profound regulatory transformations in the history of European cosmetic law. While the Directive had served as an essential foundation for harmonisation, the Regulation provided the structural, institutional, and methodological environment necessary for a mature, evidence driven regulatory system—one capable of addressing the complex safety and efficacy issues associated with modern sunscreen formulations [12].

Unlike directives, which require transposition into national law, regulations are directly applicable in all Member States, ensuring uniformity and eliminating national discrepancies. This shift was pivotal for sunscreens, whose evaluation requires highly standardised methodologies, consistent risk assessment criteria, and pan European mechanisms for surveillance and enforcement. The Regulation entered into force in 2013, after a transitional period, and its impact on photoprotection can hardly be overstated. It established the procedural and scientific infrastructure through which sunscreen filters are evaluated, listed, monitored, and reassessed. It institutionalised the Product Information File (PIF), created the Responsible Person, mandated the CPNP notification system, prohibited animal testing, and formalised the Annex VI list of authorised UV filters. In many respects, Regulation 1223/2009 translated the scientific and normative lessons embodied in the 2006 Commission Recommendation into a binding regulatory system [11,12].

The Responsible Person and the PIF: Sunscreen Compliance as a Scientific Obligation
The Regulation introduced the concept of the Responsible Person (RP)—a legal entity responsible for ensuring compliance with all aspects of the Regulation, including safety, labelling, and adherence to the authorised UV filter list. For sunscreens, the RP has particularly demanding responsibilities.

The Product Information File (PIF) must contain:

- a full toxicological safety assessment;
- exposure calculations specific to sunscreens (which involve comparatively high application quantities and frequency);
- data on photostability of filters and the finished formulation;
- compatibility information with packaging materials;
- proof of efficacy (e.g., SPF and UVA testing under recognised methods);
- justification of claimed benefits, including broad spectrum designations and water resistance.

This represents a sharp departure from pre 2009 practices, where data requirements were inconsistent and often left to national interpretation. The PIF transformed sunscreen manufacturing into a discipline of regulatory toxicology and photobiological rigor, where every UV filter must be justified through harmonised standards [12].

1.1.13 Annex VI: the Positive List of UV Filters

Regulation 1223/2009 introduced a legally binding positive list of approved UV filters—Annex VI—which today includes around 30 substances. No sunscreen may be placed on the European market using UV filters not listed in Annex VI [12].

This system accomplishes several goals:

- it ensures that all UV filters undergo a robust risk assessment by the SCCS;
- it limits market access to substances with known safety profiles;
- it provides legal certainty for manufacturers;
- it prevents the proliferation of untested or unsafe UV filter technologies.

The European model contrasts sharply with the American system (FDA OTC monograph), where approved filters are limited, outdated, and extremely slow to change, creating a regulatory bottleneck widely criticised by scientists and industry [43].

1.1.14 The SCCS: Scientific Gatekeeper of Sunscreen Safety

At the core of Annex VI oversight lies the Scientific Committee on Consumer Safety (SCCS), an independent scientific advisory body responsible for evaluating the safety of cosmetic ingredients. The SCCS assesses UV filters with an approach that is quantitative, exposure driven, and conservative, reflecting the high frequency, high quantity use of sunscreens [13].

The SCCS evaluates:

- systemic absorption and plasma concentrations;
- photostability under solar simulated irradiation;
- potential for photo toxicity and photo allergy;
- metabolism;
- endocrine potential;
- carcinogenicity, mutagenicity, and reproductive toxicity;
- nanomaterial forms and coatings;

- environmental fate (increasingly relevant, although formally outside SCCS mandate).

Its Opinion documents are highly technical, often exceeding 100 pages, and represent the most advanced non pharmaceutical risk assessment system for sunscreens globally.

Nanomaterials in Sunscreens: TiO₂, ZnO, and the New Era of Regulatory Complexity
The introduction of nanomaterial forms of titanium dioxide and zinc oxide in sunscreen formulations triggered a paradigm shift in regulatory science. Nanoparticle UV filters brought significant performance advantages:

- reduced whitening on skin;
- improved transparency and cosmetic acceptability;
- broad spectrum coverage;
- improved photostability.

Yet they also raised new questions, prompting regulators to grapple with issues rarely encountered in traditional cosmetic toxicology:

1. Dermal penetration

Studies generally show minimal penetration of TiO₂ and ZnO nanoparticles into viable epidermis [13]. However, this needed rigorous verification, given concerns about nanoparticle translocation.

2. Photocatalytic activity

Uncoated TiO₂ nanoparticles can generate free radicals under UV exposure. Regulations thus require appropriate coatings or surface treatments [7,30].

3. Inhalation hazards

Sprayable sunscreen formats raise concerns about pulmonary exposure, prompting restrictions for certain particle-size ranges.

4. Physicochemical complexity

Nanomaterials are not single substances but material systems whose safety depends on:

- size distribution,
- shape,
- agglomeration behavior,
- coating,
- crystalline form (anatase vs. rutile),
- surface charge.

European law addresses these concerns by requiring nano specific notification, detailed physicochemical characterization, and explicit “nano” labelling on ingredient lists [15,16].

The EU remains one of the only jurisdictions in the world with such detailed nanomaterial oversight for sunscreens.

1.1.15 Endocrine Disruption Debates and the Reassessment of Legacy Filters

Beginning in the late 2000s, concerns about potential endocrine disrupting properties of certain UV filters (e.g., benzophenone 3, octocrylene, homosalate) emerged from in vitro and in vivo studies. While evidence varied in robustness, the issue necessitated re evaluation under the SCCS framework [13].

This led to:

- restricted maximum concentrations for some filters;
- new data submission requirements;
- calls for extended toxicological dossiers;
- the possibility of delisting non compliant filters.

European regulators consistently adopt the precautionary approach, balancing the public health benefits of UV protection with potential systemic risks. This stands in contrast to U.S. regulation: the FDA OTC monograph system is slow to incorporate new data on endocrine or systemic effects and relies heavily on older toxicological paradigms [43].

Environmental and Ecotoxicological Considerations in Modern Photoprotection
In recent years, another dimension has been added to sunscreen regulation: the environmental fate and effects of UV filters. Although not formally under the SCCS mandate, environmental considerations increasingly influence regulatory debates. Particular attention has been paid to organic filters such as oxybenzone (benzophenone 3) and octinoxate (EHMC). Research, including the influential study by Downs et al. (2016), has suggested potential toxic effects on coral larvae, zooxanthellae, and aquatic organisms [20].

This has triggered:

- product bans in certain ecologically sensitive jurisdictions (e.g., Hawaii, Palau, parts of Australia);
- consumer concern about “reef safe” formulations;
- increased interest in mineral filters and biodegradable systems;
- industry exploration of encapsulation, polymerisation, and new-generation organic filters.

While the EU has not (yet) instituted filter bans on environmental grounds, the issue is increasingly visible in policy discussions and may shape future revisions of Annex VI [14,15,28]

Global Regulatory Fragmentation: EU vs. USA vs. Asia.

Sunscreen regulation is globally fragmented, with major jurisdictions adopting divergent approaches:

European Union

- Sunscreens are cosmetics.
- Annex VI positive list restricts active filters.

- Strong UVA requirements ($\text{PPD} \geq 1/3 \text{ SPF}$; critical wavelength $\geq 370 \text{ nm}$).
- Robust scientific gatekeeping via SCCS.

United States (FDA)

- Sunscreens are OTC drugs.
- Only ~16 filters are allowed, most dating from the 1970s–1990s.
- New filters require NDA-level evidence—almost impossible to obtain for cosmetic products.
- Result: technological stagnation, lack of modern UVA filters.

Japan

- Highly advanced UVA filter technologies.
- Many local filters remain unavailable in the EU due to regulatory classification differences.
- “PA system” widely used (PA+, PA++, etc.).

Australia

- Hybrid model: sunscreens classified as therapeutic goods if $\text{SPF} \geq 4$.
- Some of the strictest SPF testing standards in the world.

South Korea

- Dynamic innovation and creative formulation, with rapid approval of new filters.
- However, regulatory frameworks differ substantially from the EU, creating challenges for international harmonisation.

This heterogeneity complicates global sunscreen development: companies must reformulate products for different jurisdictions, and consumers face inconsistent efficacy messaging. The EU remains a global leader in scientific rigour, especially regarding UVA protection and systemic safety evaluation [11,12,43].

1.2 Structure and Physiology of Human Skin

Human skin is a highly specialised and multifunctional organ that forms the primary interface between the body and the external environment. As the largest organ of the human body, it performs essential protective, sensory, metabolic, and immunological functions. Its complex architecture and dynamic physiology allow the skin to respond to a wide range of physical, chemical, and biological stressors, including ultraviolet (UV) radiation. A clear understanding of skin structure and function is therefore fundamental to the study of photobiology and to the development of effective photoprotective strategies [3,27].

Structurally, the skin is classically divided into three main layers: the epidermis, the dermis, and the hypodermis. These compartments are functionally interconnected and contribute synergistically to tissue homeostasis and barrier integrity. The epidermis provides the principal physical and biochemical barrier, the dermis confers mechanical strength and metabolic support, and the hypodermis contributes to insulation, energy storage, and mechanical cushioning [30,31].

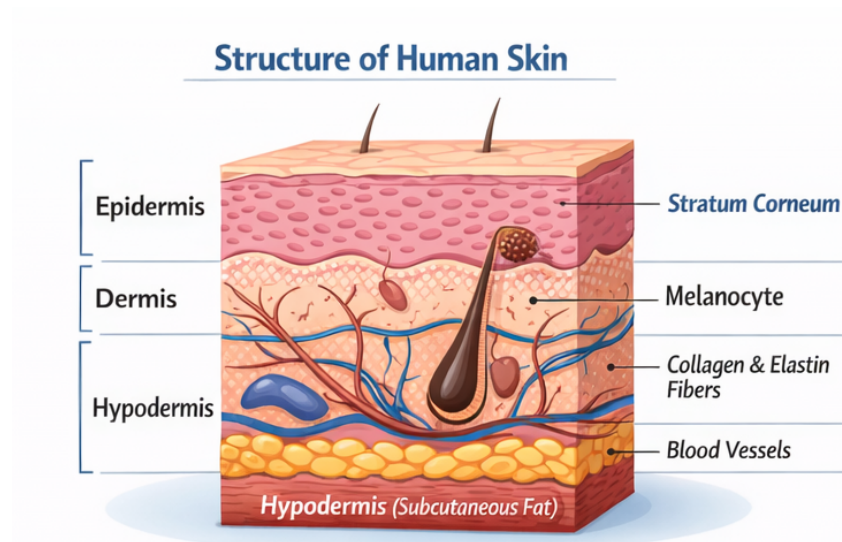


Figure 1. Schematic representation of the structure of human skin. The skin is composed of three main layers: the epidermis, dermis, and hypodermis. The epidermis, including the stratum corneum, provides the primary barrier against environmental stressors. The dermis contains structural components such as collagen and elastin fibres, blood vessels, and adnexal structures, supporting mechanical strength and metabolic functions. Melanocytes located in the basal layer contribute to pigmentation and endogenous photoprotection. The hypodermis, composed mainly of subcutaneous fat, plays a role in insulation, energy storage, and mechanical cushioning.

1.2.1 Epidermis and Barrier Function

The epidermis is a stratified squamous epithelium composed predominantly of keratinocytes at different stages of differentiation. From the basal layer, where keratinocyte proliferation occurs, to the outermost stratum corneum, epidermal cells undergo a tightly regulated process of differentiation, keratinisation, and terminal cornification. This continuous programme of renewal is essential for maintaining epidermal integrity and for forming an effective barrier against external insults [24,26]. The barrier function of the epidermis is central to limiting transepidermal water loss and restricting the penetration of exogenous substances, including chemical agents, microorganisms, pollutants, and particulate materials. Keratinocyte differentiation is accompanied by marked structural and biochemical changes, including the synthesis of keratins, the formation of the cornified envelope, and the production of specialised lipids, all of which contribute to barrier competence [26,27].

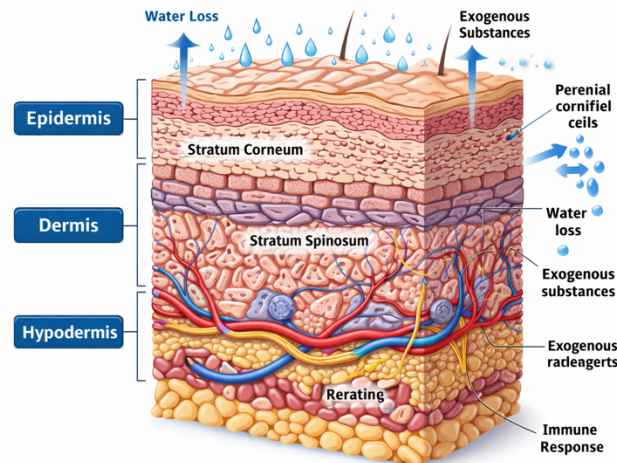


Figure 2. Anatomy and physiological role of the epidermis in skin barrier function. The epidermis is organized into distinct layers of differentiating keratinocytes, culminating in the stratum corneum, which constitutes the primary physical and biochemical barrier of the skin. The stratum corneum limits transepidermal water loss and restricts the penetration of exogenous substances through a highly organized structure of corneocytes embedded in a lipid-rich extracellular matrix. This barrier architecture supports skin hydration, mechanical protection, and initial defense against environmental stressors, including ultraviolet radiation.

The stratum corneum, representing the terminal stage of keratinocyte differentiation, is composed of flattened, enucleated corneocytes embedded in a highly organised extracellular lipid matrix. This architecture is often described by the “brick-and-mortar” model, in which corneocytes act as the structural “bricks,” while intercellular lipids—primarily ceramides, cholesterol, and free fatty acids—form the “mortar” [26,27]. The lamellar organisation of these lipids regulates permeability and confers mechanical resistance, thereby ensuring selective diffusion across the skin surface.

In addition to its barrier role, the epidermis contributes to the skin’s interaction with solar radiation. The stratum corneum provides a modest degree of passive photoprotection through scattering and partial absorption of incident UV radiation. Although this intrinsic filtering capacity is limited, it represents the first physical interface encountered by solar radiation and therefore contributes to the initial attenuation of UV exposure [1]. Disruption of epidermal barrier function, whether due to disease, ageing, or environmental damage, may increase the vulnerability of deeper skin layers to ultraviolet injury and other exogenous stressors [26].

1.2.2 Dermis and Structural Support

Beneath the epidermis lies the dermis, a highly specialised connective tissue that provides structural support, elasticity, and metabolic exchange. The dermis consists primarily of fibroblasts embedded within an extracellular matrix rich in collagen fibres, elastic fibres, proteoglycans, and glycosaminoglycans. Type I collagen is the major structural protein of the dermis and is responsible for tensile strength, whereas elastin-containing fibres provide elasticity and allow the skin to recover its shape after deformation [25,28].

The dermis also contains blood vessels, lymphatic vessels, sensory nerve endings, hair follicles, sebaceous glands, sweat glands, mast cells, macrophages, and other resident immune cells. This high degree of vascularisation and innervation makes the dermis essential not only for structural support,

but also for nutrient delivery, thermoregulation, waste removal, sensory perception, inflammatory responses, and tissue repair [25,28].

Dermal fibroblasts play a particularly important role in maintaining cutaneous homeostasis by regulating extracellular matrix synthesis and remodelling. Through the production of growth factors, cytokines, and matrix components, fibroblasts also influence keratinocyte proliferation, differentiation, and migration, underscoring the importance of epidermal–dermal crosstalk in normal skin physiology [28]. Disruption of this bidirectional communication may contribute to impaired repair processes and pathological skin conditions.

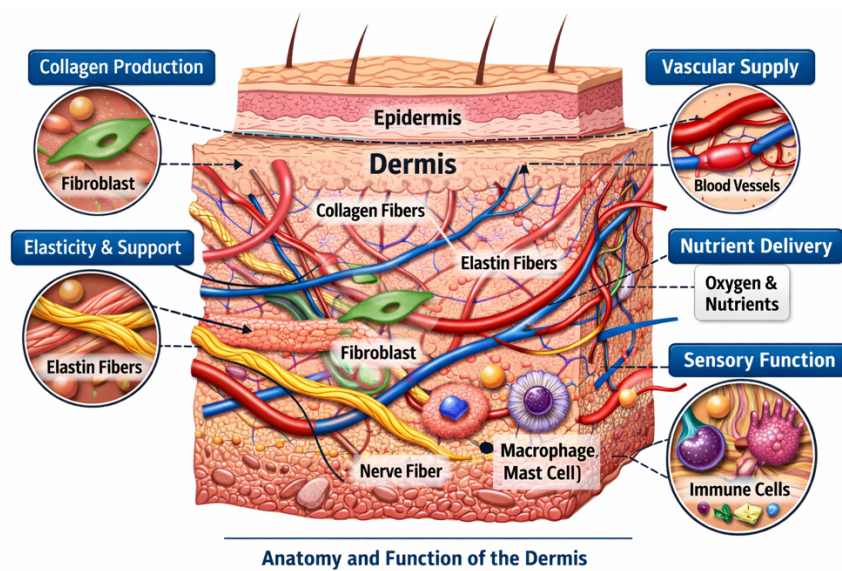


Figure 3. Anatomy and functional organization of the dermis. The dermis is a highly vascularized and innervated connective tissue layer located beneath the epidermis, composed primarily of fibroblasts embedded within an extracellular matrix rich in collagen and elastin fibers. Fibroblasts are responsible for the synthesis and remodeling of structural proteins that confer tensile strength and elasticity to the skin. The dermal vascular network ensures nutrient and oxygen delivery, thermoregulation, and waste removal, while sensory nerve fibers mediate tactile and nociceptive functions. Resident immune cells, including macrophages and mast cells, contribute to immune surveillance and inflammatory responses. Together, these components support epidermal function, mechanical stability, and long-term skin resilience to environmental stressors.

From a photobiological perspective, the dermis is especially relevant because it is the principal target of long-wavelength UVA radiation, which penetrates more deeply into the skin than UVB [8]. UVA exposure induces biological effects predominantly through indirect oxidative mechanisms, generating reactive oxygen species that damage proteins, lipids, nucleic acids, and extracellular matrix components. Chronic UVA exposure promotes collagen degradation, elastic fibre disorganisation, and dermal matrix remodelling, thereby contributing to photoageing and long-term structural deterioration of the skin [8,10].

1.2.3 Melanocytes and Pigmentation

Melanocytes are specialised dendritic cells located primarily in the basal layer of the epidermis. Their main function is the synthesis of melanin, a biopolymeric pigment derived from tyrosine and transferred to neighbouring keratinocytes via melanosomes. Within keratinocytes, melanin

accumulates above the nucleus and forms supranuclear caps that help shield DNA from ultraviolet-induced damage [29].

Two principal forms of melanin are found in human skin: eumelanin and pheomelanin. Eumelanin, which is brown to black in colour, is a highly efficient absorber of UV radiation and also possesses antioxidant properties. Pheomelanin, by contrast, is yellow to reddish in colour and provides less effective photoprotection, while under some conditions contributing to oxidative stress [29]. The balance between eumelanin and pheomelanin, rather than total pigment amount alone, substantially influences individual susceptibility to photodamage.

This variation underlies the concept of skin phototype. Individuals with lighter phototypes generally have lower intrinsic photoprotection and are therefore more susceptible to sunburn, photoageing, and skin cancer. Nevertheless, even darker skin, despite higher eumelanin content and greater baseline photoprotection, remains vulnerable to cumulative UV-induced injury. Endogenous pigmentation thus reduces, but does not eliminate, the biological need for exogenous photoprotection [29].

1.2.4 Cutaneous Immune System

The skin is also an active immunological organ containing a complex network of innate and adaptive immune cells. Langerhans cells, dermal dendritic cells, macrophages, mast cells, and resident T lymphocytes contribute to immune surveillance and to the defence against pathogens, toxins, and transformed cells [29]. These populations continuously monitor the cutaneous environment and participate in inflammatory and repair responses following tissue injury.

Ultraviolet radiation has profound effects on this system. Acute UV exposure can alter antigen presentation, modify cytokine release, and induce local and systemic immunosuppressive effects. Although some degree of immunomodulation may serve a protective role by limiting excessive inflammation, chronic UV-induced immune dysregulation compromises the ability of the skin to detect and eliminate damaged or transformed cells, thereby contributing to photocarcinogenesis [4,29].

1.2.5 Endogenous Photoprotection and Its Limitations

The skin possesses several endogenous defence mechanisms against solar radiation, including pigmentation, epidermal thickening, antioxidant systems, and DNA repair pathways. Enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, together with non-enzymatic antioxidants including vitamins C and E, help neutralise reactive oxygen species generated during UV exposure [8,10]. At the genomic level, nucleotide excision repair and related pathways contribute to the removal of UV-induced DNA lesions [7].

Despite these mechanisms, endogenous protection is inherently limited. Repeated or intense exposure can overwhelm antioxidant defences and repair capacity, leading to cumulative damage over time. This imbalance between UV-induced injury and biological defence forms the mechanistic basis for photoageing, immunological alterations, and photocarcinogenesis [3,6]. Consequently, endogenous cutaneous defence cannot be regarded as sufficient under realistic patterns of solar exposure, and exogenous photoprotective strategies, including appropriately formulated sunscreen products, remain essential [1,11].

1.3 Solar Radiation and Skin Exposure

As the primary interface between the human body and the external environment, the skin is continuously exposed to solar radiation. Although its structural organisation and endogenous defence mechanisms provide a degree of natural protection, the skin remains highly vulnerable to the biological effects of ultraviolet radiation. The interaction between solar radiation and cutaneous tissues is therefore a critical determinant of both physiological adaptation and pathological outcome [24].

Solar radiation reaching the Earth's surface encompasses a broad spectrum of electromagnetic wavelengths, including infrared radiation, visible light, and ultraviolet radiation. Among these components, UV radiation is of particular biological relevance because of its relatively high photon energy and its capacity to induce molecular and cellular alterations in skin tissues. The ultraviolet spectrum is conventionally divided into UVC, UVB, and UVA according to wavelength and biological effect [14,30].

UVC radiation, typically defined as 100–280 nm, is highly energetic and potentially cytotoxic, but under normal environmental conditions it is effectively absorbed by atmospheric gases, especially stratospheric ozone, and does not reach the Earth's surface [17,19]. Consequently, natural human exposure to solar UV radiation consists almost entirely of UVB and UVA wavelengths. These two regions of the UV spectrum differ substantially in penetration depth, molecular targets, and biological consequences [31]

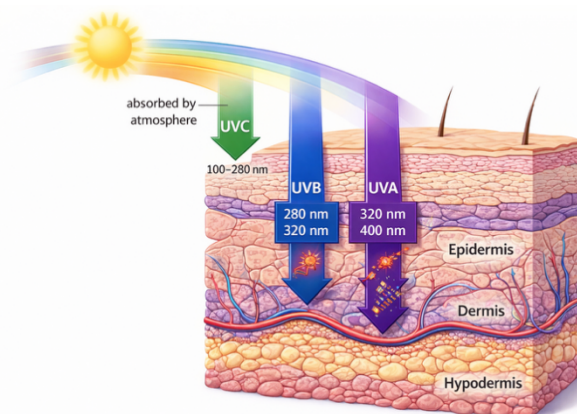


Figure 4. Wavelength-dependent interaction of solar ultraviolet radiation with human skin. Solar ultraviolet radiation is conventionally divided into UVC (100–280 nm), UVB (280–320 nm), and UVA (320–400 nm) based on wavelength and biological relevance. UVC radiation is effectively absorbed by the Earth's atmosphere and does not reach the skin under natural environmental conditions. UVB radiation penetrates predominantly into the epidermis, where it is associated with erythema and direct DNA damage. In contrast, UVA radiation penetrates more deeply into the dermis, contributing to oxidative stress and long-term structural alterations. The figure illustrates the relationship between UV wavelength, depth of skin penetration, and biological effects relevant to cutaneous photobiology.

UVB radiation, generally ranging from 280 to 320 nm, is absorbed predominantly within the epidermis. Because of its higher energy, UVB directly interacts with nuclear DNA and is strongly associated with the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts. This direct genotoxicity explains its central role in erythema, sunburn, mutagenesis, and the initiation of cutaneous carcinogenesis [6,7]. The relatively superficial penetration of UVB does not imply limited biological importance; rather, it reflects the concentration of key targets, particularly keratinocyte DNA, within the epidermal compartment.

By contrast, UVA radiation, conventionally defined as 320–400 nm, penetrates more deeply into the skin and reaches the dermis to a much greater extent than UVB. Although UVA is less efficient in producing direct DNA photolesions, it contributes substantially to skin damage through indirect oxidative mechanisms. Following absorption by endogenous chromophores, UVA promotes the generation of reactive oxygen species, which induce lipid peroxidation, protein oxidation, mitochondrial dysfunction, extracellular matrix degradation, and oxidative DNA damage [8]. For this reason, UVA is strongly implicated in photoageing, dermal remodelling, immunomodulation, and long-term carcinogenic processes [4,8].

The extent of UV radiation reaching the skin is not constant but is influenced by numerous geographical, environmental, and behavioural factors. Latitude is a major determinant, as regions closer to the equator receive more intense UV radiation due to a more direct angle of solar incidence. Altitude also plays an important role, since the thinner atmospheric layer at higher elevations results in less UV absorption and therefore greater irradiance at ground level [16]. Seasonal variation further modifies exposure patterns, with peak UV levels typically observed during summer months in temperate climates.

Atmospheric conditions add further complexity. Cloud cover can reduce UV irradiance, but not always to a degree that eliminates biologically relevant exposure. Ozone concentration, particulate matter, and air pollution also modulate the transmission of solar UV radiation through the atmosphere [16,32]. In addition, environmental reflectivity, or albedo, may significantly increase effective exposure. Surfaces such as snow, sand, and water can reflect substantial proportions of incident UV radiation, thereby increasing cutaneous exposure even in circumstances where direct sunlight appears reduced [16].

Individual behaviour is equally important in determining cumulative UV dose. Occupational exposure, recreational habits, time spent outdoors, clothing, use of shade, and intentional tanning behaviours all contribute to the overall burden of solar radiation received by the skin [12,22]. Intermittent but intense UV exposure, particularly when associated with episodes of sunburn, has been strongly linked to melanoma risk, whereas long-term cumulative exposure is more closely associated with non-melanoma skin cancers and chronic photoageing [2,12]. This distinction underscores the importance of both exposure pattern and total dose in photobiology and public health. Despite the presence of endogenous defence systems, including melanin synthesis, epidermal thickening, antioxidant responses, and DNA repair mechanisms, the adaptive capacity of the skin is limited. Cumulative damage begins early in life and may persist even when overt clinical signs are absent. Repeated exposure progressively exceeds the capacity of biological repair systems, allowing molecular lesions and structural alterations to accumulate over time [7,8]. This process is central to the delayed manifestation of photoageing and skin cancer.

The quantitative assessment of skin exposure to solar UV radiation has also driven the development of standardised photoprotection metrics. Among these, the Sun Protection Factor (SPF) remains the most widely recognised indicator of sunscreen efficacy. SPF is defined as the ratio between the minimal erythema dose on protected skin and that on unprotected skin under standardised conditions, and therefore reflects protection primarily against UVB-induced erythema [1,11]. Although SPF is often interpreted by consumers as a general measure of total sun protection, it does not directly describe protection against UVA-mediated biological effects.

For this reason, modern sunscreen evaluation also includes specific indicators of UVA protection, such as the UVA Protection Factor and related in vitro or in vivo methods [1,11]. Within the European regulatory framework, the requirement that UVA protection should be at least one-third of the labelled

SPF reflects the recognition that effective photoprotection must address the full biologically relevant UV spectrum rather than erythema alone [11]. This principle has become central to the concept of broad-spectrum protection.

Another important point is that the relationship between SPF and UV attenuation is not linear. Increases in SPF correspond to progressively smaller gains in percentage UVB absorption, meaning that the apparent difference between high-SPF products may be larger numerically than biologically [1]. This has important implications for risk communication, consumer understanding, and product labelling, since very high SPF values may create unrealistic expectations of complete protection if not accompanied by appropriate warnings and guidance on application and reapplication.

Taken together, these considerations show that solar radiation exposure is a complex and multidimensional phenomenon. It is governed not only by wavelength-specific photobiology, but also by environmental variability, behavioural patterns, and the limits of endogenous skin defence. This complexity provides the scientific rationale for external photoprotection and forms the conceptual bridge between cutaneous photobiology and the development, regulation, and evaluation of sunscreen products [1,11].

1.4 Biological Effects of UV Radiation on Human Skin

Ultraviolet radiation exerts a broad spectrum of biological effects on human skin, ranging from transient acute responses to progressive and irreversible chronic damage. These effects depend on wavelength, dose, duration, exposure pattern, and host susceptibility, and they manifest at molecular, cellular, tissue, and clinical levels [24]. The cumulative nature of UV-induced injury makes solar radiation one of the most important environmental determinants of skin pathology.

1.4.1 Acute and Chronic Effects of UV Exposure

One of the earliest and most visible consequences of excessive UV exposure is erythema, commonly referred to as sunburn. Erythema is primarily mediated by UVB radiation and results from vasodilation, inflammatory signalling, and cellular injury within the epidermis and superficial dermis. Clinically, it is characterised by redness, warmth, pain, and, in more severe cases, oedema, blistering, and epidermal necrosis [14,31]. Although sunburn is often perceived as a temporary condition, it reflects substantial underlying biological damage and is strongly associated with long-term skin cancer risk, particularly when it occurs repeatedly or early in life [2].

Repeated exposure to ultraviolet radiation also accelerates photoageing, a distinct form of extrinsic skin ageing caused by chronic solar damage. Photoaged skin is characterised by coarse wrinkling, loss of elasticity, rough texture, telangiectasia, and uneven pigmentation. Histologically, these changes are associated with collagen fragmentation, elastic fibre degeneration, solar elastosis, and remodelling of the dermal extracellular matrix [8,10]. UVA plays a major role in this process because of its deep dermal penetration and its capacity to induce oxidative stress, although UVB also contributes, particularly through cumulative epidermal damage and inflammatory pathways [8].

Ultraviolet exposure additionally promotes pigmentary alterations, including tanning, hyperpigmentation, solar lentigines, and exacerbation of disorders such as melasma. While increased melanin synthesis may represent an adaptive response to UV exposure, persistent or dysregulated pigmentation often reflects pathological changes in melanocyte activity and melanosome distribution

[10,29]. These alterations may have substantial psychosocial consequences in addition to their biological significance.

Beyond these visible manifestations, chronic UV exposure induces long-term structural and functional deterioration of the skin. Repeated injury impairs barrier integrity, alters immune responses, disrupts extracellular matrix homeostasis, and contributes to the accumulation of genomic and mitochondrial damage. These effects do not occur in isolation, but rather interact over time, producing a progressive decline in skin resilience and function [8].

1.4.2 Molecular and Cellular Mechanisms of UV-Induced Skin Damage

The harmful effects of ultraviolet radiation are mediated by a network of interconnected molecular and cellular mechanisms. One of the most important targets is DNA. UVB radiation directly induces the formation of cyclobutane pyrimidine dimers and pyrimidine-(6-4)-pyrimidone photoproducts, both of which distort the DNA helix and interfere with transcription and replication [7,31]. If not effectively repaired, these lesions may become fixed as mutations during subsequent cell division, thereby contributing to carcinogenesis.

UVA radiation contributes to genomic injury predominantly through indirect oxidative pathways. Following absorption by endogenous chromophores, UVA promotes the generation of reactive oxygen species such as singlet oxygen, superoxide anions, and hydroxyl radicals. These reactive species oxidise DNA bases, proteins, and membrane lipids, resulting in oxidative lesions including 8-oxo-7,8-dihydroguanine and other mutagenic modifications [8,10]. Mitochondrial DNA is particularly vulnerable because of its limited repair capacity and proximity to intracellular sources of oxidative stress.

At the signalling level, ultraviolet exposure activates multiple stress-responsive pathways. The tumour suppressor protein p53 plays a central role in the cellular response to UV-induced DNA damage by promoting cell cycle arrest, DNA repair, or apoptosis depending on the severity of injury [8,9]. This mechanism is essential for maintaining genomic integrity. However, chronic or excessive UV exposure can lead to p53 mutation or dysfunction, allowing damaged cells to evade apoptosis and persist within the epidermis [6].

UV radiation also activates mitogen-activated protein kinase pathways, including ERK, JNK, and p38, as well as transcription factors such as activator protein-1 and nuclear factor kappa B. These signalling cascades regulate inflammatory gene expression, apoptosis, matrix degradation, and stress adaptation [8,10]. One important downstream consequence is the induction of matrix metalloproteinases, particularly MMP-1, MMP-3, and MMP-9, which degrade collagen and other extracellular matrix components. Simultaneously, UV exposure suppresses transforming growth factor-beta-mediated collagen synthesis, resulting in a net loss of dermal structural proteins and progressive tissue remodelling [8].

Lipid peroxidation constitutes another major pathway of UV-induced injury. Reactive oxygen species attack polyunsaturated fatty acids within cellular and subcellular membranes, generating reactive aldehydes and secondary oxidative products that propagate tissue damage [3]. Protein oxidation further alters enzyme function, structural integrity, receptor signalling, and cellular metabolism. Together, these processes disrupt normal skin physiology and amplify inflammatory and degenerative responses.

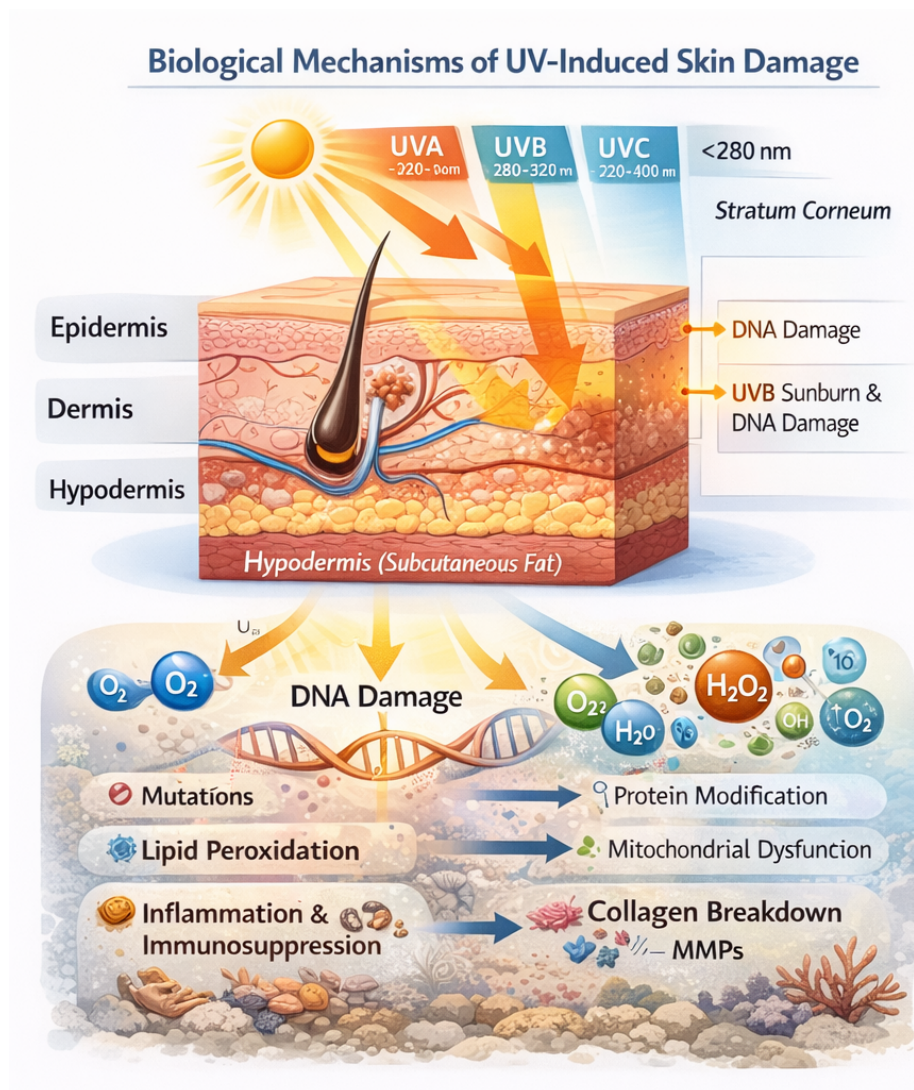


Figure 5. Biological mechanisms of UV-induced skin damage. Solar ultraviolet radiation interacts with human skin in a wavelength-dependent manner. UVB radiation is primarily absorbed within the epidermis and induces direct DNA damage, leading to erythema and mutagenesis. UVA radiation penetrates more deeply into the dermis, where it promotes the generation of reactive oxygen species (ROS) and oxidative stress. These processes result in DNA damage, lipid peroxidation, protein modification, mitochondrial dysfunction, activation of inflammatory and immunosuppressive pathways, and up-regulation of matrix metalloproteinases (MMPs), ultimately contributing to collagen degradation, photoaging, and skin carcinogenesis.

1.4.3 Immunological Effects of UV Radiation

The skin is not only a structural barrier but also an immunologically active tissue, and ultraviolet radiation has profound effects on cutaneous immune function. Both UVA and UVB can impair antigen presentation, alter cytokine profiles, reduce Langerhans cell density and function, and promote the development of a locally immunosuppressive environment [4]. This immunomodulation contributes to reduced surveillance against transformed cells and may facilitate the initiation and progression of skin tumours.

UV-induced immunosuppression also has broader implications for host defence and inflammatory regulation. While some degree of immune modulation may protect against excessive inflammation

after acute exposure, chronic immunosuppression undermines the capacity of the skin to respond adequately to pathogens and neoplastic change [4]. The immunological dimension of photodamage is therefore highly relevant to understanding why ultraviolet radiation acts not only as a mutagen, but also as a promoter of carcinogenesis.

1.4.4 UV Radiation and Skin Carcinogenesis

The classification of ultraviolet radiation as a complete carcinogen reflects its ability to act at multiple stages of tumour development. It contributes to initiation through direct and indirect DNA damage, to promotion through chronic inflammation and immunosuppression, and to progression through the accumulation of mutations and altered tissue microenvironments [4,6,7]. This mechanistic complexity helps explain the strong epidemiological relationship between UV exposure and both melanoma and non-melanoma skin cancers.

Non-melanoma skin cancers, particularly basal cell carcinoma and squamous cell carcinoma, are strongly associated with cumulative lifetime UV exposure, especially in fair-skinned populations [8,11]. Melanoma, although less common, is more strongly linked to intermittent intense exposure and repeated episodes of sunburn, especially during childhood and adolescence [2]. The different exposure patterns associated with these malignancies highlight the need for broad and sustained photoprotection across the lifespan.

1.4.5 Limits of Endogenous Repair and the Need for Photoprotection

Human skin possesses multiple endogenous protective and reparative mechanisms, including DNA repair systems, antioxidant defences, melanogenesis, and adaptive epidermal thickening. However, these responses are only partially effective under realistic exposure conditions [7,10]. Repeated UV exposure overwhelms repair capacity, allowing molecular damage to accumulate over time even when immediate clinical signs are absent.

This progressive mismatch between injury and repair is central to the long-term consequences of ultraviolet exposure. Acute damage may resolve clinically, but the biological effects often persist at subclinical levels, contributing to mutational burden, immune dysregulation, extracellular matrix degradation, and functional decline [8]. For this reason, effective photoprotection should not be understood solely in terms of preventing sunburn; it should also be evaluated in relation to its ability to limit oxidative stress, DNA damage, immunological alteration, and cumulative structural injury [1,11].

In this context, sunscreen products represent a key component of a broader photoprotective strategy. Their relevance lies not only in reducing erythema, but in attenuating the initiating events that underlie photoageing, photocarcinogenesis, and chronic cutaneous dysfunction. The biological effects of UV radiation therefore provide the essential scientific rationale for the development of broad-spectrum, safe, and effective sunscreen formulations [1,11].

1.5 Skin Cancer and Public Health Impact

Skin cancer represents one of the most significant long-term consequences of ultraviolet exposure and constitutes a major public health concern worldwide. The causal relationship between solar radiation and skin carcinogenesis is supported by epidemiological, clinical, and molecular evidence.

Despite increased awareness of sun-related risks and the wider availability of photoprotective products, the global incidence of skin cancer continues to rise in many populations, reflecting changing lifestyles, demographic trends, and cumulative patterns of environmental exposure [2,16]. Skin cancers are broadly classified into melanoma and non-melanoma skin cancers, the latter consisting mainly of basal cell carcinoma and squamous cell carcinoma. Although these malignancies differ substantially in cellular origin, biological behaviour, prognosis, and mortality, they share ultraviolet radiation as a major etiological factor [6].

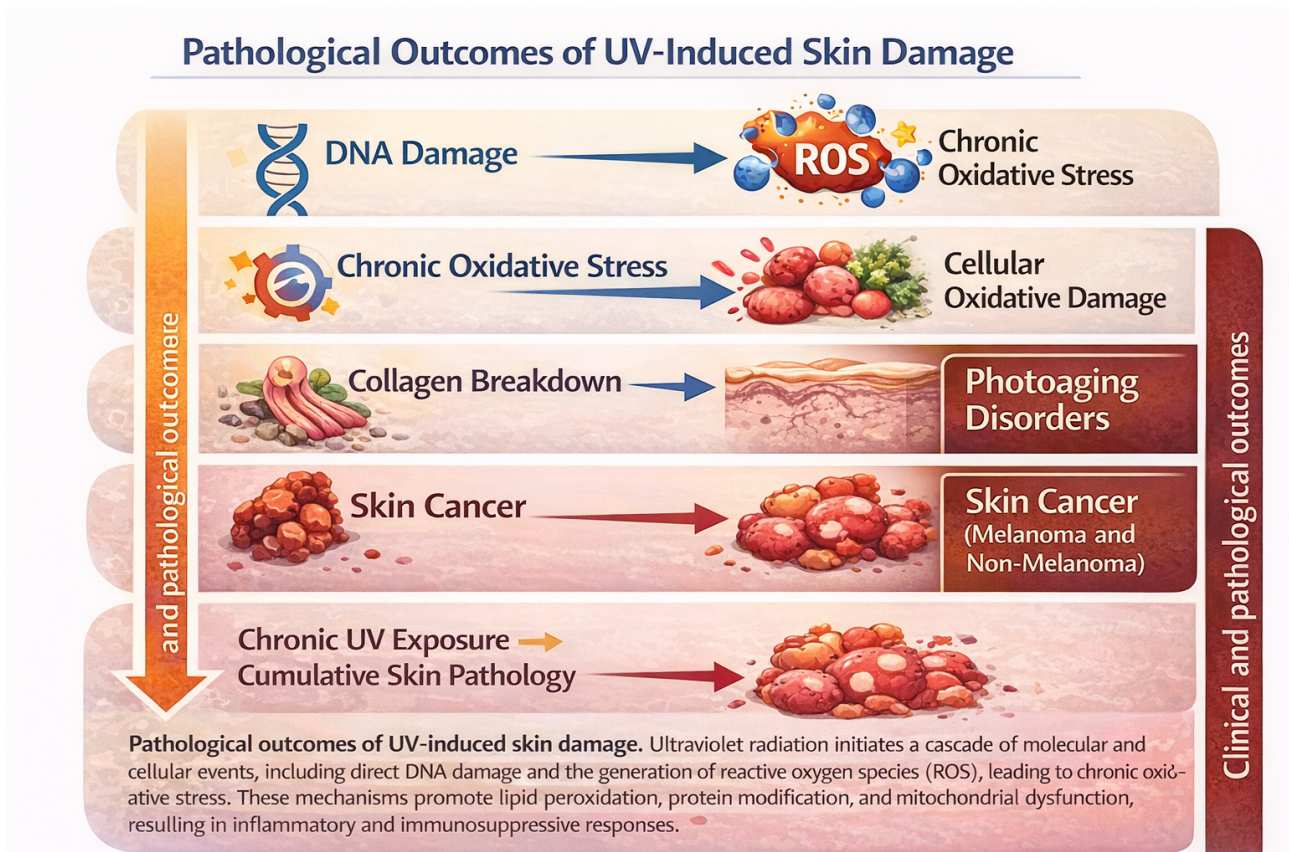


Figure 6. Pathological outcomes of UV-induced skin damage. Chronic exposure to ultraviolet radiation initiates a progressive cascade of biological alterations, beginning with DNA damage and the induction of chronic oxidative stress. Persistent oxidative injury leads to cumulative cellular damage and disruption of skin homeostasis. Degradation of dermal collagen contributes to photoaging disorders, while the accumulation of genetic and cellular alterations increases the risk of skin cancer, including both melanoma and non-melanoma skin cancers. The figure highlights the temporal and pathological progression from early molecular injury to clinically relevant skin disorders.

1.5.1 Non-Melanoma Skin Cancers

Non-melanoma skin cancers are the most frequently diagnosed malignancies in fair-skinned populations and account for the majority of skin cancer cases globally. Basal cell carcinoma arises from basal keratinocytes and is generally characterised by slow growth and very limited metastatic potential. Squamous cell carcinoma, by contrast, arises from more differentiated keratinocytes and has a greater capacity for local invasion and, in some cases, metastasis, particularly in immunocompromised individuals [6].

The development of non-melanoma skin cancers is strongly associated with cumulative lifetime ultraviolet exposure, especially chronic exposure to UVB-rich sunlight. Occupational exposure, advanced age, and residence in regions with high solar irradiance are well-established risk factors [14]. At the molecular level, these tumours often harbour characteristic UV-signature mutations, particularly in genes involved in cell cycle regulation and DNA repair, reinforcing the mechanistic link between repeated solar exposure and carcinogenesis [6,7].

Although non-melanoma skin cancers are associated with lower mortality than melanoma, their extremely high incidence imposes a substantial healthcare burden. The need for repeated diagnosis, surgical treatment, clinical follow-up, and management of recurrent lesions contributes significantly to healthcare expenditure and to reduced quality of life among affected individuals. Their epidemiological relevance is therefore considerable even when case-fatality rates are comparatively low.

1.5.2 Melanoma

Cutaneous melanoma is less common than basal cell carcinoma and squamous cell carcinoma, but it is the most aggressive and lethal form of skin cancer. Melanoma arises from melanocytes and is characterised by a marked propensity for invasion and metastasis. Its incidence has increased steadily over recent decades in many regions, especially among fair-skinned populations [2].

In contrast to non-melanoma skin cancers, melanoma risk is more strongly associated with intermittent, intense UV exposure and repeated episodes of sunburn, particularly during childhood and adolescence [2]. This exposure pattern is commonly linked to recreational sun exposure and deliberate tanning behaviours rather than to continuous occupational exposure alone. Such observations suggest that the timing, intensity, and intermittency of UV exposure may be as important as cumulative dose in melanoma pathogenesis.

Melanoma development reflects a complex interplay between ultraviolet-induced DNA damage, oxidative stress, immune dysregulation, and genetic susceptibility. While melanocytes possess endogenous protective mechanisms, including melanin production and antioxidant activity, these defences are insufficient to prevent malignant transformation under conditions of repeated or intense solar insult [3,29]. Because advanced melanoma is associated with poor prognosis, high therapeutic complexity, and substantial mortality, prevention and early detection remain central public health priorities [2].

1.5.3 Epidemiological Trends and Risk Factors

The growing incidence of skin cancer reflects the interaction of behavioural, environmental, and demographic determinants. Increased recreational sun exposure, outdoor lifestyles, changes in clothing habits, and the persistent cultural value attributed to tanned skin have all contributed to greater cumulative UV exposure in many populations [3,4]. In addition, the use of artificial tanning devices has further increased exposure to biologically active ultraviolet radiation, particularly among younger individuals [33].

Population ageing is another important factor. Because much of the carcinogenic burden of UV exposure is cumulative, longer life expectancy allows more time for latent molecular damage to manifest clinically as neoplasia. Genetic predisposition, skin phototype, pigmentation traits, immune status, and geographic residence also modulate individual and population-level susceptibility,

contributing to marked heterogeneity in skin cancer incidence across regions and demographic groups [2,14].

1.5.4 Economic and Societal Burden

The burden of skin cancer extends well beyond morbidity and mortality. Direct healthcare costs include screening, diagnostic procedures, surgical treatment, pathology, oncological care, and long-term surveillance. Indirect costs include loss of productivity, psychosocial distress, cosmetic and functional impairment, and reduced quality of life. In the case of melanoma, advanced disease imposes particularly high economic costs because of the complexity of treatment and long-term management.

From a societal perspective, the public health significance of skin cancer is amplified by the fact that a substantial proportion of cases are theoretically preventable. This makes skin cancer unusual among malignancies: it is common, costly, and linked to a largely identifiable environmental risk factor that can be mitigated through behavioural and technological interventions [16].

1.5.5 Photoprotection as a Public Health Strategy

Photoprotection represents a cornerstone of primary prevention in skin cancer control. Public health strategies aimed at reducing ultraviolet exposure typically include behavioural recommendations such as seeking shade, avoiding peak sun hours, wearing protective clothing, and applying sunscreen correctly and consistently [16]. Within this framework, sunscreen products serve as one component of a broader protective strategy rather than as a standalone solution.

The preventive value of sunscreen use has been supported by evidence showing reductions in actinic keratoses and non-melanoma skin cancer risk, as well as broader protective effects against UV-induced damage when products are used appropriately [11]. The evidence for melanoma prevention is more complex, partly because melanoma is influenced by intermittent exposure patterns and long latency periods. Nevertheless, regular and correct use of broad-spectrum sunscreen is widely regarded as an important element of melanoma risk reduction, especially when integrated into comprehensive sun-protection behaviour [2].

These considerations have important regulatory implications. Because the public health relevance of sunscreens extends beyond mere cosmetic appearance, claims relating to efficacy, broad-spectrum coverage, and correct application must be scientifically substantiated and communicated responsibly. In this sense, the burden of skin cancer provides a compelling rationale for the strict evaluation of sunscreen performance, safety, and labelling, and for continued innovation in sunscreen formulation science [11,12].

1.6 UV Filters: Classification and Mechanisms of Action

UV filters constitute the functional core of sunscreen formulations, as they are the components directly responsible for attenuating ultraviolet radiation before it penetrates the skin. Their selection, concentration, and combination determine not only the level and spectral profile of photoprotection achieved, but also the stability, safety, sensory properties, and regulatory compliance of the final product [1,11]. From a scientific and regulatory perspective, UV filters are commonly classified into inorganic and organic filters according to their chemical nature and predominant mode of action.

Although this binary classification remains useful, it should be recognised as a simplification. Both inorganic and organic systems may attenuate ultraviolet radiation through a combination of absorption, scattering, reflection, and energy dissipation mechanisms. Nevertheless, distinguishing between these two major classes remains valuable for understanding their formulation behaviour, safety considerations, environmental implications, and regulatory treatment [11,34].

1.6.1 Inorganic UV Filters

Inorganic UV filters, often referred to as mineral or physical filters, are represented primarily by titanium dioxide (TiO₂) and zinc oxide (ZnO). These materials are widely used in sunscreen formulations because of their broad-spectrum attenuation properties, high photostability, and low intrinsic chemical reactivity when appropriately formulated [11,34].

Historically, inorganic filters were described mainly as reflecting and scattering incident UV radiation. This interpretation is now considered incomplete. In addition to scattering and reflection, both titanium dioxide and zinc oxide can absorb ultraviolet photons and dissipate the absorbed energy through non-damaging pathways [11,34]. The relative contribution of these mechanisms depends strongly on particle size, crystalline form, aggregation state, surface treatment, and formulation context.

Particle size is a particularly important determinant of optical behaviour and cosmetic acceptability. Larger particles are efficient at scattering both UV and visible light, but they produce an opaque white film on the skin that is often considered cosmetically undesirable. The development of micronised and nanoparticulate forms of TiO₂ and ZnO has significantly improved transparency in visible light while maintaining high UV attenuation, thereby increasing consumer acceptability [13,34]. However, the use of finely divided and nanoscale materials has also introduced additional toxicological and regulatory concerns, particularly regarding photocatalytic activity, inhalation risk in spray formulations, and the need for detailed physicochemical characterisation [13,14].

A key issue for titanium dioxide, especially in certain crystalline forms such as anatase, is photocatalytic activity. When irradiated, uncoated TiO₂ may generate reactive oxygen species at the particle surface, which can compromise both safety and formulation stability [11,14]. For this reason, commercial sunscreen-grade mineral filters are typically surface-treated with inert coatings such as silica, alumina, or silicone-based materials to reduce photocatalytic reactivity, improve dispersion, and enhance compatibility with formulation matrices [11,34].

From a biological perspective, inorganic UV filters are generally considered suitable for sensitive skin and paediatric applications because of their high photostability and limited penetration through intact skin under normal conditions of use [13,14]. Their safety profile, however, depends not only on the bulk material itself, but also on particle size distribution, coating, aggregation behaviour, and route of exposure. As a result, inorganic filters must be assessed as material systems rather than as chemically simple ingredients [13].

1.6.2 Mechanisms of TiO₂ interaction with microorganisms in aquatic systems

The interaction between TiO₂ particles and microorganisms in aquatic environments is governed by a combination of interfacial surface chemistry, colloidal behaviour, and photocatalytic activity. Once dispersed in water, TiO₂ surfaces rapidly undergo hydroxylation, and the resulting surface hydroxyl groups may be protonated or deprotonated depending on the pH of the surrounding medium. This

gives rise to a pH-dependent surface charge, which in turn influences particle stability, aggregation, and interaction with biological interfaces [Hou et al., 2019].

These properties are particularly relevant because microbial cell envelopes are generally negatively charged under environmental conditions due to the presence of phosphate, carboxylate, and other ionisable groups in membrane and cell wall components. As a result, interactions between TiO₂ particles and microorganisms may be favoured or hindered depending on the balance of electrostatic forces, but also on aggregation state, ionic strength, and the adsorption of dissolved organic matter or other biomolecules onto the particle surface [He et al., 2016; Hou et al., 2019]. Such factors can substantially alter the effective surface reactivity of the particles and therefore their biological behaviour.

In addition to these interfacial processes, the semiconductor nature of TiO₂ plays a central role in its biological activity. Under irradiation, TiO₂ can generate electron-hole pairs that promote the formation of reactive oxygen species, including hydroxyl radicals, hydrogen peroxide, and related oxidative intermediates. These species may induce oxidative stress, membrane destabilisation, increased permeability, and loss of cellular integrity, thereby contributing to antimicrobial and toxic effects [Foster et al., 2011; Tsuang et al., 2008; Hou et al., 2019]. In bacterial systems, TiO₂-mediated toxicity has also been associated with membrane disruption and leakage of intracellular material, supporting the view that cell envelope damage is one of the main early events in the toxic response [Ranjan and Ramalingam, 2016].

Overall, the biological effects of TiO₂ cannot be explained solely by nominal concentration or particle size category, but should be understood as the result of multiple interacting variables, including surface charge, aggregation, medium composition, organic matter, and photoinduced oxidative reactivity. This mechanistic framework is important for interpreting the variability of TiO₂ effects across organisms, environmental matrices, and experimental systems [He et al., 2016; Hou et al., 2019].

1.6.3 Organic UV Filters

Organic UV filters, sometimes referred to as chemical filters, are carbon-based molecules designed to absorb radiation within specific wavelength ranges. Their structures typically include conjugated aromatic systems that enable efficient absorption of ultraviolet photons. Once excited, these molecules dissipate absorbed energy through non-radiative pathways such as internal conversion, vibrational relaxation, tautomerisation, or isomerisation, thereby reducing the amount of harmful radiation reaching the skin [11,34].

Each organic UV filter has a characteristic absorption profile. Some filters primarily cover the UVB region, whereas others provide protection in the UVA region, and some contribute to broad-spectrum protection when combined appropriately. Common UVB-oriented filters include cinnamates, salicylates, and triazines, while dibenzoylmethane derivatives and certain benzophenone-related compounds have historically been used for UVA protection [11,34]. Broad-spectrum performance is generally achieved by combining multiple filters with complementary spectral properties rather than relying on a single active molecule.

A major challenge associated with organic filters is photostability. Some molecules undergo photodegradation when exposed to sunlight, leading to reduced efficacy over time and, in some cases, the formation of reactive intermediates or degradation products [35,36]. Avobenzone is a classic example: although highly effective in the UVA range, it is intrinsically photolabile and requires

stabilisation through suitable filter combinations, antioxidants, or encapsulation strategies [11,35]. Modern sunscreen formulation therefore depends heavily on understanding filter–filter interactions and the broader photochemical environment of the finished product.

Organic filters are generally easier to formulate into transparent, cosmetically elegant products than inorganic filters. However, they are also the subject of continuing scientific and regulatory scrutiny related to dermal absorption, systemic exposure, phototoxicity, photoallergy, endocrine activity, and environmental fate [16,26]. These concerns have contributed to repeated reassessment of certain legacy filters and to growing interest in new-generation photostable filters, hybrid systems, and strategies that reduce the total active load while maintaining high efficacy [23,34].

1.6.4 Comparative Considerations

Inorganic and organic filters differ not only in chemical composition, but also in formulation behaviour, safety assessment, and consumer perception. Inorganic filters are valued for their broad-spectrum attenuation and photostability, yet they may present challenges related to whitening, dispersion, and particle-specific safety evaluation. Organic filters allow the design of lighter and more transparent products, but often require careful stabilisation and toxicological review [11,34].

From a functional perspective, neither class should be considered inherently superior in all contexts. The performance of a sunscreen depends on the finished formulation, including the concentration of each filter, the vehicle architecture, the distribution of the film on skin, photostability, resistance to wash-off, and interactions among active and inactive ingredients [1,35]. Regulatory evaluation therefore focuses not only on the properties of individual UV filters, but also on the efficacy and safety of the final product as marketed and used.

Consumer discourse frequently simplifies sunscreens into “chemical” versus “physical” categories, often with strong value judgements attached to each. Scientifically, however, such binary narratives are inadequate. Both classes involve complex photophysical and photobiological processes, and both may raise specific safety or environmental questions depending on context [16,39]. A more accurate perspective recognises sunscreen protection as the result of highly engineered systems rather than of a simple opposition between two ingredient families.

1.6.5 Toward Advanced Photoprotection Strategies

The limitations associated with both inorganic and organic filters have encouraged the development of advanced formulation strategies designed to improve efficacy while reducing potential risks. These strategies include optimised filter combinations, encapsulation technologies, the use of photostabilisers and antioxidants, safer-by-design material engineering, and the incorporation of auxiliary ingredients that enhance UV attenuation without acting as primary filters [19,35].

Within this broader context, the concept of sunscreen boosters has gained increasing attention. These are ingredients or systems capable of enhancing SPF or UVA performance indirectly, for example by improving film formation, increasing uniformity of UV filter distribution, stabilising active ingredients, or modifying the optical properties of the sunscreen layer [37,38]. Such approaches are particularly attractive because they may allow a reduction in total filter concentration while maintaining high levels of protection, thereby addressing both formulation efficiency and emerging safety or environmental concerns.

1.6.6 Recent Regulatory Scrutiny of UV Filters

Recent regulatory developments in the European Union have highlighted the dynamic nature of UV filter safety assessment. In particular, the potential endocrine activity of some organic UV filters, including certain benzophenone derivatives and other legacy substances, has prompted renewed evaluation by regulatory authorities and the Scientific Committee on Consumer Safety [13,23]. The European Commission has requested additional toxicological and exposure data for selected substances, and for some filters revised concentration limits have already been introduced [13].

These developments underscore the fact that UV filter regulation is not static. The safety of authorised filters continues to be reassessed in light of emerging toxicological evidence, evolving analytical methods, and changing exposure scenarios. This ongoing scrutiny has direct implications for formulation science, as manufacturers are increasingly required to reconcile photoprotective efficacy with stricter demands relating to systemic safety, environmental compatibility, and claim substantiation [12,13].

1.7 UV Filters in Sunscreen Products on the EU Market: Formulation Patterns and Market Perspective

Beyond the classification and mechanistic properties of individual UV filters, it is also important to consider how these substances are actually combined in finished sunscreen products placed on the market. Product-level evidence is particularly relevant because environmental exposure does not derive from isolated filters in the abstract, but from commercial formulations that are manufactured, marketed, applied to the skin, and ultimately released into environmental compartments during use. In this sense, the formulation architecture of sunscreen products provides an important bridge between the chemistry of UV filters and their real-world human and environmental relevance.

A useful snapshot of current sunscreen formulation practices within the European context is provided by the survey conducted by Pniewska and Kalinowska-Lis, who analysed sunscreen products marketed in Poland, considered by the authors as part of the broader EU market context [39]. Their study examined INCI compositions collected online between October and December 2023 for 200 sunscreen products, including 150 products intended for adults and 50 for children. Importantly, the analysis recorded the presence or absence of authorised UV filters on product labels rather than their concentration in the final formulation. Although this approach does not provide quantitative compositional data, it is nevertheless informative for identifying which filters are most frequently used and how manufacturers combine them in commercially available sunscreens.

The results indicate that both adult and children's sunscreens rely on a relatively limited set of recurrent UV filters. In products intended for children, the most frequently reported ingredient was bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT), present in 60.0% of formulations, followed by ethylhexyl triazone (EHT) at 52.0% and ethylhexyl salicylate (EHS) at 46.0%. Other filters with substantial representation included diethylamino hydroxybenzoyl hexyl benzoate (DHHB) at 38.0% and butyl methoxydibenzoylmethane (BMBM; avobenzone) at 36.0%. Mineral filters were comparatively more common in children's products, with titanium dioxide reported in 28.0% of formulations in non-nano form and 22.0% in nano form, while zinc oxide, including nano forms, appeared in 22.0% of products. In adult sunscreens, the most frequent filter was BMBM at 56.0%, followed closely by BEMT and EHS, each present in 54.7% of products, and EHT at 50.0%. Other widely used filters included DHHB at 38.0%, octocrylene at 36.7%, ethylhexyl methoxycinnamate at 24.0%, and phenylbenzimidazole sulfonic acid at 22.7%. In this adult product group, mineral filters were less prevalent, with titanium dioxide, including nano and non-nano forms,

reported in 21.3% of formulations and zinc oxide in 6.7% [39]. A schematic comparison of the most frequently used filters in adult and children's products is shown in Figure 7.

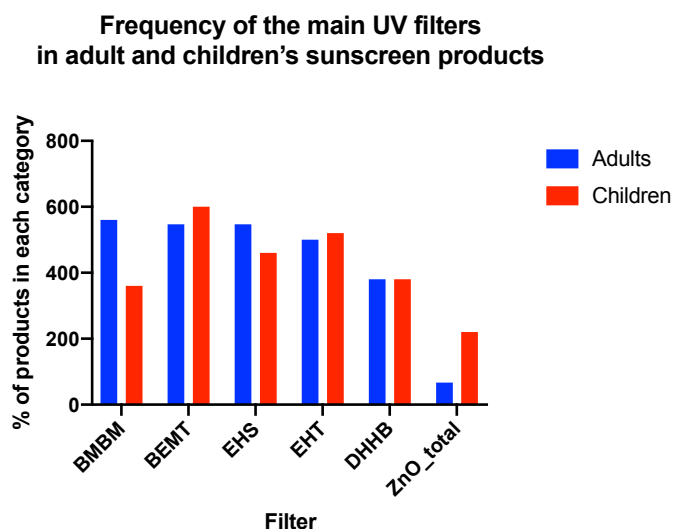


Figure 7. Frequency of the main UV filters in adult and children's sunscreen products. Graphical summary of the percentage of sunscreen products containing the most frequently reported UV filters in formulations intended for adults and children, based on products marketed in Poland and surveyed by Pniewska and Kalinowska-Lis [45]. The figure is presented as a visual synthesis of the comparative prevalence of selected filters in the two product categories.

A further point of interest in the same dataset is the concentration of formulation practices around a relatively narrow “core toolkit” of approved filters. The authors report that approximately 30% of the UV filters listed in Annex VI to Regulation (EC) No 1223/2009 did not appear in any of the tested products [39]. This suggests that, despite the broader regulatory list of authorised substances, commercial sunscreen products tend to rely repeatedly on a smaller subset of filters that are evidently favoured for reasons of efficacy, formulation compatibility, safety profile, cost, market availability, or regulatory familiarity.

The study also provides insight into formulation complexity by reporting the number of UV filters used in each product. In children's sunscreens, the largest groups contained either two UV filters or four UV filters, each accounting for 22.0% of products, whereas 18.0% contained five filters and 14.0% contained six. Adult products tended to be somewhat more complex, with the largest groups containing five UV filters (27.0%) or four UV filters (26.0%), while products with three or six filters each represented 12.7% [39]. These patterns are consistent with the functional requirement to combine filters with complementary absorption profiles in order to achieve broad-spectrum protection and high SPF performance. A visual summary of the distribution of products according to the number of UV filters per formulation is presented in Figure 8.

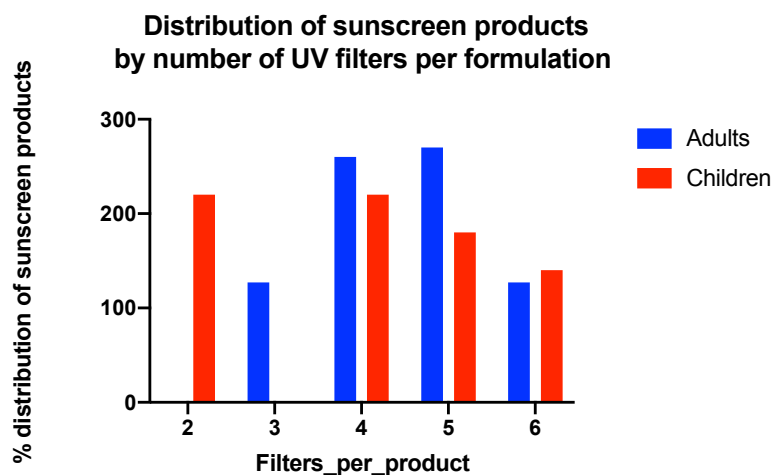


Figure 8. Distribution of sunscreen products by number of UV filters per formulation. Graphical summary of the percentage distribution of sunscreen products according to the number of UV filters declared in each formulation for adult and children's products, based on the dataset reported by Pniewska and Kalinowska-Lis [45]. The figure highlights the predominance of multi-filter systems in both product categories.

This tendency toward multi-filter design is also mechanistically meaningful. As discussed above, individual UV filters rarely provide complete and balanced protection across the entire biologically relevant UV spectrum. In practice, formulators frequently combine filters that preferentially cover UVA, UVB, or broad-spectrum regions in order to optimise overall performance. Pniewska and Kalinowska-Lis note, for example, that combinations involving avobenzone together with octocrylene, ethylhexyl salicylate, BEMT, or DHHB are commonly used to achieve broader protection and improve functional performance [39]. Interestingly, the same study also observed that many of the simplest one- or two-filter formulations were dominated by mineral filters in both adults' and children's products, and that non-nano forms appeared more frequently than nano forms within these minimal systems [39]. This observation is particularly relevant in the context of the present work, because it reinforces the idea that titanium dioxide and zinc oxide are often used within distinct formulation logics compared with multi-filter organic systems.

The SPF distribution of the surveyed products further points to a strong market orientation toward high-protection formulations. Among children's sunscreens, SPF 50 accounted for 82.0% of products, while SPF 30 represented 14.0% and SPF 20 only 2.0%. Adult products also clustered in the higher protection categories, with SPF 50 accounting for 50.0% of products, SPF 30 for 30.7%, and SPF 40 for 2.0%, whereas products labelled between SPF 6 and SPF 25 represented only 9.3% [39]. This pattern suggests that high-SPF positioning is a dominant commercial strategy in the surveyed market segment and that broad-spectrum, high-protection formulations are strongly represented in both adult and children's categories. A graphical summary of SPF distribution is shown in Figure 9.

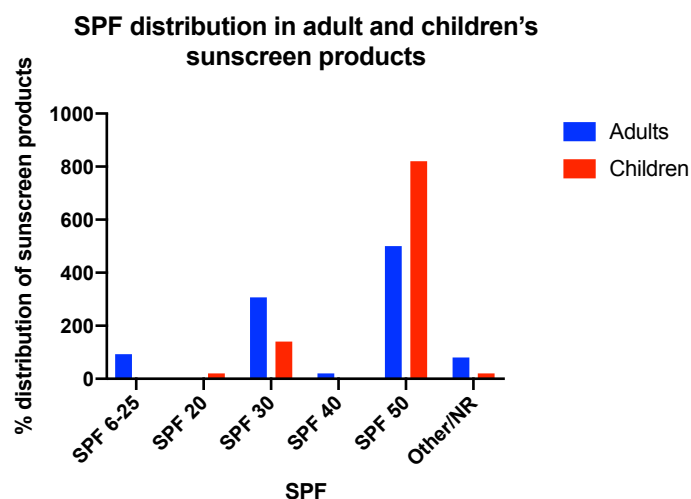


Figure 9. SPF distribution in adult and children's sunscreen products. Graphical summary of the distribution of sunscreen products across SPF categories in adult and children's formulations, based on the dataset reported by Pniewska and Kalinowska-Lis [45]. The figure shows the predominance of high-SPF products, especially in the children's category.

This finished-product evidence can be considered alongside broader ingredient-market estimates for Europe. According to a commercial market analysis by Fact.MR, the European UV filter ingredient market was valued at approximately USD 3.57 billion in 2023 and is projected to reach USD 5.77 billion by 2033, corresponding to an estimated compound annual growth rate of 4.9% over the period 2023–2033 [55]. The same source reports country-level values including Germany at USD 944.9 million in 2023 with a projected increase to USD 1.78 billion by 2033, and a projected United Kingdom demand value of USD 1.54 billion by 2033 [55]. Expressed approximately in euros, these values correspond to about EUR 3.03 billion for Europe in 2023 and EUR 4.89 billion in 2033, with Germany increasing from about EUR 800.8 million to EUR 1.51 billion, and the United Kingdom reaching about EUR 1.31 billion by 2033. A schematic overview of these market values is shown in Figure 10.

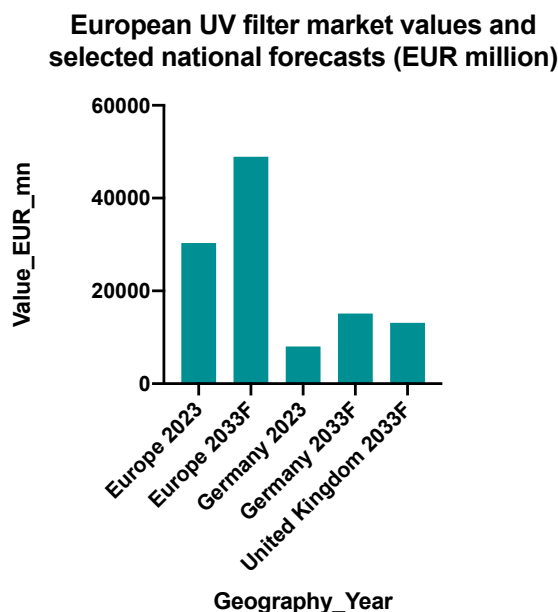


Figure 10. European UV filter market values and selected national forecasts (EUR million). Graphical overview of estimated 2023 values and forecast 2033 values for the European UV filter ingredient market, with selected national data for Germany and the United Kingdom, based on industry market estimates [61]. “F” indicates forecast.

Taken together, these data suggest that sunscreen products marketed in at least one EU sub-market are typically based on multi-filter systems, are concentrated around a relatively restricted set of frequently used UV filters, and are strongly positioned toward high-SPF protection. At the same time, the associated UV filter ingredient market in Europe appears economically substantial and is projected to continue growing. Although these sources do not allow a direct quantitative correlation between formulation architecture and market size, they nevertheless support the broader view that photoprotection in practice is shaped not only by the intrinsic properties of individual filters, but also by market preferences, formulation strategies, and the regulatory environment in which these products are developed and sold.

This context is directly relevant to the environmental evaluation of sunscreen ingredients. Aquatic exposure does not result from the abstract existence of an authorised UV filter, but from the release of specific finished formulations actually present on the market. Accordingly, environmentally relevant assessment should take into account not only the hazard profile of isolated substances, but also the formulation patterns through which those substances are combined, stabilised, applied, and washed off under realistic conditions of use.

1.8 Environmental Impact of UV Filters

The widespread and increasing use of sunscreen products has raised growing concern regarding the environmental fate and ecological effects of UV filters. Although sunscreens are widely recognised as essential tools for the prevention of ultraviolet-induced skin damage and skin cancer, their release into aquatic environments has emerged as an unintended consequence of large-scale human use. Environmental impact is therefore becoming an increasingly important dimension in the scientific and regulatory evaluation of sunscreen ingredients, alongside the traditional focus on human safety and efficacy [15,27,28].

1.8.1 Pathways of Environmental Release

UV filters may enter the environment through both direct and indirect pathways. Direct release occurs during recreational activities such as swimming, bathing, and water sports, when sunscreen ingredients are washed from the skin into marine or freshwater systems. Indirect release occurs through domestic wastewater, as filters applied during daily use are removed during showering and subsequently enter wastewater treatment systems [15,20].

Although wastewater treatment plants may partially remove certain sunscreen-derived substances, removal is often incomplete. As a result, measurable concentrations of several organic and inorganic UV filters have been detected in surface waters, sediments, and aquatic organisms in different regions of the world [15,20,28]. Coastal areas with intense tourism are considered particularly vulnerable because local concentrations may increase seasonally during periods of high sunscreen use [15,20].

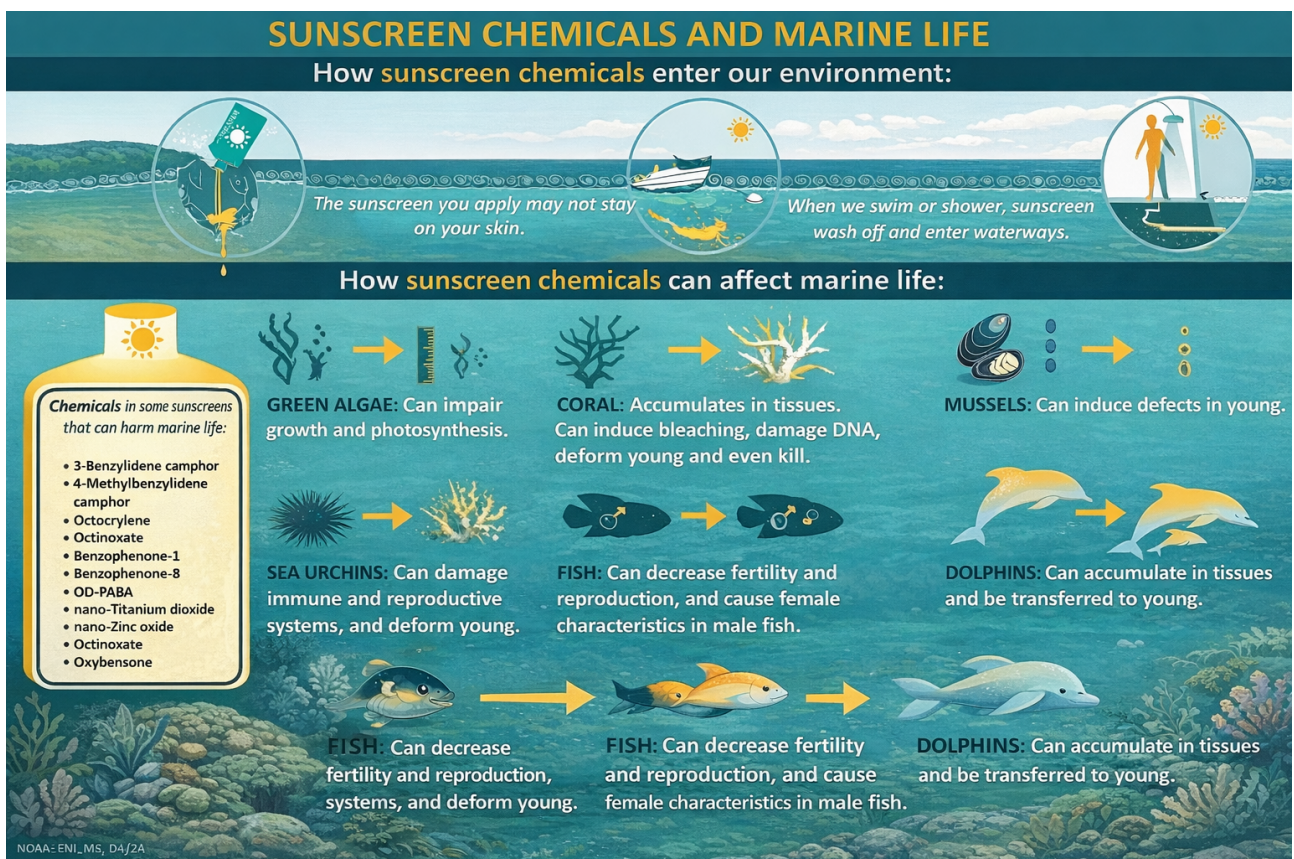


Figure 11. Pathways of sunscreen-derived UV filters into aquatic environments and their potential effects on marine organisms. Sunscreen chemicals are released into marine ecosystems through direct wash-off during recreational activities and indirect discharge via wastewater systems. Once in the aquatic environment, organic UV filters and inorganic mineral particles can disperse in the water column and interact with marine organisms. Experimental and observational studies have reported adverse effects across different taxa, including impaired growth and photosynthesis in algae, tissue accumulation and bleaching in corals, developmental alterations in invertebrates, reproductive toxicity in fish, and bioaccumulation in higher trophic levels. The figure illustrates the complexity of exposure pathways and biological responses underlying environmental risk assessment of UV filters.

1.8.2 Environmental Impact of Organic UV Filters

Organic UV filters have attracted particular attention because many of them are relatively lipophilic, may persist in environmental matrices, and may interact directly with aquatic organisms. Benzophenones, cinnamates, camphor derivatives, and related compounds have been detected in marine and freshwater environments, raising concerns about chronic exposure, sublethal toxicity, bioaccumulation, and ecosystem-level consequences [15,20].

Among the most discussed issues is the possible contribution of certain organic UV filters to coral reef damage. Experimental studies have reported adverse effects of compounds such as oxybenzone and octinoxate on coral larvae, primary coral cells, and coral-associated systems, including bleaching-like responses, DNA damage, endocrine disruption, developmental abnormalities, and altered symbiotic relationships [17,3,32]. However, the interpretation of these findings remains complex. Many laboratory studies have been conducted under controlled conditions that may not fully reproduce real environmental exposure scenarios, and the ecological relevance of reported effects may depend on local concentration, water chemistry, sunlight intensity, hydrodynamics, and co-exposure to other stressors [28,31].

Beyond corals, organic UV filters have been associated with toxic effects in microalgae, invertebrates, fish, and other aquatic organisms. Reported effects include impaired photosynthesis, oxidative stress, altered growth, reproductive disturbances, endocrine-related responses, and developmental toxicity [28,31]. These observations are especially relevant because aquatic organisms are usually exposed not to isolated substances, but to complex mixtures of filters and other contaminants, which may produce additive or synergistic effects [28].

The endocrine-active potential of certain UV filters has become a central issue in environmental risk assessment. Substances capable of interacting with hormonal signalling pathways in wildlife may produce biologically significant effects even at relatively low concentrations under chronic exposure conditions [23,28]. This dimension has strengthened calls for more comprehensive ecotoxicological and life-cycle evaluation of sunscreen ingredients.

1.8.3 Environmental Considerations Related to Inorganic UV Filters

Inorganic UV filters, primarily titanium dioxide and zinc oxide, have often been presented as environmentally preferable alternatives to some organic filters because of their mineral nature and low solubility. However, the increasing use of micronised and nanoparticulate forms has raised new questions regarding their environmental behaviour and ecological impact [7,27].

Unlike organic filters, inorganic UV filters enter aquatic systems as particulate materials. Their environmental fate is therefore governed by particle-specific properties such as size, crystalline structure, aggregation state, surface coating, sedimentation behaviour, and interactions with natural organic matter [27]. Once released, these particles may remain dispersed in the water column, aggregate, settle into sediments, undergo partial dissolution, or interact with biotic and abiotic surfaces depending on environmental conditions [7,27].

One important concern relates to photocatalytic activity. Under irradiation, certain forms of TiO₂ and ZnO may generate reactive oxygen species at the particle surface, potentially inducing oxidative stress in exposed organisms [7,27]. The extent of this effect depends strongly on particle properties and formulation design. Surface-treated particles designed for cosmetic use may differ substantially

from pristine laboratory-grade materials, and these differences must be taken into account when interpreting ecotoxicological studies [27].

Experimental work has reported adverse effects of inorganic particles on algae, invertebrates, and fish, including growth inhibition, impaired photosynthesis, oxidative damage, developmental effects, and altered physiological function [7,27]. However, the hazard profile of inorganic filters is highly context-dependent, and broad generalisations about their environmental safety should be avoided. Their ecological effects may differ substantially according to whether they are present in bulk, micronised, or nanoscale forms, and whether they are coated, aggregated, or embedded in a complex formulation matrix.



Figure 12. Environmental fate and ecological impact of physical (inorganic) sunscreen filters. Zinc oxide (ZnO) and titanium dioxide (TiO₂), used as physical UV filters in both nanoparticulate and bulk forms, enter marine environments through direct release during recreational activities and indirect discharge via wastewater systems. Once released, these particles undergo dispersion, aggregation, dissolution, and sedimentation processes, which influence their bioavailability and environmental behavior. Physical UV filters may induce oxidative stress and photosynthesis inhibition in primary producers such as microalgae, and contribute to developmental and reproductive toxicity in invertebrates and fish. The figure illustrates potential pathways leading to bioaccumulation and biomagnification across aquatic trophic levels, highlighting the importance of particle characteristics and environmental conditions in determining ecological risk.

1.8.4 Regulatory and Ecological Perspectives

Growing awareness of the environmental dimension of photoprotection has led to increased regulatory and societal attention. In some jurisdictions outside the European Union, local restrictions or bans have been introduced for selected organic UV filters, particularly in ecologically sensitive coastal regions. These measures are generally precautionary in nature and have been motivated largely by concern for coral reef conservation [17,28].

Within the European context, environmental risk remains institutionally separate from core cosmetic safety evaluation, which is primarily focused on human health. Nevertheless, the broader policy environment is moving toward greater integration of sustainability principles, chemical risk reduction, and responsible product design. As a result, environmental considerations are increasingly influencing scientific discussion, consumer expectations, and formulation strategies even when they are not yet fully embedded in cosmetic regulatory approval pathways [21,22,28].

This shift has also intensified scrutiny of environmental marketing claims. Terms such as “reef-safe,” “reef-friendly,” or “ocean-friendly” are widely used in commercial communication, yet they lack harmonised scientific or legal definitions and may therefore be misleading [21–25]. The environmental profile of a sunscreen cannot be reduced to the absence of one or two controversial filters, because ecological impact depends on multiple variables, including formulation context, environmental concentration, transformation products, persistence, and route of release. Accordingly, simplified environmental claims may contribute to greenwashing rather than informed consumer choice [21,22].

1.8.5 Implications for Future Sunscreen Development

The growing body of evidence concerning the environmental fate of UV filters has important implications for future sunscreen development. Formulators are increasingly encouraged to reduce environmental release through more substantive films, lower total filter loads, safer-by-design materials, encapsulation technologies, biodegradable excipients, and improved water-resistance strategies [27,28,37]. At the same time, these innovations must not compromise the primary public health objective of preventing UV-induced skin damage and skin cancer.

Future progress will likely require a more integrated approach to sunscreen design, combining photoprotective efficacy, human safety, and environmental sustainability. This may include the development of new filters with improved ecotoxicological profiles, more realistic ecotoxicological testing frameworks, and stronger alignment between formulation science and environmental risk

assessment [27,28]. In this context, understanding the environmental impact of UV filters is not secondary to sunscreen science; it is becoming one of its defining contemporary challenges.

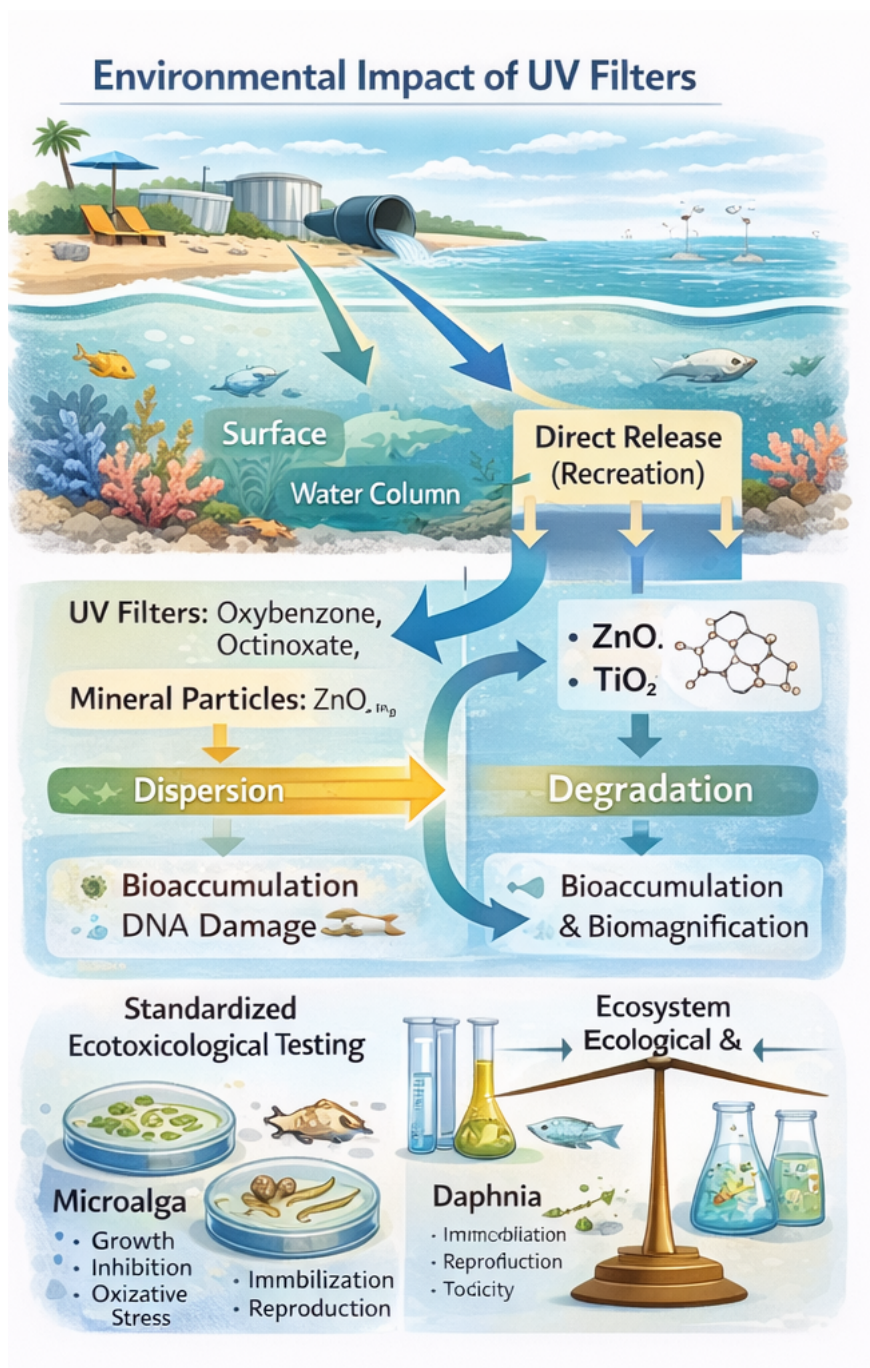


Figure 13. Environmental pathways and ecological impact of UV filters released from sunscreen products. UV filters enter aquatic ecosystems through direct release during recreational activities and indirect discharge via wastewater systems. Once in the water column, organic UV filters (e.g., oxybenzone and octinoxate) and inorganic mineral particles (e.g., zinc oxide and titanium dioxide) undergo dispersion, transformation, and degradation processes. These substances may accumulate in aquatic organisms, leading to bioaccumulation and, in some cases, biomagnification across trophic levels. The figure also illustrates standardized ecotoxicological testing approaches using representative aquatic organisms, such as microalgae and invertebrates, to assess growth inhibition, oxidative stress, immobilization, and reproductive effects, providing a basis for environmental risk assessment.

1.9 “Reef-Safe” Sunscreens: Marketing Claims versus Scientific Evidence

Growing public concern regarding the environmental effects of sunscreen ingredients has led to the rapid diffusion of terms such as “reef-safe,” “reef-friendly,” and similar expressions in commercial communication, retail positioning, and tourism-related guidance. Although these labels resonate strongly with environmentally conscious consumers, they remain scientifically and legally problematic. At present, such terms are not supported by harmonised definitions within major cosmetic regulatory frameworks, and their use often exceeds the level of certainty warranted by the available evidence [21–25].

In commercial practice, the term “reef-safe” is commonly used to describe products that exclude a limited number of specific organic UV filters, most notably oxybenzone and octinoxate. This approach derives largely from laboratory studies reporting adverse biological effects of selected filters on corals and other marine organisms under controlled experimental conditions [17,31]. These studies have contributed substantially to public awareness and to local policy responses in environmentally sensitive areas. However, the absence of one or two specific filters cannot, by itself, be equated with overall environmental safety. The ecological impact of a sunscreen depends on multiple variables, including the complete formulation, the environmental concentration achieved after release, degradation pathways, transformation products, particle behaviour, and interactions with other pollutants and stressors [24,28].

The scientific interpretation of the available ecotoxicological evidence remains complex. Laboratory studies often employ simplified exposure systems that may not reflect the dynamic conditions of natural aquatic environments, where dilution, sunlight-driven transformation, sediment interactions, organic matter binding, hydrodynamics, and mixture effects influence actual exposure and toxicity [28,31]. In addition, much of the public discourse surrounding reef protection focuses narrowly on a few organic filters, whereas inorganic filters, including titanium dioxide and zinc oxide in nano- or microstructured forms, may also raise environmentally relevant questions depending on particle characteristics, coatings, aggregation behaviour, and test conditions [9,30]. As a result, the binary distinction between “harmful chemical filters” and “safe mineral filters” is scientifically reductive. From a regulatory standpoint, environmental claims relating to sunscreen products must be truthful, clear, substantiated, and non-misleading [21,22,23]. Broad expressions implying environmental harmlessness are difficult to justify, especially when the underlying evidence base is incomplete, context-dependent, or methodologically heterogeneous. The use of terms such as “reef-safe” may therefore create a false impression of ecological neutrality and may contribute to greenwashing rather than informed consumer decision-making [21,24,25]. This issue is particularly significant in the sunscreen sector, where the public health need for effective photoprotection must be balanced against legitimate environmental concerns.

The popularity of “reef-safe” claims also reflects a broader tension between scientific complexity and consumer communication. Consumers often favour simple binary categories, whereas environmental risk assessment requires nuanced interpretation of incomplete and evolving evidence [23,28]. In the case of sunscreen products, this tension is intensified by the coexistence of urgent skin cancer prevention needs, ecological anxieties, and highly competitive marketing practices. As a result, environmental messaging may become detached from the actual state of scientific knowledge.

A more rigorous approach would avoid simplistic terminology and instead promote transparent communication grounded in measurable and verifiable criteria. This may include better disclosure of formulation choices, more realistic ecotoxicological testing strategies, life-cycle considerations, and

stronger alignment between environmental claims and substantiated evidence [24,28,33]. Within this perspective, the problem is not merely whether a sunscreen excludes a particular ingredient, but whether its overall design is compatible with both effective human photoprotection and reduced ecological burden.

The debate surrounding “reef-safe” sunscreens therefore illustrates a broader challenge in contemporary photoprotection: the need to reconcile public health, environmental stewardship, regulatory integrity, and scientific uncertainty. Rather than relying on loosely defined marketing claims, future progress in this area will depend on more robust environmental assessment methods, clearer regulatory standards, and formulation strategies capable of addressing ecological concerns without compromising sunscreen efficacy [23,28]

1.10 Formulation Science of Modern Sunscreen Products: Stability, Efficacy, Safety and Environmental Performance

Sun protection products represent one of the most scientifically complex categories in cosmetic formulation. Their design must simultaneously satisfy demanding requirements for photoprotective efficacy, physicochemical stability, toxicological safety, consumer acceptability, regulatory compliance, and, increasingly, environmental sustainability. Modern sunscreen formulation science has therefore evolved far beyond simple oil-based protective films, incorporating sophisticated multi-phase systems, combinations of organic and inorganic UV filters, advanced rheological architectures, and a growing range of delivery technologies aimed at improving film formation, substantivity, and photostability [11,34].

1.10.1 Functional Requirements of a Sunscreen Formulation

A sunscreen formulation is not merely a vehicle for UV filters; it is a functional delivery system whose purpose is to distribute active ingredients uniformly across the skin and maintain a coherent protective film during sun exposure. To achieve this objective, a sunscreen must satisfy several interdependent criteria. First, UV filters must be adequately solubilised or dispersed within the selected vehicle. Second, the formulation must remain physically and chemically stable over time, resisting phase separation, crystallisation, hydrolysis, and oxidative degradation. Third, the product must retain photostability during use, particularly when it contains filters known to undergo photolysis or structural rearrangement under irradiation. Fourth, the sunscreen film must spread evenly, adhere adequately to the skin, and resist removal by water, sweat, and friction. Finally, the formulation must maintain an acceptable toxicological profile and, increasingly, should minimise unnecessary environmental release of active materials [11,34,35].

These requirements are often in tension with one another. A formulation designed to resist wash-off strongly may improve photoprotective persistence on skin, yet the same properties may alter sensorial profile or complicate removal. Likewise, lipophilic filter systems may improve substantivity and film integrity while also influencing dermal distribution and environmental behaviour. Sunscreen design is therefore best understood as a process of optimisation across multiple performance domains rather than the pursuit of a single ideal property [34].

1.10.2 Formulation Challenges of UV Filters

Both organic and inorganic UV filters present significant formulation challenges. Organic filters may be limited by poor solubility, crystallisation tendencies, photoinstability, and undesirable interactions with other filters or excipients. Their efficacy depends not only on intrinsic absorption characteristics, but also on the microenvironment created by the formulation matrix [40,41]. Certain filters, such as avobenzone, are particularly sensitive to photodegradation and therefore require stabilisation through filter combinations, antioxidants, encapsulation, or other formulation strategies [40].

Inorganic filters such as titanium dioxide and zinc oxide are highly photostable and can provide broad-spectrum attenuation, but they present different challenges. These include dispersion instability, agglomeration, whitening on skin, and, for some particle forms, the risk of photocatalytic reactive oxygen species generation [14,34]. Surface treatments and coatings are therefore often applied to modify their physicochemical behaviour, reduce photocatalytic activity, and improve compatibility with the continuous phase of the formulation [14,34]. In practical terms, the performance of mineral sunscreens is determined not only by the identity of the active ingredient, but also by particle engineering and the rheological design of the system in which those particles are suspended.

1.10.3 Emulsions and Vehicle Architecture

Most commercial sunscreens are formulated as emulsions, typically oil-in-water or water-in-oil systems. Vehicle architecture plays a decisive role in determining filter distribution, film formation, skin feel, wash resistance, and overall product performance [34,39]. Oil-in-water emulsions generally offer lighter textures, improved spreadability, and greater cosmetic elegance, whereas water-in-oil systems often provide greater substantivity and water resistance because of their more lipophilic external phase [39].

The structure of the emulsion also affects the release and stability of UV filters. Droplet size distribution, interfacial properties, phase viscosity, and the presence of film-forming agents or structuring waxes influence how the sunscreen spreads across the microrelief of the skin and how uniformly active materials are deposited [37,39]. Because in vitro and in vivo sunscreen performance depends critically on the integrity of this film, formulation design must account not only for ingredient compatibility in bulk, but also for film behaviour after application.

Rheological modifiers are especially important in this context. Polymers and structuring agents can improve stability, increase resistance to sedimentation, enhance adherence to the skin, and influence how the sunscreen layer resists deformation during movement, perspiration, or water exposure [37,39]. Small changes in rheology may therefore alter SPF, water resistance, and filter release behaviour even when the nominal active composition remains unchanged.

1.10.4 Nanoemulsions and Nanoscale Delivery Systems

Nanoemulsions and other nanoscale delivery systems have attracted increasing attention in sunscreen formulation because they may improve the homogeneity of UV filter distribution, increase formulation transparency, and enhance spreadability [46,47]. By reducing droplet size, these systems can promote the formation of more uniform films and may increase in vitro SPF values under certain conditions [46]. They may also facilitate incorporation of poorly soluble filters and improve sensorial properties.

However, nanoscale systems are not automatically superior to conventional emulsions. Their performance depends on careful optimisation of surfactant systems, droplet size distribution, interfacial composition, and long-term physical stability [46,47]. In addition, nanoemulsions may raise specific questions concerning increased active mobility, altered skin interaction, regulatory classification, and, in some cases, environmental behaviour [47]. Their advantages therefore need to be evaluated critically rather than assumed.

1.10.5 Photostability and Filter–Filter Interactions

Photostability is a central requirement for modern sunscreen performance. A sunscreen may display excellent initial absorption characteristics, yet still fail to provide reliable protection if key filters degrade significantly during exposure. The photostability of a formulation depends not only on the intrinsic behaviour of individual filters, but also on the interactions between filters, antioxidants, solvents, polymers, and other excipients [35,36].

Some filter combinations are strongly synergistic. A photolabile UVA filter may be stabilised by another filter capable of quenching excited states or reducing degradation pathways. Conversely, some combinations may accelerate photodecomposition or reduce overall effectiveness [40,41]. The sunscreen formulation must therefore be assessed as an integrated photochemical system. This is one reason why efficacy and stability cannot be predicted reliably from the simple presence of individual filters on an ingredient list.

Encapsulation technologies, antioxidant systems, and polymer-assisted film formation have all been explored as means of improving photostability. These strategies may reduce filter mobility, isolate reactive moieties, suppress degradation pathways, or improve the persistence of a homogeneous film under irradiation [37,38]. Such approaches are increasingly important as formulators seek to maintain high broad-spectrum efficacy while limiting total filter concentration and avoiding unstable filter combinations.

1.10.6 Analytical Approaches to Sunscreen Evaluation

Contemporary formulation research relies on a broad range of analytical techniques to evaluate sunscreen performance. These include chromatographic methods to quantify active ingredient stability under stress conditions, dynamic light scattering and related tools to characterise particle or droplet size, spectrophotometric approaches to assess in vitro UV protection, and standardised water-resistance protocols to estimate film persistence [35,40,41]. In addition, optical and microscopic methods may be used to study film distribution on skin or surrogate substrates.

Such methods are important because sunscreen performance is multidimensional. A formulation may show satisfactory filter stability in bulk yet perform poorly after application because of inadequate spreading or wash-off resistance. Conversely, a cosmetically elegant product may spread well but lack sufficient photostability or broad-spectrum balance. Analytical integration is therefore essential for linking formulation structure to real-world performance [34].

1.10.7 Environmental Performance and Safer-by-Design Strategies

Environmental considerations are now influencing sunscreen formulation at an increasingly fundamental level. Traditional product development focused primarily on efficacy, safety, and aesthetics, whereas contemporary approaches increasingly aim to reduce environmental release, improve biodegradability of non-active components, and limit the ecotoxicological burden associated with active filters [27,28].

Safer-by-design strategies may involve the use of coated inorganic particles with reduced photocatalytic activity, more substantive vehicle architectures that limit wash-off, optimised filter combinations that reduce total active load, biodegradable emollients and structuring agents, and encapsulation systems that control filter mobility [27,37]. These approaches do not eliminate the need for ecotoxicological assessment, but they can contribute to lowering environmental exposure while preserving product performance.

Importantly, environmental compatibility should not be reduced to simplistic claims or to the mere exclusion of one or two controversial ingredients. A sunscreen's environmental profile is shaped by the complete formulation, its behaviour during use, its release into environmental matrices, and the persistence or transformation of its components after release [23,28]. Formulation science is therefore central to the development of genuinely more sustainable photoprotection systems.

1.10.8 Toward Integrated Sunscreen Design

The future of sunscreen formulation lies in integrated optimisation. High-performing photoprotective systems increasingly depend on the synergy between UV filter chemistry, vehicle architecture, rheology, film formation, photostability, and environmental design [11,34]. This integrated perspective reflects a broader shift in cosmetic science: sunscreens are no longer evaluated solely as products that reduce sunburn, but as multifunctional systems expected to deliver balanced broad-spectrum protection, high user compliance, acceptable safety margins, and reduced ecological impact. Emerging trends include hybrid organic–inorganic systems, advanced coating technologies, booster ingredients that enhance SPF indirectly, biomimetic approaches inspired by natural photoprotective systems, and predictive tools such as *in silico* formulation modelling and data-driven optimisation [37,38]. These developments suggest that sunscreen formulation science is becoming increasingly interdisciplinary, situated at the intersection of colloid science, photochemistry, toxicology, dermatology, and environmental assessment.

In this context, formulation science provides the essential bridge between the theoretical principles of photoprotection and the practical performance of sunscreen products in real-world use. It is also highly relevant to the aims of the present study, as understanding the environmental behaviour of TiO₂-based sunscreen systems requires attention not only to the active material itself, but also to the formulation matrix through which that material is delivered, stabilised, and potentially released into aquatic environments.

1.11 Aim of the Study

The overarching aim of this study is to advance the understanding of the environmental behaviour and ecological safety of titanium dioxide-based sunscreen formulations by developing and applying

an ecotoxicology-oriented assessment framework that more closely reflects realistic exposure scenarios in aquatic environments.

Despite the widespread use of sunscreens as an essential strategy for preventing UV-induced skin damage and skin cancer, increasing attention has been directed toward the environmental fate of UV filters released from cosmetic products during normal use. While the ecological effects of several organic UV filters have been widely debated, inorganic filters, particularly titanium dioxide in micro- and nano-structured forms, are also extensively used in modern sunscreen formulations and may enter freshwater and marine systems through direct and indirect pathways. However, their environmental behaviour and biological effects remain insufficiently characterised, especially when considered within the context of complete formulations rather than as isolated raw materials [15,27,28].

A major limitation of much of the existing literature is that ecotoxicological evaluation has often focused on pristine active ingredients tested under simplified laboratory conditions, whereas environmental exposure in real life occurs through the release of substances from finished formulations into complex aqueous matrices. This discrepancy may lead to an incomplete or misleading representation of actual ecological risk. The present study is therefore designed to address this gap by moving from ingredient-centred testing toward a formulation-aware and exposure-relevant approach.

Specifically, the objectives of the study are as follows:

To develop and implement a leachate-based exposure methodology capable of simulating the release of titanium dioxide from sunscreen formulations under conditions intended to approximate realistic wash-off during human use.

To evaluate the ecotoxicological effects of both micro-sized and nano-sized TiO₂-based UV filters, tested as isolated active ingredients and as components of complete sunscreen formulations, in representative freshwater and marine test systems.

To apply a multi-species, multi-endpoint testing strategy including bacteria, phytoplankton, and crustaceans in order to assess acute and sub-acute biological responses such as bioluminescence inhibition, growth inhibition, immobilisation, and behavioural alteration.

To quantify titanium concentrations in formulation-derived leachates and relate these measurements to observed biological effects, thereby improving the interpretation of ecotoxicological outcomes in relation to actual material release.

To contribute to the development of a more standardised and environmentally relevant framework for the assessment of inorganic sunscreen filters, with potential implications for risk assessment, formulation design, and safe and sustainable by design approaches in cosmetic science.

By integrating realistic release scenarios with ecotoxicological testing across different trophic levels and environmental compartments, this study seeks to generate scientifically robust evidence on the environmental behaviour of TiO₂-based sunscreen systems. The ultimate goal is to support the development of sunscreen products that better reconcile effective human photoprotection with the protection of aquatic ecosystems.

2. Materials and Methods

2.1 Chemicals and Test Materials

Two commercially available titanium dioxide (TiO₂)-based ultraviolet filters were selected as representative inorganic sunscreen actives with different physicochemical characteristics. Parsol® TX (CAS No. 13463-67-7; DSM Nutritional Products) consisted of rutile-phase titanium dioxide (82-

87%) coated with silicon dioxide (10.5-14.5%) and was supplied as a fine powder with particle sizes below 1000 μm . Aerodisp® W740X (CAS No. 13463-67-7; Evonik Industries) was an anatase-phase TiO_2 formulation supplied as a milky white aqueous dispersion containing 40% (w/w) titanium dioxide, with a density of 1.41 g/cm^3 and a reported mean aggregate size of ≤ 100 nm.

A stock suspension of Parsol® TX was prepared by dispersing the powder in ethanol under controlled conditions to ensure adequate homogenisation. The final ethanol concentration in test solutions did not exceed 0.02% (v/v) at the highest tested concentration and was considered non-toxic for the test organisms employed. Aerodisp® W740X was used as supplied and diluted directly in the relevant exposure media. All working test solutions were prepared by dilution of stock materials with either filtered natural seawater (salinity 37‰) or artificial freshwater, depending on the test organism and assay conditions.

2.2 Preparation of Sunscreen Cream Leachates

Experimental sunscreen formulations were provided by AHAVA Dead Sea Laboratories and manufactured in compliance with Good Manufacturing Practices. Two formulations contained TiO_2 -based UV filters at a final concentration of 5% (w/w), using either Parsol® TX or Aerodisp® W740X as the active ingredient. A control formulation without titanium dioxide (hereafter referred to as blank cream) was included in order to evaluate possible effects attributable to the cosmetic matrix itself.

To generate formulation-derived leachates, approximately 72 mg of each cream was uniformly applied to a synthetic skin substrate measuring 6×6 cm, selected to simulate sunscreen application on the skin surface. The coated substrate was then immersed in the appropriate exposure medium to allow release of cream components into the aqueous phase. Leachates were tested either undiluted or after 1:6 dilution, corresponding to final cream concentrations of 72 mg/L and 12 mg/L, respectively. Under the assumption of complete release, these conditions corresponded to nominal TiO_2 concentrations of 3.6 mg/L and 0.6 mg/L.

The experimental design was intentionally conservative and represented a worst-case exposure scenario, assuming complete transfer of the applied cream from the substrate to the surrounding aqueous medium. This approach was adopted to estimate the upper boundary of potential environmental exposure and toxicity associated with sunscreen-derived TiO_2 .

2.3 Ecotoxicological Exposure Design

Ecotoxicological testing was designed to evaluate the biological effects of titanium dioxide in both micro-sized and nano-sized forms, examined either as isolated active ingredients or as leachates obtained from finished sunscreen formulations. All assays were conducted in accordance with standardised national or international methods in order to ensure methodological robustness, comparability, and regulatory relevance.

The test battery was selected to encompass multiple trophic levels and both marine and freshwater environments. Test organisms included the marine luminescent bacterium *Aliivibrio fischeri*, the marine diatom *Phaeodactylum tricornutum*, the freshwater green alga *Raphidocelis subcapitata*, the marine crustaceans *Amphibalanus amphitrite* and *Artemia franciscana*, and the freshwater cladoceran *Daphnia magna*. This multi-species approach was intended to capture a range of ecologically relevant sensitivities and biological endpoints.

Although some of the selected invertebrate species are considered relatively tolerant compared with highly sensitive algal endpoints, they are widely recognised as standard organisms for ecotoxicological evaluation. In particular, *Daphnia magna* remains one of the most commonly used

model species in environmental hazard assessment because of its well-established test protocols, ecological relevance, ease of laboratory maintenance, and high interlaboratory reproducibility. Test solutions consisted of serial dilutions of Parsol® TX and Aerodisp® W740X, together with undiluted and diluted sunscreen cream leachates, including the blank cream control. All assays were performed in triplicate. Negative and, where appropriate, solvent controls were included in each experimental set.

2.3.1 Bacterial Bioluminescence Inhibition Assay

Acute toxicity was evaluated using the marine luminescent bacterium *Aliivibrio fischeri* according to the UNI EN ISO 11348-3 standard method. Lyophilised bacterial cultures were stored at -20 °C and reactivated immediately before use by hydration according to the manufacturer's instructions.

Bioassays were conducted in triplicate by exposing *A. fischeri* to a concentration range of Aerodisp® W740X (0.1, 1, 14.1, 141, and 1410 mg/L), Parsol® TX (0.1, 1, 12.5, and 50 mg/L), and sunscreen cream leachates obtained from blank cream, Parsol® TX cream, and Aerodisp® W740X cream at 100% and 16.6%. During exposure, samples were maintained at 15 °C on a thermostatically controlled plate.

A sodium chloride solution served as the negative control, and ethanol was used as the solvent control where relevant. After 30 min of exposure, bacterial bioluminescence was measured using a Microtox® M500 luminometer. Toxicity was expressed as the effective concentration causing 50% inhibition of bioluminescence (EC_{50}) relative to the negative control, where calculable.

2.3.2 Phytoplankton Growth Inhibition Assays

Growth inhibition tests were performed using the marine diatom *Phaeodactylum tricornutum* and the freshwater green alga *Raphidocelis subcapitata* in accordance with ISO 10253 and OECD 201 guidelines, respectively. The protocols were adapted to a 24-well plate format to facilitate replication and sample handling.

Cultures were exposed in triplicate to serial dilutions of Aerodisp® W740X (1.41, 14, and 141 mg/L), Parsol® TX (0.0001 to 50 mg/L), and formulation-derived cream leachates at 100% and 16.6%, including the blank cream control. Exposures were carried out for 72 h at 20 ± 0.5 °C under a 12:12 h light:dark photoperiod, with light intensities between 6000 and 10,000 lux.

The negative controls consisted of the relevant algal growth media, while ethanol was used as solvent control where applicable. At the end of the exposure period, algal growth was arrested by addition of Lugol's solution. Cell densities were determined using an inverted microscope and a Bürker counting chamber. Growth inhibition was calculated by comparing cell density in treated samples with that of the negative control.

2.3.3 Zooplankton Bioassays

Acute and sublethal responses were assessed in the marine crustaceans *Amphibalanus amphitrite* and *Artemia franciscana*, as well as in the freshwater crustacean *Daphnia magna*. *A. amphitrite* stage II nauplii and *A. franciscana* Instar I larvae were obtained under controlled laboratory conditions according to established culture procedures. *D. magna* neonates were obtained using standardised commercial or laboratory culture systems.

Bioassays with *A. amphitrite* were conducted according to the relevant UNICHIM standardised procedure, whereas tests with *A. franciscana* followed ISO TS/20787 guidance. Organisms were exposed in triplicate in multiwell plates containing 1 mL of test solution per well, with 10-15 organisms per well. Test solutions included serial dilutions of Aerodisp® W740X (1.41, 14.1, 141, and 1410 mg/L), Parsol® TX (0.0001 to 50 mg/L), and sunscreen cream leachates at 100% and 16.6%, including the blank cream.

Plates were incubated for 48 h in darkness at 20 ± 0.5 °C for *A. amphitrite* and 25 ± 0.5 °C for *A. franciscana*. At the end of the exposure period, immobile organisms were counted under a stereomicroscope. Immobility was defined as the absence of any movement and expressed as a percentage relative to the negative control.

In addition to immobility, swimming speed alteration was measured as a sublethal behavioural endpoint. Swimming behaviour was recorded for 3 s in darkness using a Swimming Behaviour Recorder system, and average swimming speed was compared between treated and control groups. Swimming speed alteration was expressed as the percentage difference relative to controls.

Immobility tests with *Daphnia magna* were conducted according to ISO 6341 and the procedure provided by the commercial Daphtoxkit F™ system. Assays were performed in triplicate using multiwell plates containing 9 mL of test solution and five neonates per well. Plates were incubated for 48 h at 20 ± 0.5 °C in darkness. Immobility was defined as absence of movement for at least 15 s after gentle agitation and was expressed relative to the negative control.

2.4 Titanium Quantification in Cream Leachates by ICP-MS

Total titanium concentration in undiluted cream leachates was determined by inductively coupled plasma mass spectrometry (ICP-MS) in order to assess the extent of titanium release from the sunscreen formulations into the aqueous exposure media. Analyses were performed on leachates prepared from the blank cream, the Parsol® TX-containing cream, and the Aerodisp® W740X-containing cream in the different media used for ecotoxicological testing, namely natural seawater, marine algal medium, freshwater algal medium, and *Daphnia* freshwater medium.

Before analysis, samples were collected from the aqueous phase of the leachates and prepared according to standard ICP-MS requirements. Titanium concentrations were quantified against appropriate calibration standards and expressed as µg/L of total titanium. Where measured concentrations approached the lower analytical range, values were interpreted cautiously in relation to the method quantification limits.

Because the aim of this analysis was to estimate the amount of titanium detectable in the aqueous phase under the selected leaching conditions, the measurements were used as an indicator of formulation-derived titanium transfer rather than as a direct measure of bioavailable titanium. Accordingly, ICP-MS data were interpreted in combination with physicochemical observations and ecotoxicological responses

2.5 Statistical Analysis

Ecotoxicological and physicochemical data are expressed as mean $\pm$ standard error. Statistical analyses were performed using GraphPad Prism 5. Concentration–response relationships were modelled by nonlinear regression using a log agonist versus normalised response approach with variable slope, and EC₅₀ values were calculated where appropriate.

One-way analysis of variance followed by Dunnett's multiple comparison test was used to identify statistically significant differences between treated samples and controls and to derive no observed effect concentration and lowest observed effect concentration values where applicable. For each species, two-way analysis of variance followed by Bonferroni post hoc testing was used to evaluate the effects of cream leachate type and dilution on the measured biological endpoints.

3. Results

3.1 Titanium Concentration in Formulation-Derived Leachates

As a preliminary analytical step, total titanium concentrations released from the sunscreen formulations into the aqueous media used for ecotoxicological testing were determined in order to evaluate the extent of Ti transfer from the cosmetic matrix to the exposure systems and to support interpretation of the biological data.

Titanium quantification was performed by inductively coupled plasma mass spectrometry (ICP-MS). Leachates derived from the blank cream, the Aerodisp® W740X-containing cream, and the Parsol® TX-containing cream were analysed in four aqueous media representative of the ecotoxicological exposure conditions: natural seawater, marine algal medium, freshwater algal medium, and *Daphnia* freshwater medium.

Across most tested media, measured titanium concentrations were low, generally ranging from approximately 1 to 8.6 µg/L. These values were close to the analytical limit of quantification, indicating that only a minor fraction of the nominally applied TiO₂ was detectable in the aqueous phase under the selected exposure conditions. In the *Daphnia* freshwater medium, titanium concentrations were consistently below or near the lower quantification range for all formulations, including the blank cream, suggesting either negligible detectable Ti release in this matrix or matrix-dependent analytical constraints affecting titanium determination.

Some medium-dependent variation was observed. Media characterised by higher ionic strength, such as seawater and marine algal medium, generally showed slightly higher detectable titanium concentrations than freshwater systems. The freshwater algal medium showed the greatest difference between the blank cream and the Aerodisp® W740X-containing cream, whereas seawater showed the largest difference between the blank cream and the Parsol® TX-containing cream.

Despite these differences, all measured titanium concentrations remained several orders of magnitude lower than the theoretical nominal concentration corresponding to complete release of the applied TiO₂. Overall, these data indicate limited transfer of titanium from the sunscreen formulations into the aqueous phase under the simulated exposure conditions.

Table 1. Total titanium concentration measured in undiluted cream leachates prepared in the aquatic media used for ecotoxicological testing.

EXPOSURE MEDIUM	BLANK CREAM (µG/L)	AERODISP® CREAM (µG/L)	W740X	PARSOL® TX CREAM (µG/L)
SEAWATER	3.3	2.5		6.4
MARINE ALGAL MEDIUM	5.1	5.5		6.3
DAPHNIA FRESHWATER MEDIUM	0.28	1.0		0.3
FRESHWATER ALGAL MEDIUM	1.2	8.6		1.3

3.2 Ecotoxicological Assessment

The ecotoxicological assessment combined standardised bioassays across multiple trophic levels in order to evaluate the responses of representative aquatic organisms exposed to both isolated TiO₂ actives and sunscreen-derived leachates. Endpoints included bacterial bioluminescence inhibition, phytoplankton growth inhibition, zooplankton immobility, and swimming speed alteration.

For clarity, concentration–response relationships were analysed using the full range of tested concentrations for each assay. EC₅₀ values were calculated where the response reached the required threshold within the measurable range. Where this threshold was not reached, no EC₅₀ was determined.

3.2.1 EC₅₀ Values for TiO₂ Active Ingredients and Cream Leachates

Concentration–response analysis revealed marked differences among species and endpoints, as well as between isolated TiO₂ actives and complete sunscreen formulations.

For the bacterial endpoint, no EC₅₀ values were determined for either Aerodisp® W740X or Parsol® TX within the concentration ranges evaluated. In the case of Aerodisp® W740X, concentrations above 141 mg/L could not be tested in the bacterial and phytoplankton assays because increasing opacity of the dispersion interfered with reliable photometric measurement. For Parsol® TX, concentrations above 50 mg/L were not tested because of solvent-related limitations. Within the tested range, inhibition of *Aliivibrio fischeri* bioluminescence never reached the 50% threshold required for EC₅₀ calculation.

Among the phytoplankton assays, EC₅₀ values were obtained only for Parsol® TX. The marine diatom *Phaeodactylum tricornutum* showed an EC₅₀ of 0.38 mg/L, whereas the freshwater green alga *Raphidocelis subcapitata* showed a lower EC₅₀ of 0.018 mg/L, indicating greater sensitivity of the freshwater algal species to the micro-sized TiO₂ material.

For the nano-sized active Aerodisp® W740X, EC₅₀ values were obtained only for the marine crustacean *Amphibalanus amphitrite*, for which both immobility and behavioural alteration were measurable within the tested range. No EC₅₀ values were determined for *Artemia franciscana* or *Daphnia magna*.

Importantly, no EC₅₀ values were determined for any species exposed to complete sunscreen leachates, whether derived from the blank formulation or from TiO₂-containing creams. In all cases, the tested formulation-derived leachates failed to reach the threshold required for calculation of acute median effect concentrations.

Table 2. EC₅₀ values for active ingredients and cream leachates. Values for active ingredients are expressed in mg/L; values for cream leachates are expressed as percentage of the undiluted leachate. ND, not determined within the tested concentration range.

ORGANISM	ENDPOINT	PARSOL® TX	AERODISP® W740X	BLANK CREAM	PARSOL® TX CREAM	AERODISP® W740X CREAM
<i>ALIIVIBRIO FISCHERI</i>	Bioluminescence inhibition	ND	ND	ND	ND	ND
<i>PHAEODACTYLUM TRICORNUTUM</i>	Growth inhibition	0.38	ND	ND	ND	ND
<i>RAPHIDOCELIS SUBCAPITATA</i>	Growth inhibition	0.018	ND	ND	ND	ND
<i>AMPHIBALANUS AMPHITRITE</i>	Immobility	>50	84.6	ND	ND	ND
<i>AMPHIBALANUS AMPHITRITE</i>	Swimming speed alteration	>50	229.7	ND	ND	ND
<i>ARTEMIA FRANCISCANA</i>	Immobility	>50	>1410	ND	ND	ND
<i>ARTEMIA FRANCISCANA</i>	Swimming speed alteration	>50	>1410	ND	ND	ND
<i>DAPHNIA MAGNA</i>	Immobility	>50	>1410	ND	ND	ND

3.2.2 Bioluminescence Inhibition in *Aliivibrio fischeri*

The inhibition of bacterial bioluminescence after 30 min of exposure to isolated TiO₂ actives and sunscreen-derived leachates was used as a rapid screening endpoint for acute marine toxicity (Figure 14).

Within the tested concentration ranges, neither Aerodisp® W740X nor Parsol® TX induced marked reductions in luminescence. Across all concentrations evaluated, bioluminescence inhibition remained limited, with maximum effects generally ranging between approximately 15% and 20%. A comparable response pattern was observed for cream-derived leachates, including those obtained from formulations containing TiO₂.

No clear concentration–response profile consistent with acute toxicity was observed in this assay. Even at the highest testable concentrations, the level of inhibition remained well below the threshold required for EC₅₀ estimation. Solvent controls produced effects below 10%, confirming that the modest reductions observed in treated groups were not attributable to solvent interference.

Overall, the bacterial endpoint displayed limited sensitivity to both isolated titanium dioxide materials and complete sunscreen leachates under the experimental conditions employed.

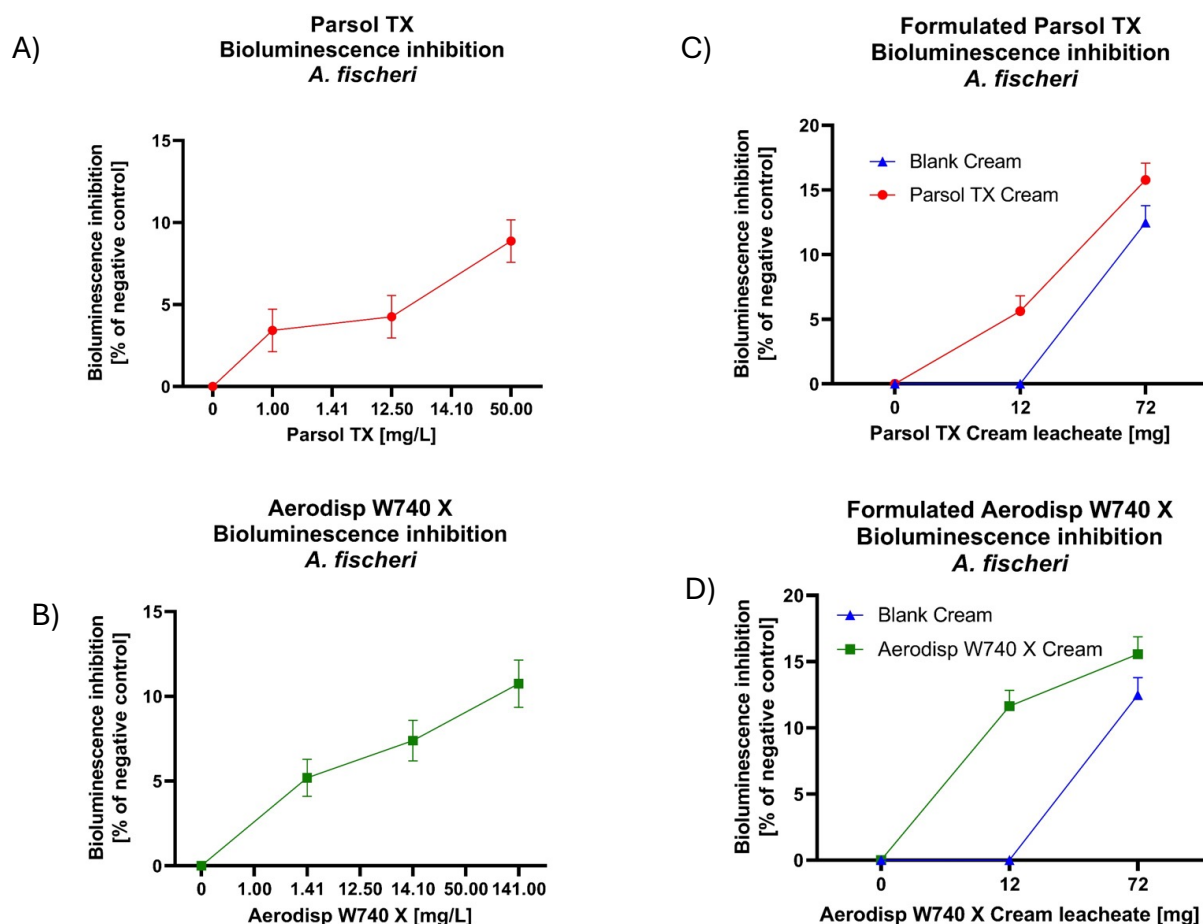


Figure 14. Bioluminescence inhibition in *Aliivibrio fischeri* following exposure to the TiO₂ active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The bacterial response was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg).

3.2.3 Growth Inhibition in Phytoplankton

Growth inhibition assays revealed that phytoplankton represented one of the most responsive trophic levels within the test battery, with clear differences between the marine and freshwater species and between the two TiO₂ materials.

In *Phaeodactylum tricornutum*, exposure to Aerodisp® W740X produced statistically significant reductions in algal growth beginning at the lowest tested concentration of 1.41 mg/L. However, an EC₅₀ could not be calculated because concentrations above 141 mg/L could not be reliably assessed owing to sample opacity. Parsol® TX produced a more pronounced effect in this species, with significant inhibition from 1 mg/L onward and an EC₅₀ value of 0.38 mg/L (Figure 15).

The freshwater species *Raphidocelis subcapitata* showed greater overall sensitivity than the marine diatom. Exposure to Aerodisp® W740X resulted in significant growth inhibition from 1.41 mg/L upward, with a clear concentration-dependent trend across the tested range, although no EC₅₀ could

be determined within the measurable window. Parsol® TX induced a stronger inhibitory response, with significant effects already evident at low concentrations and an EC₅₀ value of 0.018 mg/L (Figure 16).

When phytoplankton were exposed to formulation-derived leachates, no EC₅₀ values were reached at either tested dilution. In the marine diatom, growth responses to blank cream and TiO₂-containing cream leachates did not differ significantly. In the freshwater alga, by contrast, cream leachates produced measurable growth inhibition, and the Parsol® TX cream induced approximately 40% inhibition at both tested dilutions. Statistical comparison indicated significant differences between the blank cream and TiO₂-containing formulations, suggesting that the formulation context contributed measurably to biological response in this species (Figure 15 and 16).

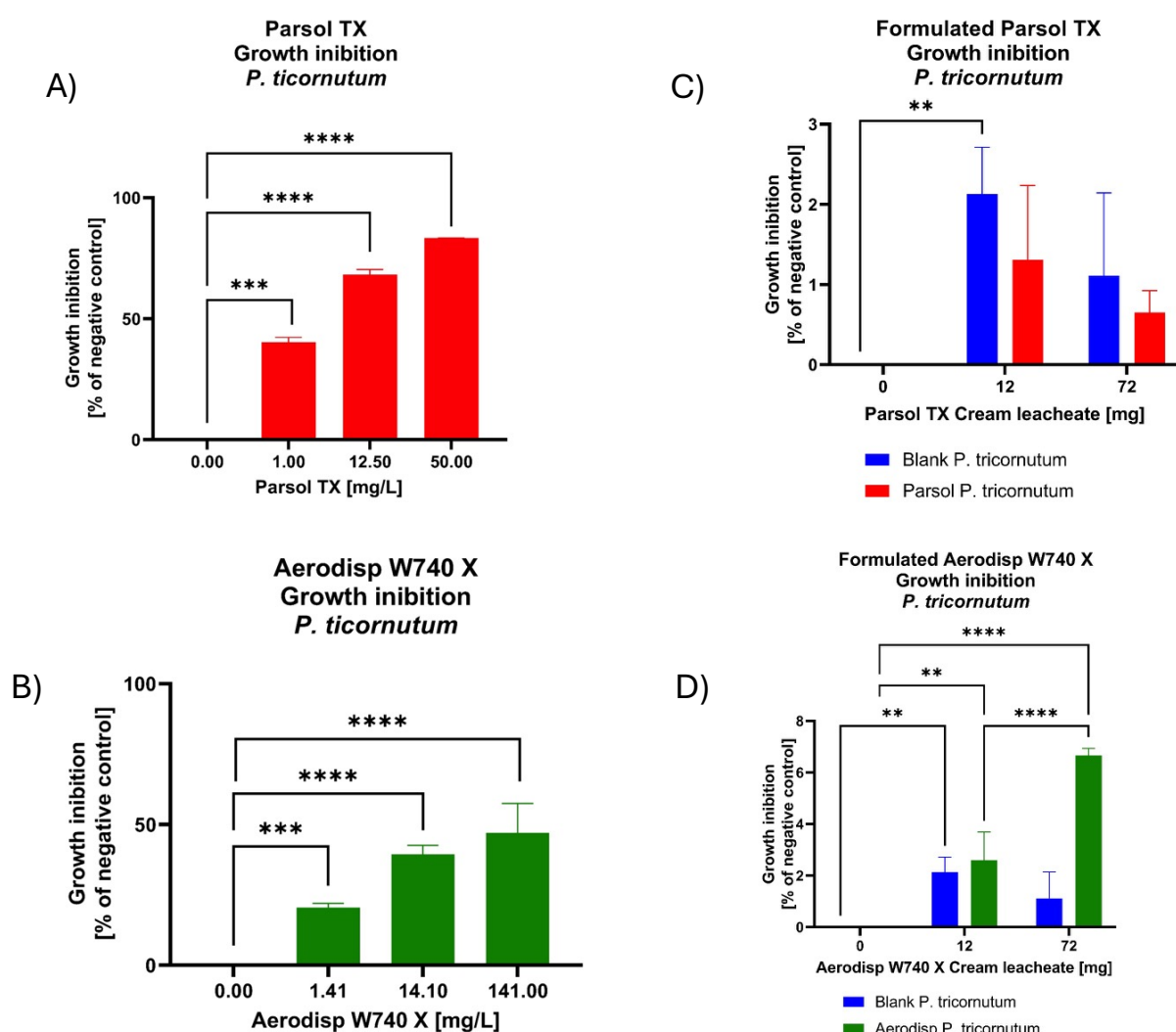


Figure 15. Growth Inhibition in *Phaeodactylum tricornutum* following exposure to the TiO₂ active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The Growth Inhibition in *P. tricornutum* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

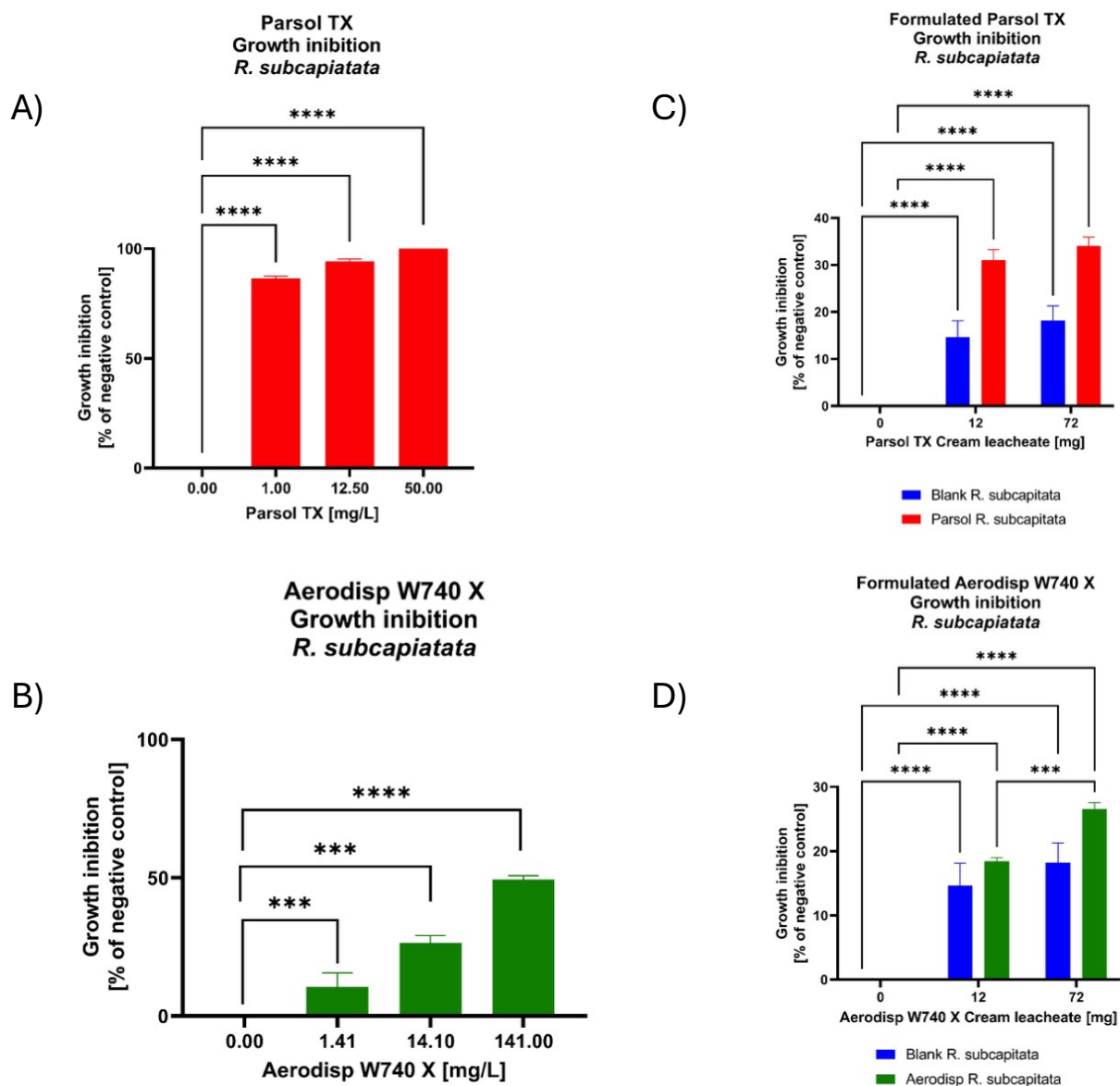


Figure 16. Growth Inhibition in *Raphidocelis subcapitata* following exposure to the TiO_2 active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The Growth Inhibition in *R. subcapitata* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean $\pm$ SEM ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

3.2.4 Effects on Zooplankton Immobility and Behaviour

Zooplankton assays showed that organismal sensitivity varied strongly by species and endpoint. The marine crustacean *Amphibalanus amphitrite* was the most responsive zooplankton species in the test battery, whereas *Artemia franciscana* and *Daphnia magna* showed limited sensitivity under the tested conditions.

The freshwater zooplankton organism *Daphnia magna* showed no significant mortality or immobility alterations following exposure to either TiO₂ active ingredient. In particular, the Aerodisp® W740X active ingredient alone did not produce any measurable response in terms of mortality or immobility, changes under the tested conditions (data not shown). Overall, the observed effects in this species remained below 5%, indicating the absence of meaningful acute or sublethal toxicity within the tested concentration range.

When complete cream leachates were evaluated, *Daphnia magna* did not exhibit statistically significant mortality or immobility changes at the lowest tested concentration. At the highest concentration (72 mg), a statistically significant difference was detected; however, the observed values remained below 7.5 % across treatments (Figure 17 and 18).

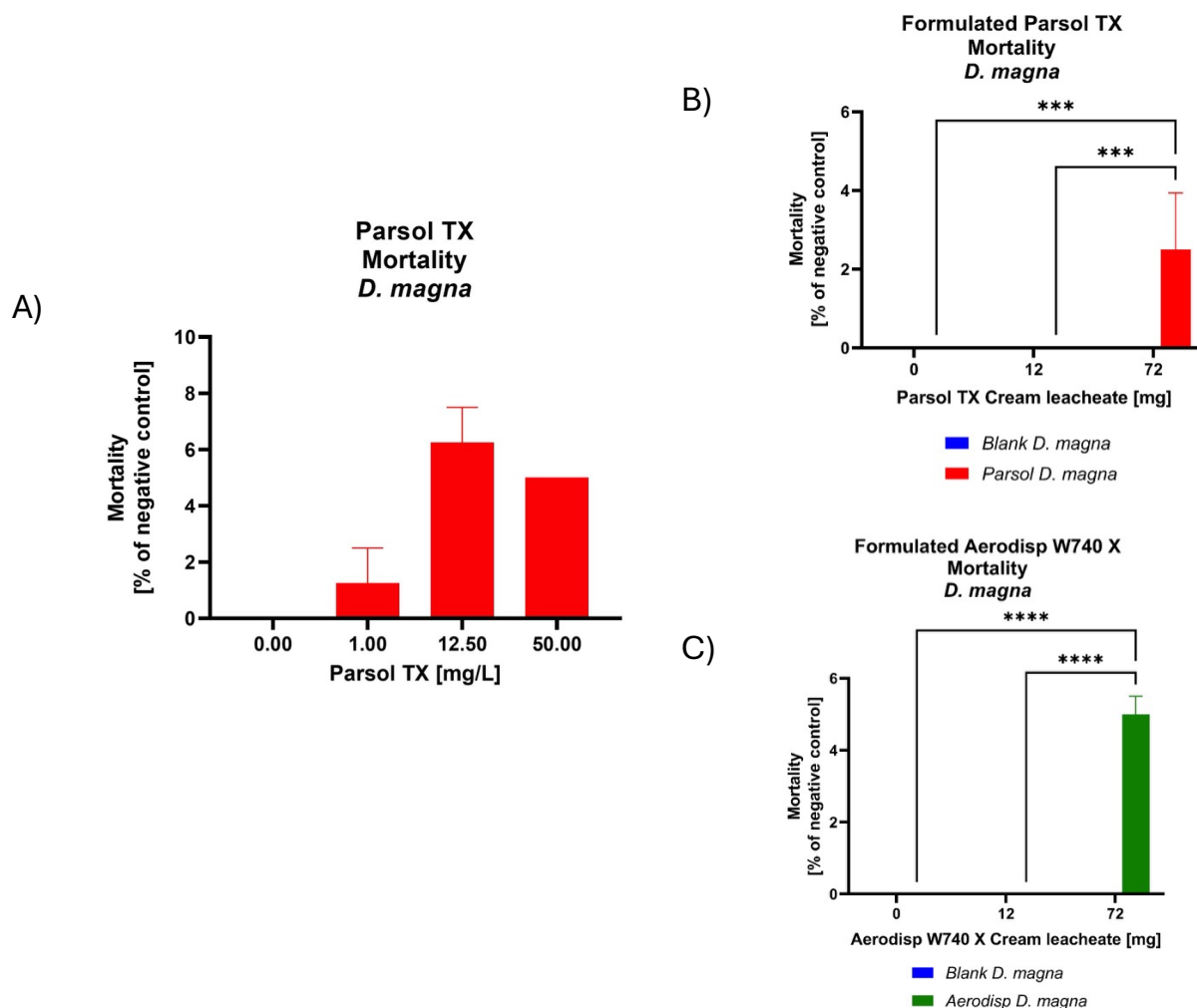


Figure 17. Mortality in *Daphnia magna* following exposure to the TiO_2 active ingredients Parsol® TX (panel A). The Mortality in *D. magna* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel B) and Aerodisp® W740X (panel C), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean $\pm$ SEM ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

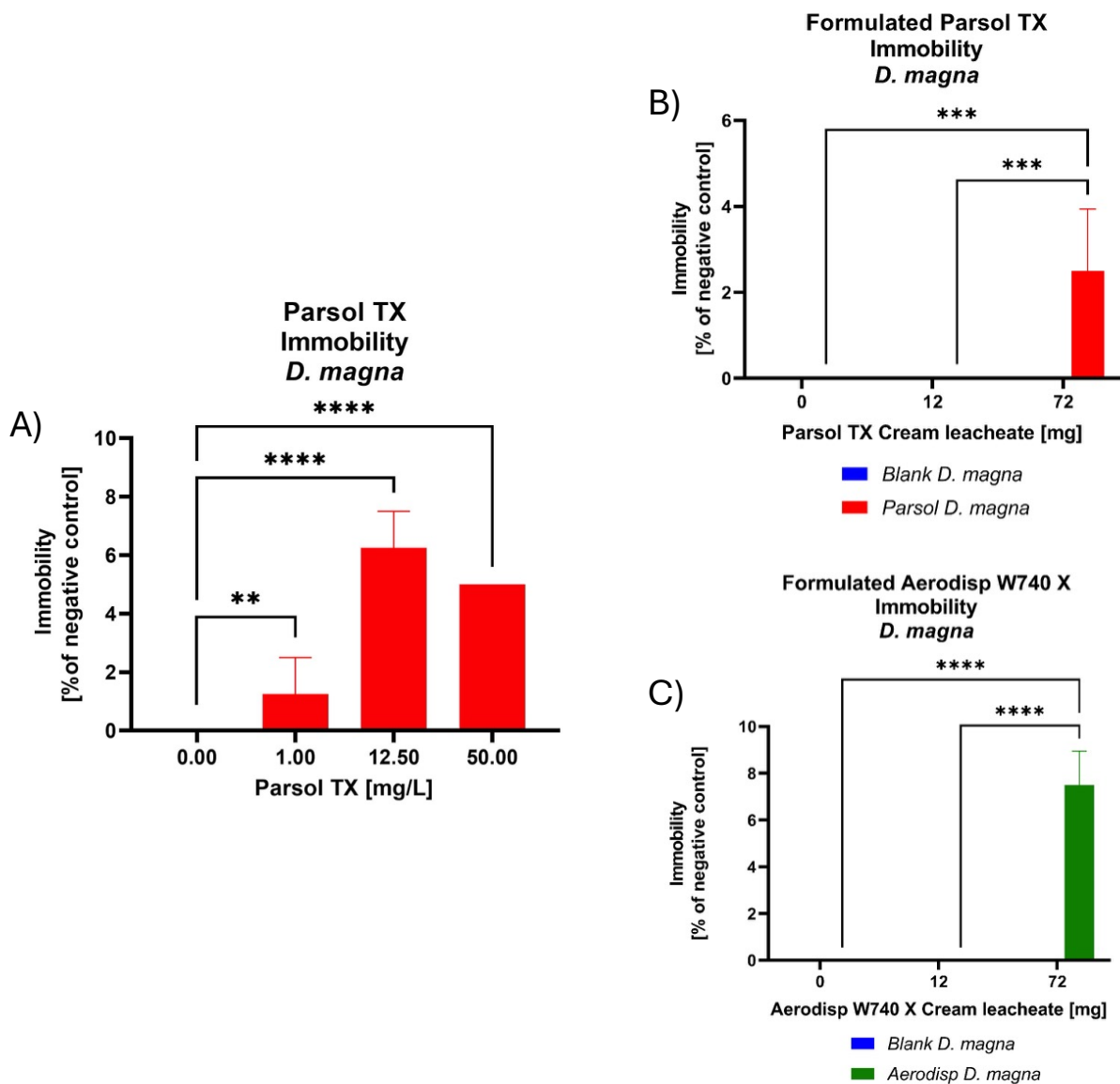


Figure 18. Immobility in *Daphnia magna* following exposure to the TiO_2 active ingredients Parsol® TX (panel A). The Immobility in *D. magna* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel B) and Aerodisp® W740X (panel C), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean $\pm$ SEM ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

Overall, neither the active ingredients nor the leachates derived from Parsol® TX and Aerodisp® W740X treatments produced substantial effects on the marine zooplankton species *Amphibalanus amphitrite* and *Artemia franciscana*, although some statistically significant differences were observed. (Figure 19)

On the other hand, *A. amphitrite* exposed to Aerodisp® W740X showed a substantial decrease in immobility beginning at 1.41 mg/L and significant swimming speed alteration from 14.1 mg/L onward. At the highest tested concentration, complete immobility was observed. For Parsol® TX, both immobility and swimming speed alteration became significant from 1 mg/L, although the magnitude of the response remained more limited than for Aerodisp® W740X, and immobility did not exceed approximately 40% even at the highest tested concentration. (Figure 20 and 21)

By contrast, *Artemia franciscana* showed no significant immobility or behavioural changes in response to either active ingredient. In these species, observed effects remained below 10% and did not indicate meaningful acute or sublethal toxicity within the tested concentration range.

When complete cream leachates were tested, both of the zooplankton species displayed drastic, even if statistically significant, immobility or swimming speed alteration at either the undiluted or diluted leachate condition. Immobility values remained below 20% across treatments, and behavioural responses did not reveal clear formulation-related toxicity.

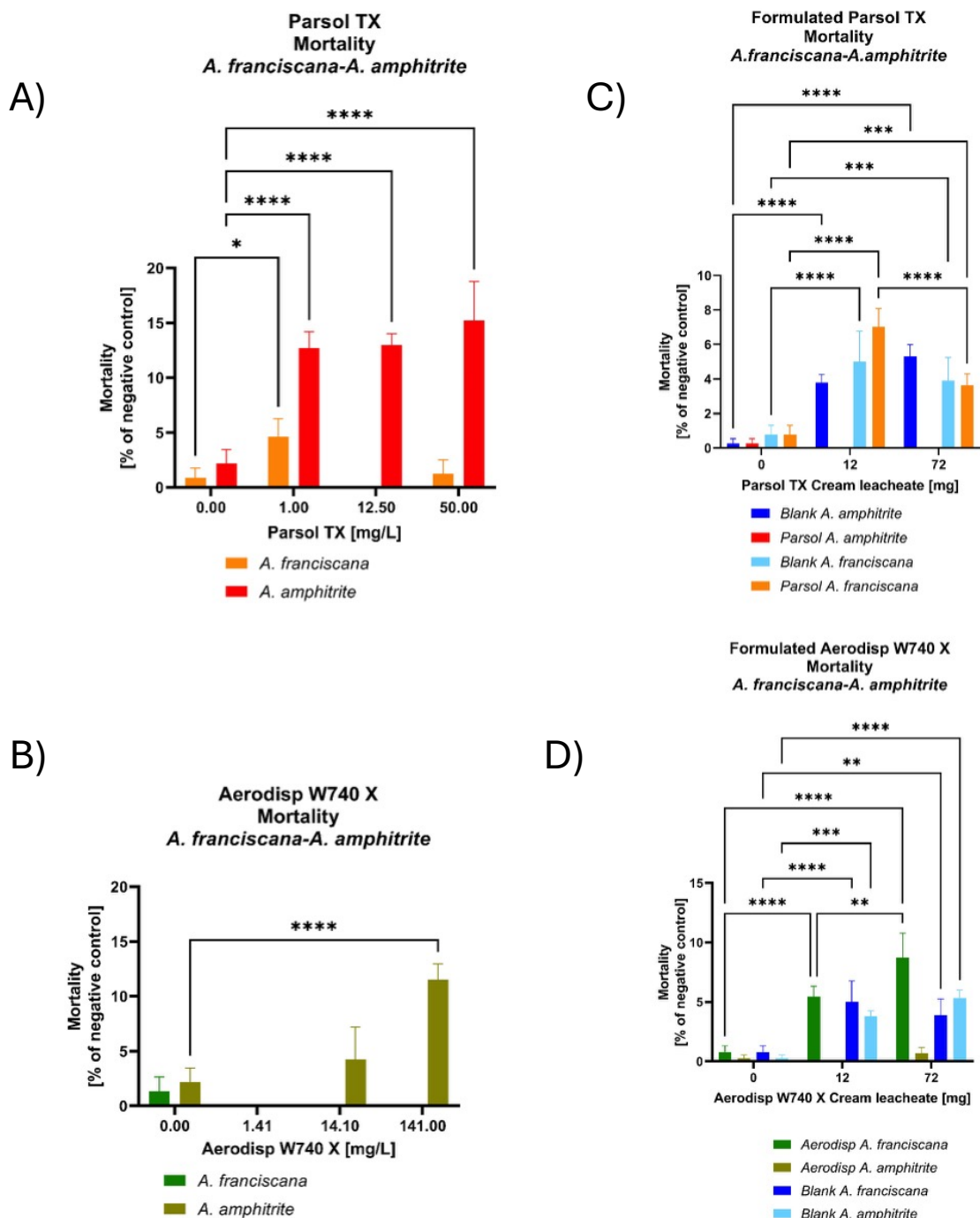


Figure 19. Mortality in *Amphibalanus amphitrite* and *Artemia franciscana* following exposure to the TiO₂ active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The Mortality in *Amphibalanus amphitrite* and *Artemia franciscana* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

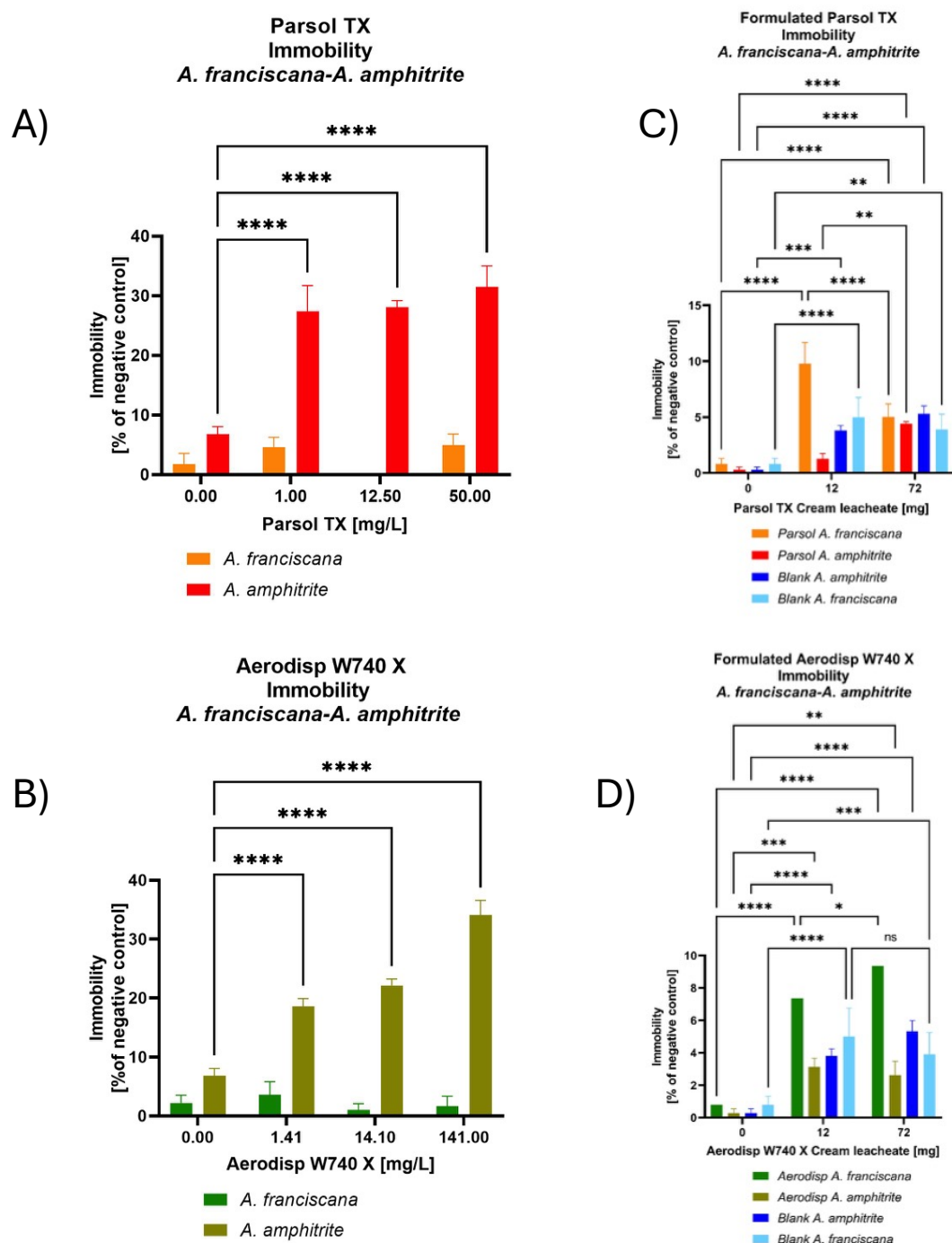


Figure 20. Immobility in *Amphibalanus amphitrite* and *Artemia franciscana* following exposure to the TiO_2 active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The Immobility in *Amphibalanus amphitrite* and *Artemia franciscana* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean $\pm$ SEM ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

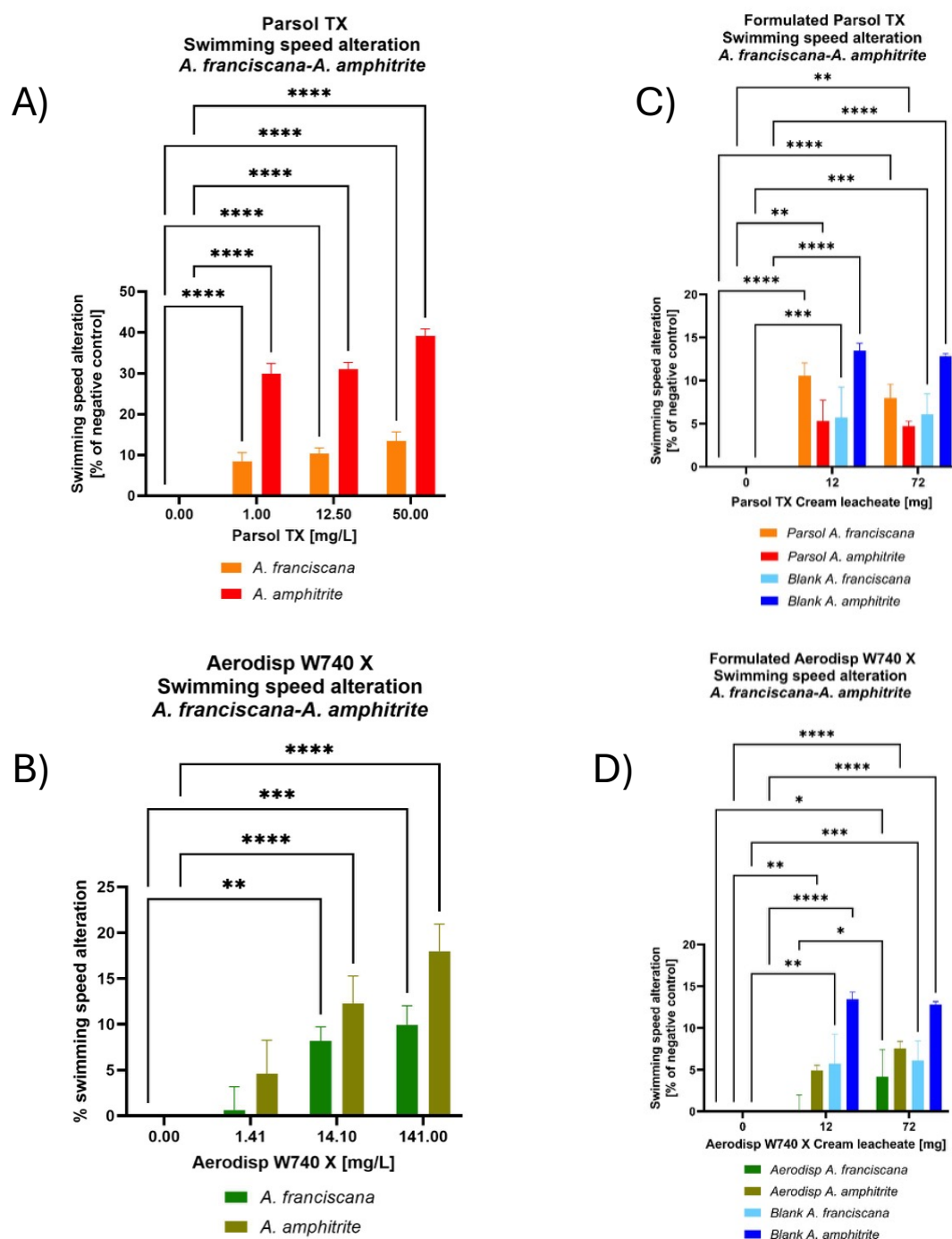


Figure 21. Behaviour in *Amphibalanus amphitrite* and *Artemia franciscana* following exposure to the TiO_2 active ingredients Parsol® TX (panel A) and Aerodisp® W740X (panel B). The Behaviour in *Amphibalanus amphitrite* and *Artemia franciscana* was assessed both for the active ingredients directly dispersed in the exposure medium and for leachates obtained from sunscreen formulations containing Parsol® TX (panel C) and Aerodisp® W740X (panel D), prepared from two different quantities of cream (12 mg and 72 mg). Data are mean $\pm$ SEM ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ vs. control (One-way ANOVA + Dunnett for actives; Two-way ANOVA + Bonferroni for leachates). No asterisk indicates no statistical significance.

3.2.5 Overall Ecotoxicological Pattern

Taken together, the ecotoxicological results revealed a species-dependent and material-dependent response pattern.

Phytoplankton, particularly the freshwater species *Raphidocelis subcapitata*, showed the highest sensitivity to Parsol® TX, indicating that primary producers were the most responsive trophic level to the micro-sized TiO₂ material. Among zooplankton species, *Amphibalanus amphitrite* was the most sensitive, especially to the nano-sized Aerodisp® W740X formulation. By contrast, the bacterial endpoint and the freshwater crustacean *Daphnia magna* showed limited responsiveness to both isolated actives and complete cream leachates.

A consistent finding across the test battery was that complete sunscreen formulations did not induce acute toxicity sufficient to yield EC₅₀ values under the tested exposure conditions. This result contrasts with the responses observed for isolated active ingredients and suggests that formulation context and limited titanium release into the aqueous phase substantially influenced the biological outcomes.

4. Discussion

The present study contributes to the growing body of literature addressing the environmental implications of sunscreen-derived UV filters by focusing on titanium dioxide-based materials in both micro-sized and nano-sized forms and, importantly, by evaluating them not only as isolated active ingredients but also as components of complete sunscreen formulations. This distinction is highly relevant because environmental exposure does not occur through contact with idealised pristine raw materials, but through the release of formulation-associated substances during product use and wash-off. The findings therefore support an important methodological shift in sunscreen ecotoxicology: from substance-centred testing toward more realistic, formulation-aware exposure models [27,28].

A first key result of the study is the limited detectable transfer of titanium from the sunscreen formulations into the aqueous phase under the selected leaching conditions. Across the tested media, measured titanium concentrations in undiluted cream leachates remained low, generally in the range of approximately 1 to 8.6 µg/L, and thus several orders of magnitude lower than the theoretical nominal concentration corresponding to complete release of the applied TiO₂. This finding suggests that only a minor fraction of the nominally present titanium dioxide became analytically detectable in the water phase during the experimental exposure scenario. Such a result is important because it indicates that nominal formulation content cannot be assumed to correspond to actual environmentally available exposure. It also supports the view that the cosmetic matrix strongly constrains particle release, at least under the present conditions.

At the same time, the titanium measurements must be interpreted cautiously. Total titanium concentration in the aqueous phase does not necessarily correspond to the biologically available fraction, since titanium may be present as suspended particles, aggregates, sedimented material, or matrix-associated forms. This is particularly relevant for TiO₂-based materials, whose environmental behaviour depends strongly on aggregation state, surface treatment, ionic strength, and interactions with organic components [7,27]. The analytical data are therefore most informative when considered together with the ecotoxicological endpoints and physicochemical observations rather than in isolation.

In interpreting the biological responses observed in this work, it is also necessary to consider the known mechanisms governing TiO₂ interaction with microorganisms and aquatic biota. In aqueous

systems, TiO₂ surfaces become hydroxylated and acquire a pH-dependent charge, while microbial cell envelopes are generally negatively charged because of phosphate, carboxylate, and related functional groups. As a result, adhesion between particles and biological surfaces may be favoured or hindered depending on pH, ionic strength, aggregation state, and the presence of dissolved organic matter or other adsorbed biomolecules. In addition, the semiconductor nature of TiO₂ enables the photoinduced generation of reactive oxygen species, which may promote oxidative stress, membrane destabilisation, increased permeability, and cellular damage [27,28 56-60]. The biological effects of TiO₂ should therefore be interpreted not as a simple function of nominal concentration alone, but as the outcome of interfacial chemistry, colloidal behaviour, and photocatalytic reactivity acting together.

A second major finding is the clear contrast between the responses observed for isolated active ingredients and those observed for formulation-derived leachates. The active materials, particularly Parsol® TX in the phytoplankton assays and Aerodisp® W740X in *Amphibalanus amphitrite*, produced measurable biological effects within the tested concentration ranges. By contrast, none of the complete cream leachates induced effects sufficient to generate EC₅₀ values in any of the tested organisms. This difference strongly suggests that the formulation context substantially modifies effective exposure and, consequently, biological response. In finished products, TiO₂ particles are not present as freely available raw materials but are embedded within a complex matrix of emulsifiers, oils, dispersants, thickeners, and other excipients that may reduce particle mobility, alter aggregation, limit aqueous transfer, or otherwise affect bioaccessibility [27,28,37].

This result has important methodological implications. Ecotoxicological studies based exclusively on isolated UV filters may be useful for identifying intrinsic hazard mechanisms, but they may overestimate or misrepresent the exposure profile associated with real sunscreen use if they do not consider formulation-dependent release. The present data therefore support the use of leachate-based testing strategies as a more environmentally relevant complement to conventional ingredient-centred assessment. Such approaches are particularly valuable for particulate materials, for which the distinction between nominal content, measurable release, and biologically effective dose is especially critical.

The species-dependent nature of the responses observed in this study further emphasises the need for a multi-species framework. Among the tested organisms, phytoplankton proved to be the most sensitive trophic level overall, particularly the freshwater green alga *Raphidocelis subcapitata*, which showed an EC₅₀ of 0.018 mg/L for Parsol® TX. The marine diatom *Phaeodactylum tricornutum* was also responsive, with an EC₅₀ of 0.38 mg/L for the same material. These findings are consistent with the known vulnerability of primary producers to particulate UV filters and metal oxide materials, which may impair growth through mechanisms including shading, adsorption to cell surfaces, oxidative stress, and disturbance of photosynthetic function [7,27,28]. The greater sensitivity of *R. subcapitata* compared with *P. tricornutum* may reflect both species-specific physiology and differences between freshwater and marine exposure media.

The sensitivity of phytoplankton can also be interpreted mechanistically. Because these organisms remain suspended in the water column and present a high surface-area-to-volume ratio, they may be especially exposed to particle–cell contact and to local oxidative processes occurring at the cell interface. Adsorption of TiO₂ aggregates onto algal surfaces may reduce light availability, interfere with nutrient exchange, and enhance membrane-level stress, while under irradiated conditions photocatalytically generated reactive oxygen species may further contribute to growth inhibition [61-65]. In this sense, the responses of primary producers are compatible with an exposure scenario in which both physical and oxidative mechanisms operate simultaneously.

By contrast, the nano-sized active Aerodisp® W740X did not yield measurable EC₅₀ values in the phytoplankton assays within the tested range, although statistically significant growth inhibition was observed from the lowest tested concentrations. Interpretation of this result is constrained by the fact that higher concentrations could not be reliably assessed because increasing sample opacity interfered with measurement. This highlights a common methodological challenge in nanoparticle ecotoxicology: physical properties of the test suspension may limit both exposure characterisation and endpoint quantification. Even so, the observed inhibition indicates that absence of a calculable EC₅₀ should not be interpreted as absence of biological activity.

Among zooplankton, *Amphibalanus amphitrite* emerged as the most responsive species. Exposure to Aerodisp® W740X induced significant immobility beginning at 1.41 mg/L and behavioural alteration from 14.1 mg/L onward, with complete immobility at the highest tested concentration. Parsol® TX also affected this species, though less strongly. In contrast, *Artemia franciscana* and *Daphnia magna* showed very limited responsiveness under the tested conditions. This divergence among crustacean models illustrates the importance of species biology in determining nanoparticle sensitivity. Factors such as body size, feeding mode, surface interactions, behavioural patterns, and the degree of contact between suspended particles and exposed epithelia may all influence exposure and effect. It also shows that reliance on a single standard invertebrate species would have provided an incomplete picture of hazard.

The bacterial assay using *Aliivibrio fischeri* showed only modest inhibition, with effects generally remaining below approximately 20% even at the highest testable concentrations. This limited sensitivity suggests that short-term bacterial bioluminescence inhibition may not be the most responsive indicator for TiO₂-based sunscreen materials under the present conditions. While this assay remains useful as a rapid marine screening tool, the results indicate that primary producers and selected invertebrate endpoints may provide greater discriminatory power for this class of materials. At the same time, previous mechanistic studies on bacteria indicate that TiO₂ can damage cell envelopes through reactive oxygen species generation and membrane disruption, especially when close contact between the particle surface and the microorganism is achieved [56-60]. The limited response observed here may therefore reflect the specific exposure conditions of the assay rather than the absence of antibacterial potential per se.

The contrast between freshwater and marine systems also deserves attention. The results suggest that environmental matrix plays an important role in shaping TiO₂ behaviour and apparent toxicity. In saline media, elevated ionic strength may promote aggregation and sedimentation by compressing the electrical double layer, thereby potentially reducing stable suspension in the water column while increasing localised deposition [34]. In freshwater systems, greater colloidal stability may persist under some conditions, potentially prolonging exposure of suspended organisms such as phytoplankton. Moreover, dissolved organic matter may either stabilise or partially passivate particle surfaces, thereby modifying particle–cell interactions and oxidative behaviour [56-60]. The higher sensitivity observed in the freshwater algal assay is consistent with the idea that matrix-dependent particle behaviour can strongly influence biological outcome. However, these patterns should be interpreted cautiously, as species-specific sensitivity and test design also contribute.

The study also informs the broader debate surrounding the environmental positioning of mineral sunscreens. In public discourse, inorganic UV filters are often framed as environmentally preferable alternatives to controversial organic filters. The present findings support a more nuanced interpretation. Although titanium dioxide differs fundamentally from organic UV absorbers in chemistry and mechanism, its environmental profile cannot be assumed to be benign simply because it is “mineral” in origin. Hazard depends not only on nominal material class but on particle engineering, surface treatment, aggregation behaviour, formulation matrix, release scenario, and

receiving environment [9,30]. Replacing organic filters with mineral ones may therefore change the type of environmental question being asked, but it does not eliminate the need for careful ecotoxicological assessment.

From a methodological standpoint, the work also highlights limitations of conventional test systems when applied to particulate cosmetic materials. Standard ecotoxicological protocols were largely developed for soluble chemicals and may not fully capture time-dependent aggregation, sedimentation, shading, or matrix-driven changes in particle availability. This can complicate dose definition and interpretation, particularly when nominal concentrations differ sharply from measurable aqueous-phase release. The integration of leachate generation, particle characterisation, titanium quantification, and multi-species bioassays therefore represents a useful step toward more realistic assessment, even though further refinement is still needed. Future research would benefit from more standardised protocols for sunscreen leachate preparation, better distinction between dissolved and particulate fractions, and broader investigation of chronic, repeated, and sediment-associated exposure scenarios.

The implications of these findings extend beyond hazard identification to formulation science. If environmental release and effective exposure are strongly shaped by the formulation matrix, then environmental performance becomes not only an ingredient issue but also a formulation design issue. Strategies such as improved particle coating, more substantive film-forming systems, optimised rheology, and reduced wash-off potential may help decrease environmental exposure while preserving photoprotective efficacy [30,42]. This perspective is consistent with safer-by-design approaches and is preferable to simplified binary narratives that classify ingredients as categorically safe or unsafe without regard to formulation context.

At the same time, the findings must be placed within the broader public health importance of sunscreens. Effective photoprotection remains essential for the prevention of sunburn, photoageing, immunosuppression, and skin cancer [1–4]. Environmental concerns, although highly relevant, should not translate into discouragement of sunscreen use where protection is medically necessary. Rather, they should motivate better product design, more realistic environmental assessment, and more responsible communication regarding both efficacy and sustainability.

Some limitations of the study should also be acknowledged. Laboratory test systems inevitably simplify the complexity of natural environments, where organic matter, suspended particles, microbial communities, sunlight, hydrodynamics, and co-contaminants may all modify TiO₂ behaviour and biological response. In addition, the tested endpoints mainly address acute and sub-acute responses and do not capture possible chronic, reproductive, community-level, or sediment-associated effects. The conservative leaching design was useful as a worst-case approximation, but it cannot reproduce all relevant real-world use conditions. These limitations do not invalidate the findings, but they indicate that the present results should be interpreted as part of a broader and evolving environmental evidence base.

Overall, the study shows that realistic environmental evaluation of TiO₂-based sunscreens cannot rely on simplified assumptions based only on particle size category or isolated ingredient identity. What matters is the interaction between material properties, formulation matrix, release scenario, and receiving environment. By adopting a formulation-aware and ecotoxicologically integrated approach, this work helps clarify the conditions under which titanium dioxide-based sunscreen systems may contribute to aquatic exposure and biological effects, and it supports the development of more robust assessment frameworks for future sunscreen design and regulation.

5. Conclusions

This study investigated the environmental behaviour and ecotoxicological effects of titanium dioxide-based UV filters in sunscreen systems, with particular attention to the differences between micro-sized and nano-sized materials and, crucially, between isolated active ingredients and complete formulations. The results show that the environmental assessment of inorganic sunscreen filters cannot rely solely on nominal particle size or on testing pristine raw materials, because ecological behaviour is strongly influenced by formulation context, release dynamics, and the physicochemical characteristics of the receiving environment.

A key finding of the study is that measurable titanium release from complete sunscreen formulations into the aqueous phase was low under the selected experimental conditions. Across the tested media, total titanium concentrations in undiluted cream leachates remained in the low $\mu\text{g/L}$ range and were far below the theoretical nominal concentrations expected under complete release. This indicates that the nominal TiO_2 content of a finished product cannot be assumed to represent the fraction that is actually released, dispersed, and environmentally available.

The ecotoxicological results further showed a clear distinction between isolated active ingredients and formulation-derived leachates. The raw TiO_2 materials produced species-dependent biological responses, with particularly high sensitivity observed in phytoplankton exposed to Parsol® TX and in *Amphibalanus amphitrite* exposed to Aerodisp® W740X. By contrast, complete sunscreen leachates did not induce effects sufficient to yield EC_{50} values in any of the tested species. This contrast indicates that the cosmetic matrix substantially modifies effective exposure and biological response, likely through combined effects on release, dispersion, aggregation, sedimentation, and bioaccessibility.

The comparison between micro- TiO_2 and nano- TiO_2 also showed that the distinction between these categories, although relevant, is not sufficient in itself to predict environmental hazard. Particle behaviour in aquatic media is dynamic and depends on multiple interacting variables, including aggregation state, ionic strength, medium composition, dissolved organic matter, and species-specific biology. In addition, the biological effects of TiO_2 are linked not only to particle presence, but also to interfacial interactions with cells and to oxidative processes that may contribute to membrane stress and altered cellular function. Accordingly, broad generalisations regarding the environmental safety of “mineral” or “nano” sunscreens are scientifically inadequate.

By applying a multi-species test battery and integrating biological endpoints with physicochemical characterisation and titanium quantification, this work highlights the importance of environmentally relevant and formulation-aware assessment strategies. Bacteria, phytoplankton, and crustaceans did not respond uniformly to TiO_2 -based exposures, confirming that no single test system is sufficient to capture the ecological relevance of sunscreen-derived particulate contamination. At the same time, the stronger responses observed in selected species support the view that sensitivity is shaped by both organism-specific traits and exposure conditions, including the extent of particle–cell contact and the effective bioavailable fraction.

More broadly, the findings support a shift from simplified ingredient-centred ecotoxicology toward product-relevant exposure models that better reflect real sunscreen release scenarios. Such an approach is essential not only for more accurate environmental risk assessment, but also for the development of safer-by-design sunscreen formulations in which photoprotective efficacy, human safety, and reduced ecological burden are considered together.

In conclusion, this study contributes to the advancement of sunscreen ecotoxicology by showing that meaningful environmental evaluation of TiO₂-based sunscreen systems must be formulation-aware, physicochemically informed, mechanistically grounded, and biologically integrated. Only by considering actual release, particle transformation in the receiving medium, and the processes governing biological interaction can risk assessment move beyond simplified assumptions based on nominal composition alone. This perspective is necessary to support more robust environmental evaluation, more responsible scientific communication, and the future design of sunscreen products that reconcile effective human photoprotection with improved environmental compatibility.

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