

Comparative *Danio rerio* (Hamilton, 1822) embryo toxicity of three sunscreen products: mortality, coagulation, and morphological outcomes

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Abstract. Three commercial sunscreen formulations, organic (A), mineral (B), and hybrid (C), containing UV filters and cosmetic additives, were evaluated for developmental toxicity in zebrafish, *Danio rerio* (Hamilton, 1822) embryos. The bioassay was conducted for 120 hours on post-fertilization stages. A broad concentration range of 1 to 1,000 mg L⁻¹ was tested for effects on mortality, coagulation, and morphology. Each tested sunscreen displayed distinct toxicity profiles. Sunscreen A caused mostly sublethal effects, leading to developmental changes, whereas the mineral sunscreen B displayed the highest acute toxicity, reaching ~40% mortality at 1,000 mg L⁻¹. However, the mineral sunscreen C exhibited non-monotonic toxicity, with the strongest effects observed at the lowest concentrations tested. In contrast, organic sunscreen A caused mild changes in shape and behavior at high concentrations and exhibited very low toxicity. Threshold values for effect (NOEC, LOEC) for all tested effects were determined, and mortality proved to be the most sensitive effect. Despite a non-linear toxicity profile for the chemical Sunscreen C, significant risks to aquatic ecosystems at environmentally relevant exposure levels are expected, attributable to endocrine effects on sexual characteristics. The increased toxicity at lower concentrations indicates that high-dose toxicity testing may grossly underestimate the potential effects of such chemicals on ecosystems. Evaluating whole commercial formulations rather than individual ingredients and assessing environmental hazards of sunscreen pollution from the perspective of the final formulated product (FP) rather than individual chemicals can better predict ecological risks. An evaluation of the potential ecological risks to aquatic and terrestrial ecosystems from the human use of sunscreens, along with consideration of mixture toxicity effects and non-monotonic dose-response relationships, may be warranted. The possible consequences of sunscreen pollution are discussed to inform the development of greener alternatives and formulations that pose lower environmental hazards and are safer for the environment than current products.

Keywords: aquatic risk, coagulation, *Danio rerio* embryo, developmental toxicity, mortality, sunscreen.

Introduction. Personal care products, such as sunscreens, are widely used to protect human skin from ultraviolet radiation and reduce the risk of sunburn, photoaging, and skin cancer. Many personal care products have been recognized as emerging contaminants because of their widespread use and continuous release into aquatic environments (Butt & Christensen, 2000). However, they are considered emerging environmental pollutants, entering the environment through wastewater discharge from sewage treatment plants, as well as from recreational activities in surface waters such as swimming, boating, and personal watercrafts. Many UV filters have been detected in various aquatic environments, including surface waters. These chemical sunscreens are generally highly photostable and show environmental persistence. There is also potential for bioaccumulation of these compounds. Many studies have reported ecological effects (Tsui et al., 2014; Gago-Ferrero et al., 2015). Previous studies have demonstrated that sunscreen ingredients may

contribute to coral bleaching and alter microbial communities in marine ecosystems (Danovaro et al., 2008; Downs et al., 2016). Recreational activities were argued to have contributed substantially to the direct release of sunscreen-derived contaminants into coastal ecosystems, particularly during peak tourism seasons (Sánchez-Quiles & Tovar-Sánchez, 2015).

Most aquatic contaminants exhibit rapid acute toxicity to cells through their roles in biological processes and have thus received extensive attention in ecotoxicity studies. In contrast, concerns have been increasingly raised regarding the effects of chemical contaminants that function as endocrine-disrupting chemicals (EDCs) and interfere with gene expression. Sunscreens containing chemical UV filters are among the typical pollutants released into aquatic environments. Exposure to UV filters has been shown to affect growth, developmental rate, reproductive success, behavior, and other ecologically relevant traits in aquatic organisms (Fent et al., 2008; Mitchelmore et al., 2019). Effects are generally most sensitive during early stages, where slight changes in environmental conditions can alter recruitment, survival, and long-term population dynamics.

Some studies on UV filters have begun to investigate their potential ecological effects. To date, however, nearly all these ecotoxicological studies have been conducted using single active ingredients, whereas in real life, mixtures of several active ingredients, preservatives, emulsifiers, antioxidants, and other stabilizers together make up complete sunscreen products. The potential for interactive effects, which can be additive, synergistic, or antagonistic, must not be ignored, as they can produce environmental toxicity that cannot be predicted from single-compound studies (Kortenkamp et al., 2009; Backhaus & Faust, 2012). Several UV filters have been reported to induce toxicity in corals, fish, and other aquatic organisms, raising concerns regarding the ecological consequences of sunscreen contamination (Sieratowicz et al., 2011; Downs et al., 2016; Lozano et al., 2020).

The zebrafish, *Danio rerio* (Hamilton, 1822) embryo model is widely used for assessing developmental toxicity in aquatic environments (Organization for Economic Co-operation and Development, 2013). The test protocol using *D. rerio* embryos was formalized in OECD Test Guideline 236 (Organization for Economic Co-operation and Development, 2019). The *D. rerio* embryo model offers several advantages, including rapid development, optical transparency, and high sensitivity to toxic substances that can induce lethal and sublethal effects, such as mortality, coagulation, and morphological abnormalities (He et al., 2019; Nataraj et al., 2020). *D. rerio* embryos are particularly useful for assessing the toxicity of complex contaminant mixtures during early developmental stages.

The present study extends existing knowledge by evaluating the toxicity of selected commercial sunscreen formulations, rather than isolated UV filters, under controlled laboratory conditions. Three widely used sunscreen types, organic (A), mineral (B), and hybrid (C), were tested across environmentally relevant and elevated concentrations using *D. rerio* embryo assays. By examining mortality, coagulation, and morphological outcomes, this study aims to provide a more realistic assessment of ecological risk associated with sunscreen pollution.

This work attempts to connect laboratory toxicology experiments to real-world environmental exposures by addressing the effects of sunscreen toxicity. These findings can be used to improve ecological risk assessments and design greener sunscreens.

Material and Method

Test organisms and ethical considerations. *D. rerio* were selected as the test organism for this study due to their extensive application in developmental toxicology and environmental safety assessments. The Organization for Economic Co-operation and Development (OECD) Test Guideline 236 (Organisation for Economic Co-operation and Development, 2019) has standardized the model. This guideline outlines the steps for acute toxicity tests on fish embryos. *D. rerio* embryos offer numerous advantages, including rapid external development, chorion transparency, and significant genetic and physiological homology with other vertebrates, including humans. These characteristics render them

especially effective for detecting developmental disturbances and fatal consequences resulting from chemical exposures (Nataraj et al., 2020; He et al., 2019).

Adult *D. rerio* were acclimated for two to three weeks and kept in laboratory aquaria with a constant flow of air, a water temperature of 28°C, and a 14:10 h light-dark cycle. To keep the fish healthy for breeding, they were fed a mix of flake food and live brine shrimp twice a day. We obtained embryos by placing male and female *D. rerio* together in breeding tanks with mesh bottoms, which protected the eggs from predators. Fertilized embryos were collected within two to four hours post-fertilization (HPF), washed with embryo medium, and examined microscopically to verify their developmental stage and viability.

There are no defined life stages for *D. rerio* between 120 HPF and adult. However, embryonic life stages used as surrogates for adult organisms in testing and evaluation should not be ignored in ethical considerations regarding their use. All experiments were conducted in accordance with the general principles of humane care and use of laboratory animals (Fong et al., 2016).

Experimental design and exposure setup. This study was conducted in accordance with the OECD Test Guideline 236 (TG 236) testing methodology for Commercial Soluble Substances and Mixtures (Organisation for Economic Co-operation and Development, 2019). For the Static test, embryos were held at a single-embryo-per-well in 24-well plates to prevent density-dependent effects. For each test material solution concentration and each negative control solution, a minimum of 20 embryos were used for test and control exposures on separate plates. For the negative control, embryos were maintained in untreated embryo medium. Solvent controls were not conducted because the test formulations were dissolved directly in water for testing.

For *D. rerio* embryo tests in this study, four nominal test concentrations (1, 10, 100, and 1,000 mg L⁻¹) were used. These test concentrations included environmentally relevant levels measured in recreational water environments (Rohée et al., 2019), as well as higher concentrations used in laboratory-based tests to determine hazard thresholds. Embryo exposures started from 2-4 HPF and continued until 120 hpf. By the end of the test period, the test fish had undergone several important developmental stages, including gastrulation, organogenesis, and hatching.

Embryos were checked regularly with a stereomicroscope for survival, coagulation, and severe malformations. Dead embryos were removed from the test solution as soon as possible to prevent them from rotting and to avoid contamination of the test solution with their contents. For the endpoints, embryos were assessed at 24, 48, 72, 96, and 120 HPF. This is in line with the OECD guidelines for testing chemicals on *D. rerio* (Organisation for Economic Co-operation and Development, 2013) and with other research on the toxicity of sunscreen to early life stages of fish (Kunz et al., 2006; Fent et al., 2008).

Verification of exposure concentrations. No verification of actual exposure concentrations was made in this study. Since sunscreen compounds are complex mixtures of partially lipophilic molecules, they may exist in aggregated states. Also, some of the individual constituents may adsorb onto plastic surfaces from the solution. To limit photodegradation effects, exposure solutions were renewed daily. All sunscreen exposures were reported as nominal.

To enhance the interpretation of biological effects from BMAA exposure, future studies should include chemical analyses, such as HPLC or LC-MS, to determine actual exposure concentrations and assess test solution stability (t_{1/2}). Such analyses would provide a more accurate toxicity threshold and a more robust ecological risk assessment based on measurable exposure concentrations.

Sunscreen products and concentration selection. Three different commercial sunscreen formulations - organic Sunscreen A (SPF 50), mineral sunscreen B (SPF 35), and hybrid (chemical & mineral) sunscreen C (SPF 100) for testing. Each product was purchased from a Watsons boutique in Iligan City, and selected them to demonstrate the different combinations of organic and inorganic UV filters used in everyday consumer

goods. The exact ingredient lists could not be shared due to privacy concerns. However, each formulation contained at least one standard organic UV filter (such as oxybenzone, octocrylene, or octinoxate), as well as stabilizers, emollients, and preservatives, including parabens, which are often found in consumer goods.

Stock solutions were prepared by adding sunscreen samples directly to the embryo medium and gently shaking to ensure thorough mixing. To enhance the solubility of these complex, lipid-heavy formulations in water, 0.5% dimethyl sulfoxide (DMSO) was utilized as a negative solvent control, a concentration well within the recognized safety thresholds for *D. rerio* embryos (Christou et al., 2020). We chose concentrations of 1, 10, 100, and 1000 mgL⁻¹ based on previous studies that found UV filters in the environment at levels that can reach mgL⁻¹ in surface waters near areas with much use (Rohée et al., 2019) and on previous *D. rerio* tests that showed toxic effects in similar ranges (He et al., 2019; Nataraj et al., 2020). The selected solvent concentration is well below levels known to induce developmental toxicity in *D. rerio* embryos (Christou et al., 2020).

No analytical verification of exposure concentrations was performed; therefore, nominal concentrations may differ from actual dissolved fractions due to limited solubility, aggregation, or degradation. This represents a limitation and should be addressed in future studies through chemical analysis.

Exposure conditions. In sterile 24-well plates, embryos were kept in 2 mL of test solution per well. The plates were maintained at 26-28°C and exposed to alternating light and darkness for 14 hours and 10 hours, respectively, which simulates their natural environment. A daily renewal of stock solutions wherein exposure solutions were changed was specifically utilized to mitigate the rapid photodegradation of organic UV filters, to prevent the accumulation of embryonic metabolic waste, and to ensure that the sunscreen chemicals do not adhere to the walls of the wells, thereby maintaining the nominal exposure concentrations as accurately as possible. The embryo medium was composed of buffered water adjusted to the correct conductivity and hardness levels for *D. rerio* culture. We monitored the dissolved oxygen and pH levels periodically to ensure the water quality remained within acceptable ranges. This daily renewal of exposure solutions was performed to reduce variability caused by photodegradation, adsorption, and accumulation of metabolic waste, thereby helping to maintain relatively stable exposure conditions.

Incubations were conducted on covered plates in a humidified incubation room to prevent evaporation or external contamination. Each test formulation was manufactured separately to prevent cross-contamination, and all test equipment was thoroughly washed and rinsed between each processing event. For each test concentration, 4 embryos were randomly placed into wells on a plate. This was done to avoid potential biases arising from positional variability in exposure and/or between clutches of embryos. The likelihood of obtaining a reproducible fish embryo assay (Organization for Economic Co-operation and Development, 2013) was therefore improved.

Endpoints and scoring criteria. Embryos were assessed at 24, 48, 72, 96, and 120 HPF using a stereomicroscope. Endpoints included mortality, coagulation, and morphological abnormalities, in accordance with OECD Test Guideline 236, with additional structured scoring criteria (Organization for Economic Co-operation and Development, 2019).

Mortality was defined by the presence of one or more of the following: coagulated embryo, absence of somite formation, non-detachment of the tail, or absence of heartbeat.

We have monitored coagulation as an early indicator of toxicity. Embryos were scored as 100% opaque, disorganized, and lacking normal development. Coagulation was scored as either present or absent for each embryo.

Morphological abnormalities (malformations) were scored based on surviving embryos. The following developmental defects were considered: Pericardial effusion: Fluid builds up in the space around the heart (pericardium). Also called fluid around the heart, Yolk sac edema - The yolk sac for this stage is larger than normal and has a "swollen" appearance, Scoliosis - curvature of the spine in a sideways direction, Craniofacial malformations - abnormalities of the shape of the head and facial bones, and Internal mold

- an event where the insect enters its next growth stage prior to fully completing the current form, often causing irreversible injury.

Embryos were scored for the presence or absence of each abnormality category, and for each replicate, the percentage of abnormal embryos was calculated.

All experiments were performed in accordance with OECD guidelines for visually assessing toxicity in *D. rerio* using standardized visual readouts (Fong et al., 2016; He et al., 2019). Prior to data collection, all observers received training and utilized reference images to score for endpoints of interest. Ambiguous cases were reviewed independently to ensure accuracy and reduce observer bias.

No weighted scoring system was utilized. Embryonic malformations were considered categorical endpoints to maximize reproducibility and minimize variability due to observer error.

Data treatment, threshold determination, and limitations. The data were presented as percentages of embryos impacted per concentration for each endpoint. To determine the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC), we employed conservative thresholds. LOEC was the lowest concentration that caused an increase of at least 10 percentage points above the control mean. NOEC had the highest concentration, which was lower than the LOEC. This rule-based method is suggested for screening-level hazard identification when detailed statistical modeling is impractical (Organisation for Economic Co-operation and Development, 2019).

There are, however, some limitations to the data. The research used summary-level datasets, presenting effects as percentages by group rather than individual embryo outcomes. The research is presented as summary-level data, expressed as percentage effects by group rather than by individual embryo. While some statistical tests could be performed, more advanced modeling techniques, such as generalized linear mixed models or probit regression, cannot be applied due to a lack of replicate variance data (He et al., 2019). Therefore, EC_x values and associated confidence intervals, and LOEC/NOEC values cannot be determined from this data and should be used with caution. The 'nominal' concentrations do not account for solubility, partitioning, or degradation; therefore, the actual exposure may differ from the nominal concentration. Even though there are some limitations to this dataset, it can still provide useful information regarding the toxicity of various sunscreen products.

Quality assurance and guideline compliance. All procedures on fish embryos were performed in accordance with OECD TG 236 for purposes of comparison to other fish embryo toxicity tests. In the negative control (0.5% DMSO), all embryos developed normally and were therefore considered viable. The use of replicates across plates and the random allocation of embryos to wells within each plate reduced variability.

All Positive controls, like 3,4-DCD, were used for embryo toxicity in this test (Organisation for Economic Co-operation and Development, 2013), positive controls were not used in this study, as the test facility is not equipped to use them. However, several commercial sunscreen products were tested to allow for the development of internal controls. It is recommended that future tests of this nature include positive controls and chemical analyses of the exposure media to improve test conditions.

To achieve the study objectives, conventional *D. rerio* embryo tests were adapted to evaluate commercial sunscreens, with modifications to the test protocols. The test protocols not only addressed problems related to setting up an experimental design using nominal concentrations, but they also enabled discrimination among test products based on their toxicity profiles, which showed marked differences. The test methodologies can be applied to environmental safety assessments of consumer products that may be exposed to aquatic environments.

This study lacked analytical verification of insecticide exposure, and future studies would ideally include some form of chemical monitoring of exposure media. For complex commercial insecticides, the nominal concentration of the technical material in bait, aqueous residues, surfaces, and air may not accurately reflect the actual amount available for exposure.

Results. Tables 1 to 3 present results from *D. rerio* embryo toxicity tests conducted with the three tested sunscreens (A, B, and C). Mortality, coagulation, and malformation percentages are expressed as the proportion of affected embryos among the total number of embryos at each concentration. The percentages of mortality and malformation are the means of replicating tests, in accordance with OECD criteria. The percentages of malformation include only surviving embryos and represent the proportion of affected surviving embryos.

For each treatment, the percentage of surviving embryos showing some form of malformation (termed herein as 'morphological defect (%)') was determined. As shown in Table 2, the most toxic of the sunscreens tested, Sunscreen A (SC1), had the highest percentage of embryonic malformations occurring at approximately 10% at a concentration of 10 mg L⁻¹. In contrast, the other two sunscreens (SC2, Sunscreen B, and SC3, Sunscreen C) induced minimal observable malformations; this apparent decrease in the percentage of malformations may be due to increased embryonic lethality in these two treatments, such that many of the exposed embryos did not survive to the time of gross examination.

Identifying LOEC/NOEC. We used a conservative threshold approach to find the LOEC and NOEC for each sunscreen and endpoint. The LOEC was the lowest concentration that raised the control mean by at least 10 percentage points. NOEC was defined as the highest concentration level below the LOEC. Table 1 summarizes the results.

Table 1 shows that Sunscreen B had the lowest NOEC across all mortality endpoints, whereas Sunscreen A had higher NOECs, indicating that the effects were mild. Sunscreen C could not be accurately characterized because of inconsistent responses.

Table 1

Lowest observed effect concentrations and no observed effect concentrations for *Danio rerio* embryos exposed to three commercial sunscreens (SC1-SC3)

Sunscreen	Endpoint	Control (%)	Threshold (%)	NOEC (mg L ⁻¹)	LOEC (mg L ⁻¹)
SC1	Coagulation	none	none	none	none
SC1	Morphological defects	0	10	1	10
SC1	Mortality	none	none	none	none
SC2	Coagulation	none	none	none	none
SC2	Morphological defects	0	10	none	1
SC2	Mortality	10	20	none	1
SC3	Coagulation	0	10	none	1
SC3	Morphological defects	0	10	1000	none
SC3	Mortality	6.67	16.67	none	1

Note: SC1 - organic sunscreen, SC2 - mineral sunscreen, SC3 - hybrid sunscreen, LOEC - lowest observed effect concentration, NOEC - no observed effect concentration; *Thresholds were determined using descriptive criteria: the LOEC was defined as the lowest concentration producing at least a 10% increase above control levels, and the NOEC was defined as the highest concentration without an observable effect. Mortality was the most sensitive endpoint, while coagulation and morphological defects exhibited higher thresholds or no clear responses.

Mortality outcomes. Results for moderate toxicity for Sunscreen A (SC1) and strong acute toxicity for Sunscreen B (SC2) are shown in Figure 1.

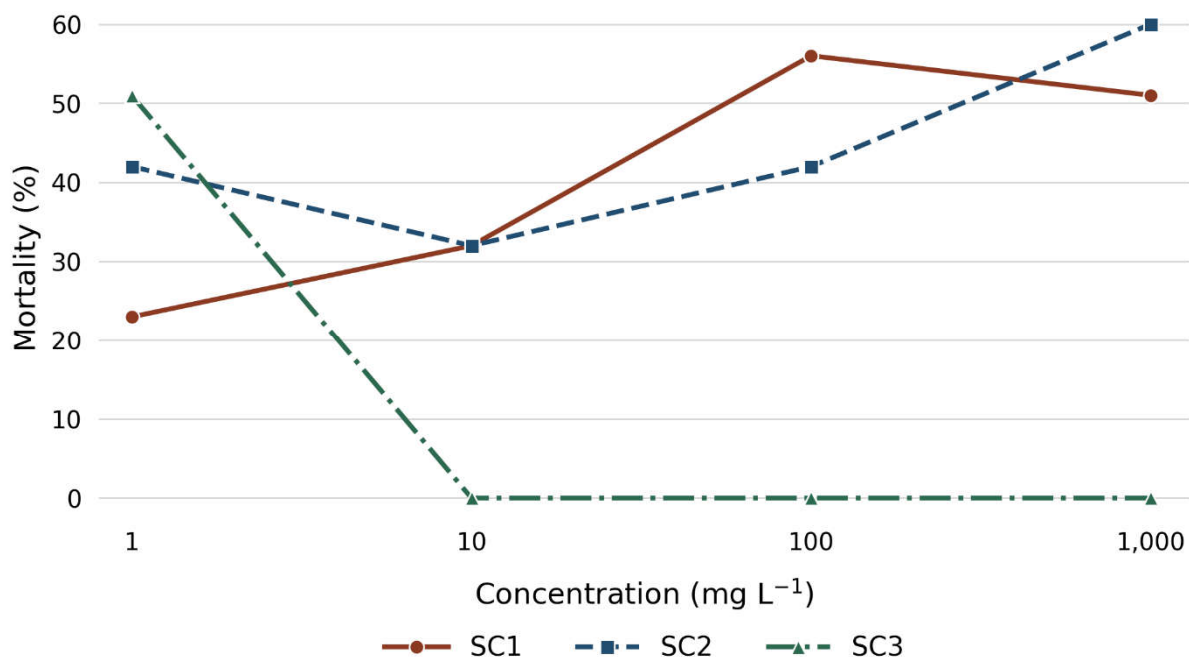


Figure 1. Comparative dose-response profiles for mortality in *Danio rerio* embryos exposed to Sunscreens A (SC1), B (SC2), and C (SC3).

Table 2 presents summary results based on dose-response relationships for mortality, coagulation, and morphological changes from *D. rerio* embryo bioassays of Sunscreens A (SC1), B (SC2), and C (SC3). From the table, it can be shown the values representing mean percentages of affected embryos at each nominal concentration (1, 10, 100, and 1000 mg L⁻¹). Negative control baselines are included for comparison. In all tests performed, mortality was the most sensitive and consistent parameter for assessing the toxic effects of the sunscreens studied.

Table 2

Dose-response effects of commercial sunscreens on *Danio rerio* embryo development

Concentration	Mortality SC1 (%)	Mortality SC2 (%)	Mortality SC3 (%)	Coagulation SC1 (%)	Coagulation SC2 (%)	Coagulation SC3 (%)
Positive control	0.887	51.330	37.330	37.330	42	37.330
1,000 mg L ⁻¹	51.333	60.667	1.400	18.667	32.667	1.400
100 mg L ⁻¹	56	42	1.400	56	14	1.070
10 mg L ⁻¹	32.667	32.667	1.027	28	4.666	0.746
1 mg L ⁻¹	23.333	42	51.333	18.667	0	51.333
Negative Control	4.667	14	9.333	28	0	0

Note: SC1 - organic sunscreen, SC2 - mineral sunscreen, SC3 - hybrid sunscreen.

From the table, Sunscreen C (SC3) exhibited an atypical non-monotonic mortality pattern. Mortality was unexpectedly high at the lowest concentration, 1 mg L⁻¹, at 51.33% ($p = 0.012$). However, at intermediate and high concentrations (10 to 1000 mgL⁻¹), mortality plummeted to near-zero levels (1.02% to 1.4%), dropping well below the negative control baseline. This non-monotonic anomaly indicates that SC3's toxicity is driven by highly sensitive, low-dose mechanisms, such as endocrine disruption, rather than standard acute chemical toxicity.

Table 3 summarizes the maximum lethal values (LC) of the three sunscreens studied. The values are expressed as a percentage effect on the organism for the total

invertebrate community. LC (L50) values for SC2 were the highest among the compounds studied (60.67% kill at 1,000 mg L⁻¹), while those for SC1 were greater than expected for a non-toxic substance (maximum effect 56% at 100 mg L⁻¹). This compares poorly with SC3, which had the lowest LC(L)50 value among the test compounds (1 mg L⁻¹) and was therefore the most toxic on a per-mass basis.

Table 3

Maximum mortality and coagulation responses in *Danio rerio* embryos exposed to commercial sunscreens (SC1-SC3)

Sunscreen	Max mortality (%)	Max coagulation (%)
SC1 (A)	56	56
SC2 (B)	60.667	42
SC3 (C)	51.333	51.333

Note: SC1 - organic sunscreen, SC2 - mineral sunscreen, SC3 - hybrid sunscreen.

The comparative evaluation of the bioassay data from tests on these three sunscreens presented in Tables 1-3 indicates that each sunscreen had a unique toxicity profile. The most acutely lethal sunscreen was sunscreen B (SC2) with strong dose-response relationships for both lethal and sublethal effects. In contrast, the toxicity of moderately toxic sunscreen A (SC1) was mainly manifest as strong sublethal effects on development. Surprisingly, the toxicity of sunscreen C (SC3) exhibited a nonmonotonic profile, with maximum toxicity at the lowest tested concentration.

Coagulation outcomes. Coagulation, an early marker of developmental disruption and severe cellular toxicity, showed variable responses across formulations. Coagulation % for SC1 (Sunscreen B) varied at different test concentrations (Figure 2, Table 2). It increased from 18.67% (1 mgL⁻¹) to 56% (100 mg L⁻¹) and then declined to 100 mg L⁻¹. Coagulation % for SC2 (Sunscreen A) increased in a dose-dependent manner (Table 2). It was negligible (0%) at the lowest test concentration, increased to 14% (100 mg L⁻¹), and peaked at 32.67% ($p < 0.05$) at 1000 mg L⁻¹. The trends in coagulation % and acute mortality for SC2 at high concentrations were similar.

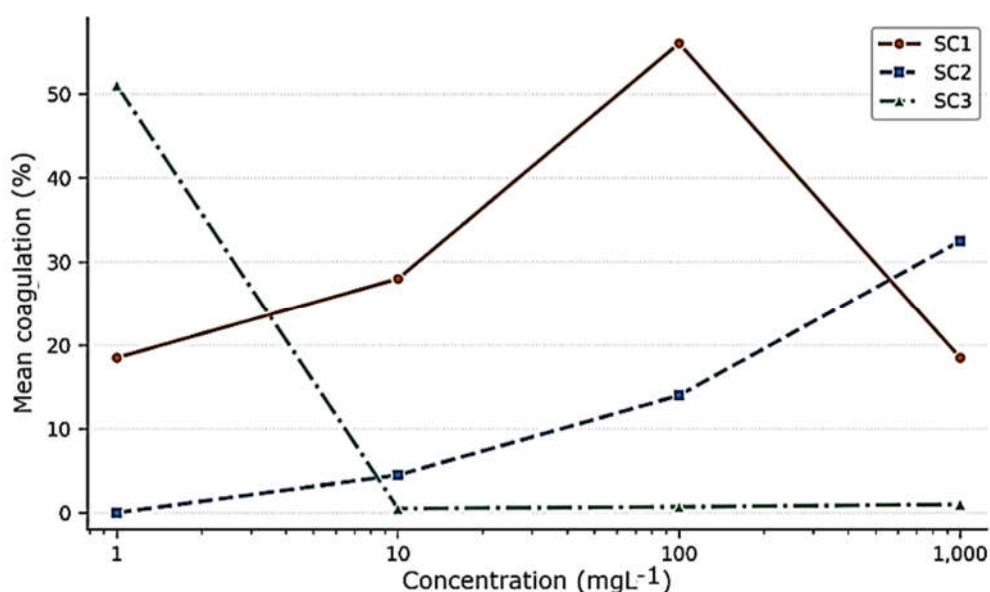


Figure 2. Dose-response profiles for coagulation in *Danio rerio* embryos exposed to Sunscreens A (SC1), B (SC2), and C (SC3).

Coagulation responses were greatest for SC3, while SC1 and SC2 were near control levels at all concentrations tested. Coagulation spiked to 51.33% only at the lowest concentration (1 mg L⁻¹), then dropped to negligible levels (< 1.5%) at all higher concentrations. This reinforces the observation that SC3 exerts its most severe teratogenic and lethal effects almost exclusively at trace environmental concentrations.

Table 3 shows the maximum coagulation responses recorded for each product. This again highlights that SC1 achieved the highest level of coagulation, 56%, at 100 mg L⁻¹. SC3 achieved a similar maximum of 51.33% at the lowest dose of 1 mg L⁻¹. SC2 had the highest level of overall lethal impact, but its maximum coagulation response was only 32.67% at the highest dose tested. The results show that coagulation is a product-specific effect, produced by different mechanisms of action.

Morphological defects. Although less prominent than toxicity and effects on coagulation, effects on morphology were observed for all tested compounds. However, there were marked differences in severity among the test chemicals (Figure 3, Table 3). The most teratogenic sunscreen tested was SC1, showing slight but consistent malformations, including pericardial edema and tail curvature, with the highest incidence (about 10%) observed at the intermediate concentration of 10 mg L⁻¹ (Kruskal-Wallis test, $p = 0.034$). However, higher concentrations of SC1 were associated with a reduced incidence of malformations (Figure 3). The most severe effects of SC1 on development occurred at sub-lethal concentrations.

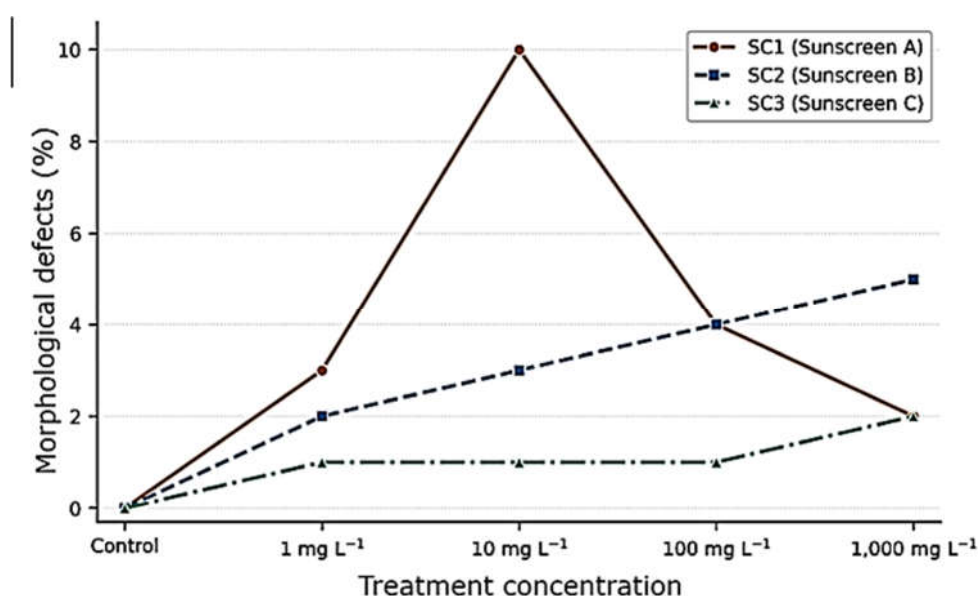


Figure 3. Graphical profile of comparative dose-response profiles for morphological defects in *Danio rerio* embryos exposed to Sunscreens A (SC1), B (SC2), and C (SC3).

The maximum percentage affected was 5% in SC2, and all SC3 test groups were below 2% (Table 3). Although there was little effect, it was far from safe and was associated with high acute toxicity of the test sunscreens. Because mortality and early embryonic coagulation were the dominant endpoints for these two formulations, the embryos perished before sub-lethal teratogenic effects could manifest. Therefore, while SC1 exhibited the highest relative teratogenicity, it is largely because its lower lethality permitted embryos to survive long enough to develop these physical abnormalities.

Comparative profiles and thresholds. The integrated analyses of hazards for product-related toxicity revealed distinct profiles for mortality, coagulation defects, and morphological defects for SC1, SC2, and SC3. SC2 showed the highest hazard for acute mortality with concentration-dependent effects exceeding 60% mortality (Figure 1; Table 1). SC3 showed an unusual non-monotonic hazard for mortality and coagulation, with massive increases at the lowest concentration tested, 1 mg L⁻¹ (Figures 2 and 3). SC1 showed moderate hazards for effects on mortality and coagulation at an intermediate concentration of 100 mg L⁻¹, as well as a unique sub-lethal hazard for malformations that peaked at 10 mg L⁻¹.

Threshold analyses for effects on endpoints tested across surfactant concentrates were determined in a separate test (Table 1). For SC2, the most sensitive endpoint was

mortality, with an LOEC of 100 mg L⁻¹. SC3 showed non-monotonic effects, with an LOEC for mortality of 1 mg L⁻¹, consistent with results from previous tests at trace concentrations (Table 2). SC1 was found to be a developmental disruptor, with an LOEC for morphological effects of 10 mg L⁻¹.

These results indicate that mortality is the most effective endpoint for identifying the acute hazards of SC2, whereas coagulation serves as a highly sensitive early indicator of the low-dose toxicity of SC3. Morphological malformations, though less frequent overall, serve as a critical product-specific endpoint for SC1. Ultimately, this comparative data underscores the severe toxicological variability among commercial formulations: SC2 demonstrates standard dose-dependent lethality, SC1 presents notable teratogenicity, and SC3 reveals unpredictable, non-monotonic hazards at trace environmental levels. A summary of the sunscreen dominant effects is shown in Table 4.

Table 4

A summary of sunscreen dominant effects on *Danio rerio*

<i>Sunscreen</i>	<i>Dominant effect</i>	<i>Dose pattern</i>	<i>Key observation</i>
SC1 (A)	Malformations	Moderate, peak at mid-dose	Sub-lethal developmental toxicity
SC2 (B)	Mortality	Monotonic (dose-dependent)	Highest acute toxicity
SC3 (C)	Mortality & coagulation	Non-monotonic	Highest toxicity at lowest dose

Note: SC1 - organic sunscreen, SC2 - mineral sunscreen, SC3 - hybrid sunscreen.

Discussion. The assessment of the developmental toxicity of three commercial sunscreen formulations (SC1-SC3) in *D. rerio* embryos, using OECD TG 236 protocols, revealed unique, product-specific hazard profiles. Sunscreen B (SC2) showed concentration-on-exposure-related acute toxicity with a peak mortality of more than 60% at 1000 mgL⁻¹. In contrast, Sunscreen C (SC3) exhibited severe non-monotonic effects, with increased mortality and coagulation at the lowest concentration of 1 mg L⁻¹, whereas no such effects were observed at higher concentrations. Sunscreen A (SC1) induced moderate mortality and the highest rates of morphological malformations (c. 10% at 10 mgL⁻¹). The tested compounds affected aquatic organisms in highly distinct ways. Our results document the complex risk potential of commercial sunscreen substances for aquatic life (Chatzigianni et al., 2022).

Ecological significance of non-monotonic toxicity. The most novel finding of this study was the pronounced non-monotonic dose-response relationship shown by Sunscreen C. In contrast to the classical toxicological paradigm, in which toxicity is expected to increase with concentration, Sunscreen C produced the highest mortality (51.33%) and coagulation responses at the lowest tested concentration (1 mg L⁻¹), with toxicity decreasing markedly at higher concentrations. Such an abnormal response suggests that its toxicity is not due to conventional cytotoxic mechanisms and may be mediated by endocrine-mediated and/or other receptor-mediated biological pathways.

Chemicals that act as endocrine disrupting chemicals (EDCs) can exhibit non-monotonic dose-response relationships. These unusual relationships can result from the chemical's unique interaction with endocrine receptors, and the subsequent endocrine-related feedback mechanisms, receptor desensitization, and the compensatory capabilities of endocrine-related physiological processes (Beausoleil et al., 2013; Jaskulak et al., 2025; Vandenberg et al., 2012). Sunscreen C is a commercial product formula consisting of more than one chemical ingredient. Thus, based on the results obtained for individual chemical ingredients of the formula, no conclusion can be drawn regarding the endocrine activity of this product formula. Interactions of UV filters with each other as well as with other formulation ingredients (e.g., preservatives, emulsifiers, antioxidants) cannot be predicted from the effects of individual chemicals (Kortenkamp et al., 2009; Backhaus & Faust, 2012; Caloni et al., 2021).

This work has significant ecological implications. First, even at very low constant concentrations of individual chemicals from sunscreen, releases to water from recreational

as well as from wastewater activities can give rise to environmentally toxic concentrations. These chemical concentrations at environmentally relevant exposure conditions are below the limits of detection by typical laboratory acute toxicity tests that have been conducted at high concentrations, and which have assumed a simple monotonic (i.e., increasing) dose-response relationship. To address these ecological risks, new methods for ecological risk assessment developed by the National Academies of Sciences, Engineering, and Medicine (2022) and by Raimondo (2024) need to be improved by using low-dose exposure tests, assessing toxicity of mixture toxicity, and accounting for non-linear biological effects.

Embryos and very young organisms can be sensitive to endocrine disrupting compounds since the process of embryogenesis is dependent on endocrine signals for organogenesis as well as for the formation of the cardiovascular, nervous, and reproductive systems. Therefore, toxicity to the embryo or very young organism can cause immediate death of the embryo or fetus, but also cause effects on growth and development, which could be long-term. These effects on growth and development could also express themselves in altered behavior and reduced reproduction with consequent decreased population size in subsequent generations. As has been noted for a number of chemical the potential for toxicity at low-dose exposures could be of greater concern than conventional concentration-dependent toxicity descriptions (Mitchelmore et al., 2019; Caloni et al., 2021; Jaskulak et al., 2025). Thus, the observed low-dose toxicity of Sunscreen C of potential ecological concern is of greater concern than the other investigated sunscreen formulations for which concentration-dependent toxicity was only found at high concentrations.

The data obtained for Sunscreen C show the difficulties in assessing the hazards of commercial sunscreen products using a dose-toxicity approach or by focusing on individual active chemical ingredients. Formulations are to be tested as complex mixtures, using environmentally relevant exposure concentrations. A mechanistic approach measuring the parent substances and their metabolites in conjunction with a set of molecular biomarkers, i.e., the ecotoxicogenomics toolbox (Chatzigianni et al., 2022; National Academies of Sciences, Engineering, & Medicine, 2022; Chavda et al., 2023), will be necessary to improve assessments of the ecological hazards of sunscreen active ingredients and design of environmentally safe chemicals for sun protection that follow principles of green chemistry.

Mechanistic insights within the aquaculture framework. While it is interesting to note, it is more important to understand the mechanisms by which these effects occur in embryos and larvae (i.e., whether they die). Commercial sunscreen is a complex mixture of substances, making it difficult to test individual components. Mortality in SC2 was largely dose-dependent due to the toxic effects of both organic and inorganic UV filters at high concentrations. Teratogenic effects in SC1 are suspected to result from endocrine disruption by octocrylene, another commonly used UV filter found in many commercial sunscreens. These effects may still be expressed in aquaculture systems and ultimately result in decreased production. Octocrylene is a widely used sunscreen chemical that may cause oxidative stress and alter gene expression, particularly in the hypothalamic-pituitary-gonadal (HPG) axis. Toxicity studies on octocrylene have suggested its developmental toxicity. In the *D. rerio* assay, octocrylene caused pericardial edema and tail curvature (He et al., 2019). In addition to octocrylene's potential toxic effects, parabens, often used as preservatives in sunscreens, cosmetics, and food additives, act as xenoestrogens and cause developmental toxicity during critical periods of embryonic development (Kunz et al., 2006). Commercial sunscreen mixtures comprise up to 12 individual chemicals, and their potential for enhanced toxicity in combination warrants consideration according to the principles of mixture toxicity (Backhaus & Faust, 2012; Kortenkamp et al., 2009). Exposure to environmental contaminants may reduce larval survival and productivity in aquaculture species, highlighting broader implications for aquatic food production (Alday-Sanz et al., 2020).

Positioning within existing literature. Previous studies on UV filters in sunscreen products have been identified to exhibit developmental toxicity, endocrine-disrupting activities, and oxidative stress in aquatic organisms (Fent et al., 2008; He et al., 2019; Mitchelmore et al., 2019). However, nearly all published research has focused on individual active ingredients, rather than on the complex chemical mixtures provided by manufacturers in pre-mixed form as commercial sunscreen products. Consequently, there is limited understanding of the combined and/or interactive effects of individual UV filter chemicals in most commercial sunscreen products.

Most commercial mixture formulations exhibit variable and unpredictable toxicity profiles, including non-monotonic effects that are not detected in single-chemical toxicity assessments. A case study of the recreational sunscreen C reveals potential low-density toxicity. Toxicity assessments of complex chemical mixtures and their effects on mixture composition should be considered, because toxicity predictions for mixtures based on the toxicity values of individual chemicals are generally unreliable (Kortenkamp et al., 2009; Backhaus & Faust, 2012).

Implications for ecological risk assessment and environmental protection. The findings of this study have direct implications for environmental risk assessment and regulatory frameworks. Current risk assessment approaches are largely based on the assumption of monotonic dose-response relationships and typically emphasize high-dose toxicity thresholds (Lagarde et al., 2015; National Academies of Sciences, Engineering, and Medicine, 2022). This ecological toxicity study on the effects of Sunscreen C on living organisms and ecosystems is relevant to environmental risk assessment and to future legislation and regulations governing the release of chemical substances into the environment (National Academies of Sciences, Engineering, and Medicine, 2022; Raimondo, 2024). The current risk assessment methods assume monotonic dose-response relationships and toxic effects showing a threshold for high-dose toxicity. However, the results obtained with Sunscreen C suggest that effects may occur at concentrations below the level predicted by current risk assessment methods, and thus risks might be underestimated, especially at ecologically relevant concentrations (Vandenberg et al., 2012; Beausoleil et al., 2013; Lagarde et al., 2015).

Future directions for regulators and environmental managers may include the use of low-dose testing approaches and non-linear dose-response models in hazard and risk assessments (National Academies of Sciences, Engineering, and Medicine, 2022; Raimondo, 2024). Assessments should ideally be based on the full chemical formulation rather than on individual active ingredients, because in the environment, exposures will occur as complex mixtures that may interact (synergistically or antagonistically) across a variety of biological compartments (Kortenkamp et al., 2009; Backhaus & Faust, 2012; National Academies of Sciences, Engineering, and Medicine, 2022).

Biodiversity conservation should consider the effects of contaminants on species, especially during sensitive life stages, since effects on individuals can translate into population-level consequences, including decreased recruitment, demographic shifts, and ecosystem impacts (Fent et al., 2008; Mitchelmore et al., 2019; Caloni et al., 2021). Previous studies have shown that two commonly used sunscreen chemicals, oxybenzone and butylparaben, can adversely affect *D. rerio* during early stages. Embryos exposed for four days to low environmental concentrations of either chemical exhibited decreased median survival, increased occurrence of behavioral aberrations, and decreased emergence of juveniles. Exposure also led to persistent growth and olfactory deficits lasting into adulthood (Kim et al., 2014; Jaskulak et al., 2025). Given the high frequency of recreational water use at lakes, rivers, and coastal sites worldwide, there is potential for continuous exposure of species to sunscreen chemicals in anthropogenically altered environments (Tsui et al., 2014; Gago-Ferrero et al., 2015; Caloni et al., 2021; National Academies of Sciences, Engineering, and Medicine, 2022).

Implications for product design and green chemistry. Toxicity data revealed significant variability among sunscreen products, with some formulations having potential hidden effects due to nonmonotonic or low-dose effects (Vandenberg et al., 2012; Beausoleil et al., 2013; Jaskulak et al., 2025). Utilization of green chemistry methods, which have been successfully applied in other sectors, could provide new approaches for developing alternative, safer sunscreens (Ahmed et al., 2017; National Academies of Sciences, Engineering, and Medicine, 2022; Chavda et al., 2023). Potential green chemistry strategies to create new, safe sunscreens include using chemical ingredients that are biodegradable, non-bioaccumulative, and lack evidence of endocrine activity (Ahmed et al., 2017; Chatzigianni et al., 2022; Jaskulak et al., 2025). Previous studies have also demonstrated that sunscreen ingredients may contribute to coral bleaching and alter microbial communities in marine ecosystems (Danovaro et al., 2008; Downs et al., 2016).

Future directions. Although existing data provide important insights into the developmental toxicity of complete sunscreen formulations, further research is required to validate environmental exposure concentrations using analytical methods. More information is currently available on the developmental toxicity of sunscreen formulations in higher organisms intended for human skin application than on their ecological risks. However, evidence suggests that many of these formulations are already entering aquatic environments, although much of this information is derived from interviews and surveys and requires validation through environmental monitoring.

Sunscreen products consist of complex chemical mixtures that may interact synergistically with each other and with other environmental contaminants, and may also exhibit synergistic or antagonistic effects in combination with endocrine-disrupting chemicals (Kortenkamp et al., 2009; Backhaus & Faust, 2012). Advanced analytical techniques such as high-performance liquid chromatography (HPLC) and, in particular, liquid chromatography-mass spectrometry (LC-MS), are suitable for assessing environmental exposure and investigating photodegradation and biotransformation processes of UV filters, which may exert toxicity through their metabolites (Gago-Ferrero et al., 2015; Ahmed et al., 2017). There is an urgent need for long-term exposure studies to evaluate the effects of UV filters as endocrine disruptors on fish reproduction over extended periods ranging from months to years (Fent et al., 2008). In addition, monitoring studies on the environmental occurrence of UV filters are essential for conducting realistic ecological risk assessments (Tsui et al., 2014). Recent advances in sunscreen formulation also emphasize balancing effective photoprotection with reduced environmental impacts (Fivenson et al., 2021). Environmental persistence and chronic exposure to UV filters remain important considerations for ecological risk assessment (Carvalho et al., 2014).

Conclusions. The toxicity of commercial sunscreen-containing products was evaluated using developing *D. rerio* embryos. Three commercial formulations (sunscreen A, B, and C) were tested, and significant developmental toxicity was observed. The LC₅₀ for acute toxicity of sunscreen B was 16 mg/L. Sunscreen A induced moderate sublethal effects across all tested concentrations, while sunscreen C exhibited a non-monotonic dose-response pattern, with higher toxicity observed at lower concentrations.

The results highlight the importance of low-dose exposure effects and suggest that current risk assessment approaches, largely based on monotonic dose-response assumptions and high-dose testing, may not fully capture the environmental risks posed by sunscreen-related contaminants. Non-monotonic responses, potentially associated with endocrine-disrupting activity, may contribute to toxicity at environmentally relevant concentrations.

These findings underscore the importance of incorporating mixture toxicity, low-dose exposure effects, and non-monotonic dose-response relationships into future ecological risk assessment frameworks for sunscreen products. Mixture toxicity remains a major challenge because interactions among formulation components may alter toxicological responses in aquatic organisms. Regulatory agencies should consider evaluating complete commercial formulations in addition to individual UV-filter ingredients, as formulation-specific interactions may significantly influence environmental toxicity.

From an industrial perspective, the development of next-generation sunscreen products should prioritize green chemistry principles by using biodegradable, non-bioaccumulative, and environmentally safer ingredients with minimal endocrine-disrupting potential. Collectively, these approaches will support evidence-based environmental policies, promote sustainable product innovation, and contribute to the long-term protection of aquatic biodiversity while maintaining effective photoprotection for human health.

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