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Ecotoxicological Effects of Cosmetic Formulas Containing Chemical and Mineral UV Filters on *Seriatopora hystrix* Fragments

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Abstract

Background: Over the last few years sunscreen products have been suspected to be harmful to corals, especially because of their putative negative impact on symbiotic microalgae housed by these cnidarians. Previous publications reported that minerals or chemical UV filters could induce the release of microalgae from corals inducing their bleaching. The study of the ecotoxicity of finished cosmetic products containing these filters is important. Objectives: We sought to assess *ex vivo* the toxicity of five emulsions containing UV-filters on coral cuttings of *Seriatopora hystrix*. Materials and Methods: Coral cuttings were put in contact with 5 different emulsions containing UV-filters. The toxicity readout was the ability to induce polyp retraction and/or fragment bleaching of the coral cuttings of *Seriatopora hystrix*. Results: In our experimental conditions, none of the five tested formulas neither induced any significant polyp retraction nor triggered fragment bleaching of the coral. Conclusions: The five tested emulsions containing UV-filters did not modify coral cuttings. *In vivo*, larger tests are necessary to verify the results of this *ex vivo* pilot study.

Keywords

Sunscreens, Chemical UV Filters, Mineral UV Filters, Coral, Polyp Retraction, Coral Bleaching, *Seriatopora hystrix*, Ecotoxicology

1. Introduction

Corals are cnidarians living in symbiosis with microalgae that they host. These

microalgae ensure coral to flourish and they give to them their colors. Over the past few years, coral reefs were reportedly affected by the presence of chemical compounds contained in sunscreens. Significant amounts of sunscreen components were found at sea surface [1] and/or in sea waters surrounding coral reefs [2] [3] [4] [5] [6]. Several studies concerning the effect of UV filters on coral bleaching report a detrimental action of chemical and/or mineral UV filters used in cosmetic products, notably on microalgae living in symbiosis with corals. Different experimental approaches were used in these studies: assessment of a direct cytotoxic and/or bleaching effect of four benzophenone UV filters on two coral species [7]; analysis of other components than UV filters contained in the sunscreen formulas that could damage the environment by increasing the bioavailability of UV filters to coral and exacerbate their toxicity [8]; other authors showed that sunscreen products caused coral bleaching by promoting viral infections [9].

However, due to the heterogeneity of the experimental designs, evaluating the actual impact of sunscreen products' dispersion into seawater and their precise action on coral reefs bleaching and trophicity remains a difficult task.

Based on the literature showing that several ingredients present in sunscreen formulas may exert synergistic toxic effects, we chose to use an experimental approach that could evaluate at their best their ecotoxicity.

Objectives: To assess *ex vivo* several formulations of external photoprotection products containing UV-filters on the coral *Seriatopora hystrix.*

2. Materials and Methods

We assess 5 sunscreens-emulsions containing different combinations of 10 UV-filters (8 chemical filters and 2 mineral filters) emulsified with excipients, focusing on two parameters: polyp retraction and bleaching of a widespread coral species, *Seriatopora hystrix* (native to the Indo-Pacific region which extends from East Africa, Madagascar and the Red Sea through the Indian Ocean to tropical Australia, Japan, the South China Sea and the island groups in the West and Central Pacific). We exposed coral fragments to very high products' concentrations (compared to those reported in seawater of different regions of the world) (1 - 6); these high concentrations used in this study allowed us to also evaluate the effect of a possible bioaccumulation of UV filters in corals.

3. Tested Emulsions INCI Composition

UV filters are highlighted

Emulsion 1 (3 chemical filters)

Aqua (water) - dicaprylyl carbonate - methylene bis-benzotriazolyl tetramethylbutylphenol[nano] - butyl methoxydibenzoylmethane - ethylhexyl triazone - nylon-12 - c20-22 alkyl phosphate - glycerin - c20-22 alcohols - decyl glucoside - butylene glycol - dimethicone - xanthan gum - chlorphenesin - parfum (fragrance) - triacontanyl pvp - benzoic acid - tetrasodium edta - o-cymen-5-ol - tocopheryl

acetate - sodium hydroxide - propylene glycol - citric acid - glycyrrhiza inflata root extract.

Emulsion 2 (5 chemical filters)

Aqua (water) - dicaprylyl ether - octocrylene - ethylhexyl methoxycinnamate - butyl methoxydibenzoylmethane - ethylhexyl salicylate - nylon-12 - peg-30 dipolyhydroxystearate - cyclopentasiloxane - cyclohexasiloxane - bis-ethylhexyloxyphenol methoxyphenyl triazine - glycerin - sodium chloride - phenoxyethanol - glucose - chlorphenesin - tetrasodium edta - citric acid - hydrogenated polydecene - tocopheryl acetate - trehalose - ascorbyl tetraisopalmitate - bht - polyquaternium-51.

Emulsion 3 (5 chemical filters)

Aqua (water) - diethylamino hydroxybenzoyl hexyl benzoate - ethylhexyl methoxycinnamate - ethylhexyl triazone - isodecyl neopentanoate - dicaprylyl carbonate - ethylhexyl salicylate - bis-ethylhexyloxyphenol methoxyphenyl triazine - glycerin - triacontanyl pvp - decyl glucoside - dimethicone - c20-22 alkyl phosphate - butylene glycol - phenoxyethanol - c20-22 alcohols - glucose - chlorphenesin - parfum (fragrance) - xanthan gum - hydrogenated polydecene - o-cymen-5-ol - tocopheryl acetate - trehalose - sodium hydroxide - propylene glycol - ascorbyl tetraisopalmitate - bht - ci75470 (carmine) - citric acid - tocopherol - sodium citrate - polyquaternium-51.

Emulsion 4 (6 chemical filters)

Dicaprylyl carbonate - isohexadecane - diethylamino hydroxybenzoyl hexyl benzoate - ethylhexyl methoxycinnamate - butyl methoxydibenzoylmethane - ethylhexyl salicylate - ethylhexyl triazone - bis-ethylhexyloxyphenol methoxyphenyl triazine - hydrogenated polydecene - parfum (fragrance) - tocopheryl acetate - tocopherol - ascorbyl tetraisopalmitate - bht.

Emulsion 5 (2 mineral filters)

Dicaprylyl carbonate - titanium dioxide[nano] - aqua (water) - zinc oxide[nano] - neopentyl glycol diheptanoate - peg-30 dipolyhydroxystearate - sodium chloride - butylene glycol - nylon-12 - polyglyceryl-3-diisostearate - alumina - stearic acid - dimethicone - glycerin - octyldodecanol - magnesium sulfate - triethoxycaprylylsilane - cera alba (beeswax) - glucose - xanthan gum - disteardimonium hectorite - hydrogenated polydecene - tocopheryl acetate - trehalose - propylene carbonate - ascorbyl tetraisopalmitate - citric acid.

4. Coral Assay

- Specie: *Seriatopora hystrix*, Dana, 1846. This specie is used as cuttings (3 cm long meaning a hundred of polyps).

Assay conditions

- Test duration: 96 h.
- Renewal of tested solutions: at 48 h.
- Medium: Synthetic seawater.
- Temperature: 25˚C ± 1˚C.

- Lightning: Day/night cycle 12 h/12 h.
- Aeration: None.
- Recipe and test volume: Plastic recipes; 200 ml by assay condition.
- Number of cutting per loading rate: 3 cuttings spread in 3 replicates of 1 cutting.

The assay contains 7 loading rates following a geometrical serie (see below).

Evaluation of polyp retraction and fragment bleaching

Organisms' preparation

Seriatopora hystrix cuttings were first placed in aquarium containing the same water which is used for the test. We wait that cuttings recover from possible stress related to transport or water change, the recovery was seen by re-blooming of polyps, at 3 hours.

After recovery, the cuttings were placed one by one in the test media in the absence (control), or in the presence of increasing concentrations (0, 17, 23, 33, 48, 64, 82 and 100, 33 µg/L) of $CuSO_4$ used as a positive control, or of increasing concentrations (0; 3.2; 5.6; 10, 18, 32, 56 et 100 mg/L) of the five tested emulsions.

Tested recipes were not watertight in order to allow the light and CO_2 penetration they were also covered to prevent contamination from air and to reduce the evaporation of water (which is done by using Petri dish lids).

The containers were then put in an oven at 25˚C, with a lighting set on a day/night cycle 12/12 h.

Observations and data treatment

Before the experiment (Th0 and after 48 hours (T48 h) and 96 hours (T96 h), cuttings were observed with a binocular magnifying glass to identify those with retracted polyps, and cuttings with whitening. Photos were taken to facilitate the data treatment.

From the repeated test data for each loading rate, were determined the Lowest Observed Effect Concentration (LOEC1) and No Observed Effect Concentration (NOEC2) for the two parameters evaluated, namely the retraction of the polyps and the whitening of the cuttings.

The two parameters taken into account focused on evaluation of two types of sublethal responses: an early response by observing the retraction of polyps, and a late response corresponding to coral bleaching, which can lead to death.

Calculation method:

For the determination of the LOEC and NOEC were used Bonferroni tests and Toxcalc™ software (Tidepool Scientific®).

5. Results and Discussion

As shown in Table 1, in the selected experimental conditions and regardless of the tested concentration (3.2 to 100 mg/ml), none of the five emulsions induced any polyp retraction of the *Seriatopora hystrix* fragments. In the same experimental conditions, $CuSO_4$ used as a positive control, induced the polyp retraction of the *Seriatopora hystrix* fragments from a concentration of 33 µg/L.

Table 1. Effect of the 5 tested emulsions on the polyp retraction of *Seriatopora hystrix* after an incubation of 96 hours. NOEC = No Observed Effect Concentration. LOEC = Lowest Observed Effect Concentration. $CuSO_4$ was used as a "positive control".

Ecotoxicological Descriptor	Emulsion 1	Emulsion 2	Emulsion 3	Emulsion 4	Emulsion 5	$CuSO_4$
NOEC	100 mg/L	100 mg/L	100 mg/L	100 mg/L	100 mg/L	33 µg/L
LOEC	>100 mg/l	>100 mg/l	>100 mg/l	>100 mg/l	>100 mg/l	33 µg/L

As shown in **Table 2** and in **Figure 1**, in the selected experimental conditions and regardless the tested concentration (3.2 to 100 mg/ml), none of the five emulsions induced any bleaching of the *Seriatopora hystrix* fragments. In the same experimental conditions, $CuSO_4$ used as a positive control, induced the bleaching of the *Seriatopora hystrix* fragments from concentrations of 33 µg/ml.

These results showed the non-cytotoxicity of the five tested emulsions on the coral specie used in our tests.

Moreover, the final UV-filters concentrations used our tests were very high compared to those reported in seawater of different regions of the world. So we can also discuss that in this *ex vivo* model, the deleterious effects following bio-accumulation of UV filters in these emulsions on coral was absent.

Our results are similar with those published by other authors who notably detect bleaching and cytotoxic effects of UV filters used alone or incorporated in cosmetic formulas.

In order to explain the differences observed between our results and those published (1 - 6), we can first recall that we did not use the same coral specie than them. However, as we tested UV filters in the five emulsions at concentrations 100,000 to 200,000-fold higher than that it was measured in several coral reefs by several teams [2] [3] [4] [5] [6], it seems difficult to explain such a difference only by the coral specie we used. There are no data showing that this specie of coral would be less sensitive than others.

On another hand, we tested UV filters in emulsions which were subsequently diluted in water. He et al used basically the same protocol in 2019 [8]. Surprisingly they observed opposite effects and concluded to deleterious effect of the products they tested, even when their products were two-fold less concentrated than ours. The underlying cause of this discrepancy could be that UV-filters containing emulsions do not display the same quality in term of ecotoxicology. Excipients and other factors could play an important part.

In this study we considered as important 1) to evaluate the ecotoxicological impact of UV filters within emulsions and not by testing them alone, and 2) that all the UV filters emulsions do not display the same quality. Finally, it seems also difficult to affirm that cosmetic sunscreen products could be responsible, alone, for the bleaching and the dieback of coral reefs; various environmental factors and/or other pollutants could also be incriminated. We also are aware of the fact that the *ex vivo* results of this pilot study cannot be completely extrapolated to *in vivo*, more complex environmental conditions.

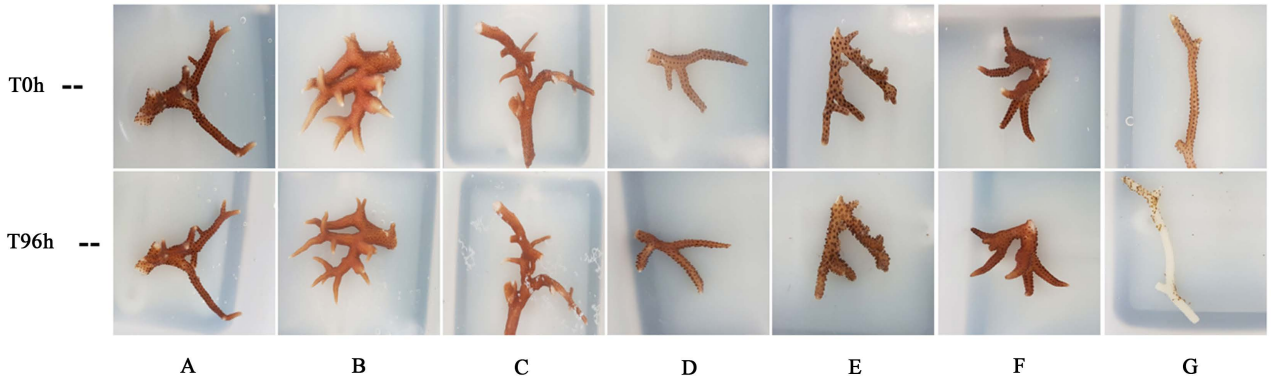

Figure 1. Effect of the 5 tested emulsions on the bleaching of *Seriatopora hystrix* fragments after an incubation of 96 hours. A = Control, B to F = The 5 tested emulsions (1 to 5, respectively), G = Positive control ($CuSO_4$ at 33 mg/L).

Table 2. Effect of the 5 tested emulsions on the bleaching of *Seriatopora hystrix* fragments after an incubation of 96 hours. NOEC = No Observed Effect Concentration. LOEC = Lowest Observed Effect Concentration. $CuSO_4$ was used as a "positive control".

Ecotoxicological Descriptor	Emulsion 1	Emulsion 2	Emulsion 3	Emulsion 4	Emulsion 5	$CuSO_4$
NOEC	100 mg/L	100 mg/L	100 mg/L	100 mg/L	100 mg/L	23 µg/L
LOEC	>100 mg/l	>100 mg/l	>100 mg/l	>100 mg/l	>100 mg/l	33 µg/L

6. Conclusion

In this *ex vivo* study, the contact of five emulsions containing 8 chemical UV filters and 2 mineral UV-filter did not modify coral cuttings of *Seriatopora hystrix*. *In vivo*, larger tests are necessary to verify the results of this *ex vivo* pilot study.

Conflicts of Interest

These studies were funded by Laboratoires Dermatologiquesd'Uriage, France.

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