Journal of Cosmetics, Dermatological Sciences and Applications, 2024, 14, 98-111
https://www.scirp.org/journal/jcdsa
ISSN Online: 2161-4512
ISSN Print: 2161-4105

New Insights in the Biodegradability and the Ecotoxicological Effects of Solar Products Containing Mineral and Chemical UV-Filters on Marine Zoo- and Phytoplanktons: An *in silico* and *in vitro* Study

Jean-Claude Hubaud[1], Karim Mekideche[2], Jean-Eric Branka[2], Luc Lefeuvre[3*]

[1]HELIOSCIENCE, Cité de la cosmétique, 2 rue Odette Jasse, Marseille, France
[2]EPHYSCIENCE, 498ter route de Saint Joseph de Porterie, Nantes, France
[3]Laboratoires Dermatologiques d'Uriage, Neuilly-sur-Seine, France
Email: *luc.lefeuvre@uriage.com

How to cite this paper: Hubaud, J.-C., Mekideche, K., Branka, J.-E. and Lefeuvre, L. (2024) New Insights in the Biodegradability and the Ecotoxicological Effects of Solar Products Containing Mineral and Chemical UV-Filters on Marine Zoo- and Phytoplanktons: An *in silico* and *in vitro* Study. *Journal of Cosmetics, Dermatological Sciences and Applications*, **14**, 98-111.
https://doi.org/10.4236/jcdsa.2024.141007

Received: February 27, 2024
Accepted: March 25, 2024
Published: March 28, 2024

Abstract

Background: Cosmetic formulations, and particularly solar products which contain mineral and chemical UV-filters, are often suspected of causing harmful effects on marine fauna and flora. After the publication of our work in 2019 concerning the ecotoxicological effects of such formulations on corals (*Seriatopora hystrix*), we here provide some new information about the biodegradability and the ecotoxicological effects of these products on marine zoo- and phytoplankton. Therefore, we choose to realize *in silico* and *in vitro* studies of the biodegradability of several solar products but also to evaluate the ecotoxicological effects of these products on one phytoplankton, *i.e. Phaeodactylum tricornutum*, and one zooplankton, *i.e. Acartia tonsa*, of a great importance for sea species survival (notably as sources of food). **Materials and methods:** Two different approaches were used to study the biodegradability of the tested products: One *in silico* method and an *in vitro* one. 2 solar products were involved in the *in silico* study which consisted in the determination of the degradation factor (DF) of each ingredient of the tested formulas in order to finally obtain their estimated biodegradability percentage. Already available data concerning each ingredient coupled to a computer model developed with one of our partners were used to achieve this study. The *in vitro* study involved 8 formulas containing UV-filters and was led by following the OECD 301 F guidelines. Ecotoxicological studies of 7 of the formulas containing UV-filters were for their part realized by following the

ISO 10253 guidelines for the experiments led with *Phaeodactylum tricornutum*, and the ISO 14669 guidelines for the experiments led with *Acartia tonsa*. In these studies, the effect of each tested product on crustaceans' mortality and algal growth inhibition was assessed. **Results:** The *in silico* study predicted that formulas containing chemical UV-filters display a high biodegradability (superior to the threshold value of 60% given by the OECD 301 F guidelines). In the *in vitro* part of our work, the 8 tested formulas showed a biodegradability slightly inferior to the one predicted in the *in silico* experiments. Therefore, in order to evaluate if these calculated biodegradability value could have significant harmful effects on zoo- or phytoplankton, we studied the effect of our products regarding the growth inhibition on *Phaeodactylum tricornutum* and the mortality on *Acartia tonsa*. In this last part of the study, all the tested products were classified as "non ecotoxic" following an internal classification based on Part 4 entitled "Environmental Hazards" of Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 9th edition (2021). **Conclusions:** These results are notably in line with those published by our teams in 2019 on the effects of solar cosmetic products on corals and seem to confirm that formulas containing mineral and chemical UV-filters can be daily used without displaying significant noxious effects on marine fauna and flora.

Keywords

UV-Filters, Chemical UV-Filter, Biodegradability, Ecotoxicology, *in silico*, *in vitro*, Zooplankton, Phytoplankton, *Phaeodactylum tricornutum*, *Acartia tonsa*

1. Introduction

During the last decades, UV radiations have shown numerous noxious effects on human skin because of their ability to generate reactive oxygen species (ROS) [1] in cutaneous tissues. As a consequence, they largely contribute to various skin pathologies including inflammation, degenerative aging and cancer (for a review, see [2]). For these reasons, a large number of products containing UV-filters (mineral and/or chemical) have been developed by the dermo-cosmetic industry. Nevertheless, even if these products are able to protect (at various levels following their efficacy) our skin and more largely our whole organism (for a review, see [3]), they are also suspected of causing harmful effects on marine fauna and flora [4] [5] due to coastal tourism. However, *in vitro* studies reporting these noxious effects of UV-filters are still small and remain difficult to analyze because of the variability of the experimental models used and of the tested UV-filters concentrations; it is indeed very difficult to estimate the concentration of these filters in the seawater.

After the publication in 2019 of our work concerning the ecotoxicological effect of mineral and chemical UV-filters on *Seriotopora Hystrix* coral fragments [6], we choose to bring here additional information concerning these effects on

other micro-organisms: a zooplankton (*Acartia tonsa*), and a phytoplankton (*Phaeodactylum tricornutum*) of great importance in sea species survival (notably as sources of food).

Acartia tonsa is a cryptogenic species with an almost worldwide distribution in coastal subtropical and temperate waters. This copepod is often a dominating species in coastal and estuarine plankton which feeds on nauplii of other copepods and on phytoplankton including diatoms as well as flagellates [7]. This micro-organism so largely contributes to estuary and coastal seawater ecological equilibrium and it is then very often included in ecotoxicological studies concerning marine environment.

Notably due to its ease of culture and accessibility to reverse genetics approaches, *Phaeodactylum tricornutum* has become a very widespread experimental model used to better understand the biology of diatoms [8]. And because of its great importance in sea species survival, it is also chosen by numerous researchers to study the putative impact of chemicals substances on marine ecosystems.

We so choose here to pursue the work realized by our teams in 2019 with UV-filters and corals by evaluating the biodegradability of solar products containing mineral and chemical UV-filters and their impact on marine ecosystems by using experimental models calling for *Acartia tonsa* and *Phaeodactylum tricornutum*. Thus, after evaluating the biodegradability of cosmetic products containing chemical UV-filters through *in silico* and *in vitro* studies, we studied the ecotoxicological effects of this type of products on *Phaeodactylum tricornutum* and *Acartia tonsa* by following ISO guidelines N˚ 10253 and 14669, respectively.

2. Materials and Methods

2.1. Tested Formulas Composition

Formula 1

C12-15 alkyl benzoate, dicaprylyl carbonate, dibutyl adipate, diethylamino hydroxybenzoyl hexyl benzoate, ethyl hexyl triazone, heptyl undecyclenate, bis-ethylhexyloxyphenol methoxyphenyl triazine, fragrance, tocopheryl acetate, tocopherol, haematococcus pluvialis extract, olea europaea fruit oil, glycine max oil

Formula 2

C12-15 alkyl benzoate, dicaprylyl carbonate, dibutyl adipate, diethylamino hydroxybenzoyl hexyl benzoate, ethyl hexyl triazone, diethylhexyl butamido triazone, heptyl undecyclenate, bis-ethylhexyloxyphenol methoxyphenyl triazine, fragrance, tocopheryl acetate, tocopherol, haematococcus pluvialis extract, olea europaea fruit oil, glycine max oil

Formula 3

Aqua, dicaprylyl carbonate, methylene bis-benzotriazolyl, tetramethylbutylphenol[nano], ethylhexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol, dicaprylate/dicaprate, C12 - C15 alkyl benzoate, di-

isopropyl adipate, bis-ethylhexylophenol, methoxyphenyl triazine, heptyl undecylenate, triacontanyl PVP, glycerin, C20 - C22 alkyl phosphate, C20 - C22 alcohols, decyl glucoside, butylene glycol, glucose, fragrance, benzoic acid, tetrasodium EDTA, xanthan gum, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, trehalose, propylene glycol, sodium hydroxide, citric acid, ascorbyl tetraisopalmitate, tocopherol, spirulina platensis extract, polyquaternium-51

Formula 4

Aqua, dicaprylyl carbonate, methylene bis-benzotriazolyl, tetramethylbutylphenol[nano], ethylhexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol, dicaprylate/dicaprate, silica, C12 - C15 alkyl benzoate, diisopropyl adipate, bis-ethylhexylophenol, methoxyphenyl triazine, triacontanyl PVP, glycerin,, C20 - C22 alkyl phosphate, C20 - C22 alcohols, decyl glucoside, butylene glycol, glucose, xanthan gum, fragrance, coco-glucoside, benzoic acid, tetrasodium EDTA, coconut alcohol, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, trehalose, propylene glycol, sodium hydroxide, citric acid, ascorbyl tetraisoplamitate, tocopherol, spirulina platensis extract, polyquaternium-51.

Formula 5

Aqua, dicaprylyl carbonate, methylene bis-benzotriazolyl, tetramethylbutylphenol[nano], ethylhexyl triazone, butyl methoxydibenzoylmethane, dimethicone, diisopropyl sebacate, propylene glycol, dicaprylate/dicaprate, C12 - C15 alkyl benzoate, diisopropyl adipate, bis-ethylhexylophenol, methoxyphenyl triazine, silica, triacontanyl PVP, glycerin,, C20 - C22 alkyl phosphate, ascorbyl tetraisopalmitate, C20 - C22 alcohols, decyl glucoside, maltodextrin, butylene glycol, glycyrrhetinic acid, glucose, xanthan gum, coco-glucoside, sucrose dilaurate, benzoic acid, tetrasodium EDTA, coconut alcohol, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, sodium cocoyl glutamate, trehalose, propylene glycol, pisum sativum extract, citric acid, sodium hydroxide, tocopherol, spirulina platensis extract, polyquaternium-51.

Formula 6

Aqua, dicaprylyl carbonate, methylene bis-benzotriazolyl, tetramethylbutylphenol[nano], ethylhexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol, dicaprylate/dicaprate, hydrogenated palm kernel glycerides C12 - C15 alkyl benzoate, disopropyl adipate, bis-ethylhexylophenol, methoxyphenyl triazine, silica, triacontanyl PVP, Cl 77492 (iron oxides), glycerin,, C20 - C22 alkyl phosphate, C20 - C22 alcohols, decyl glucoside, butylene glycol, hydrogenated palm glycerides, glucose, Cl 77491 (iron oxides), xanthan gum, fragrance, coco-glucoside, benzoic acid, tetrasodium EDTA, coconut alcohol, triethoxycaprylylsilane, hydrogenated polydecene, tocopheryl acetate, sucrose dilaurate, benzoic acid, o-cymen-5-ol, Cl 77499 (iron oxides), trehalose, propylene glycol, citric acid, sodium hydroxyde, ascorbyl tetraisopalmitate, tocopherol, spirulina platensis extract, polyquaternium-51.

Formula 7

Aqua, dicaprylyl carbonate,, C12-C15 alkyl benzoate, diethylamino hydroxybenzoyl hexyl benzoate, ethylexyl triazone, diethylhexyl butamido triazone,

heptyl undecylenate, dimethicone, silica, bis-ethylhexyloxyphenol methoxyphenyl triazine, Polyglyceryl-6 polyhydroxystearate, glycerin, magnesium stearate, polyglyceryl-6 polyricinoleate, hydroxyacetophenone, glucose, benzoic acid, glyceryl behenate, tocopheryl acetate, trehalose, sodium hydroxide, tocopherol, spirulina platensis extract, haematococcus pluvialis extract, olea europaea fruit oil, ascorbyl palmitate, glycine max oil.

Formula 8

Aqua, dicaprylyl carbonate,, methylene bis-benzotriazolyl tetramethylbutylphenol[nano], ethylexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol dicaprylate/dicaprate, C12 - C15 alkyl benzoate, diisopropyl adipate, bis-ethylhexyloxyphenol methoxyphenyl triazine, heptyl undecylenate, triacontanyl PVP, glycerin, C20 - C22 alkyl phosphate, C20 - C22 alcohols, decyl glucoside, butylene glycol, glucose, fragrance, benzoic acid, tetrasodium EDTA, xanthan gum, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, trehalose, propylene glycol, sodium hydroxide, citric acid, ascorbyl tetraisopalmitate, tocopherol, spirulina platensis extract, polyquaternium-51.

Formula 9

Aqua, dicaprylyl carbonate,, methylene bis-benzotriazolyl tetramethylbutylphenol[nano], ethylexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol dicapylate/dicaprate, C12 - C15 alkyl benzoate, diisopropyl adipate, bis-ethylhexyloxyphenol methoxyphenyl triazine, heptyl undecylenate, triacontanyl PVP, glycerin, C20 - C22 alkyl phosphate, C20 - C22 alcohols, decyl glucoside, butylene glycol, glucose, fragrance, benzoic acid, tetrasodium EDTA, xanthan gum, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, trehalose, propylene glycol, sodium hydroxide, citric acid, ascorbyl tetraisopalmitate, tocopherol, spirulina platensis extract, polyquaternium-51.

Formula 10

Octyldodecanol, titanium dioxide[nano], synthetic wax, bis-diglyceryl polyacyladipate-2, butylene glycol cocoate, zinc oxide[nano], beeswax, titanium dioxide, hydrogenated castor oil, dipentaerythrityl tetrahydroxystearate/tetraisostearate, alumina, aqua, mica, ricinus communis (castor) seed oil, tocopheryl acetate, butyrospermum parkii (shea) butter, iron oxides, triethoxycaprylylsilane, iron oxides, ascorbyl palmitate.

Formula 11

Aqua, dicaprylyl carbonate, methylene bis-benzotriazolyl tetramethylbutylphenol[nano], ethylhexyl triazone, butyl methoxydibenzoylmethane, diisopropyl sebacate, propylene glycol dicaprylate/dicaprate, silica, C12 - C15 alkyl benzoate, diisopropyl adipate, bis-ethylhexyloxyphenol methoxyphenyl triazine, triacontanyl PVP, glycerin, C20 - C22 alkyl phospate, C20 - C22 alcohols, decyl glucoside, butylene glycol, glucose, xanthan gum, fragrance, cocoglucoside, benzoic acid, tetrasodium EDTA, coconut alcohol, hydrogenated polydecene, tocopheryl acetate, o-cymen-5-ol, trehalose, propylene glycol, sodium hydroxide, citric acid, ascorbyl tetraisopalmitate, tocopherol, spirulina platensis extract, polyquaternium-51, octyldodecanol, titanium dioxide[nano], synthetic wax, bis-diglyceryl

polyacyladipate-2, butylene glycol cocoate, zinc oxide[nano], beeswax, titanium dioxide, hydrogenated castor oil, dipentaerythrityl tetrahydroxystearate/tetraisostearate, alumina, aqua, mica, ricinus communis (castor) seed oil, tocopheryl acetate, butyrospermum parkii (shea) butter, iron oxides, triethoxycaprylylsilane, iron oxides, ascorbyl palmitate.

2.2. Method Used in the *in silico* Biodegradability Studies

2.2.1. Principle

The principle of our *in silico* biodegradability study was to identify the Degradation Factor (DF) of each ingredient present in the tested formula in order to obtain estimated biodegradability percentage using a computer model developed with one of our partners.

2.2.2. Methodology

Data on the biodegradability of substances are compiled into a database and are obtained from: - OECD 301 and/or OECD 310 ready biodegradability tests and/or OECD 302 inherent biodegradability tests (empirical data);

- QSAR models (predictive data)
- The functionality of the substance when no empirical or predictive data exist.

These data make it possible to obtain the DF of each substance present in the tested formulas.

The definition of DF is based on the European Ecolabel and can take 4 values:

- 0.05 for ready biodegradable substances,
- 0.15 for biodegradable substances,
- 0.5 for inherent biodegradable substance,
- 1.0 for persistent substances.

If the tested formula contains inorganic ingredients like TiO2 and/or ZnO, the value 1.0 will be applied to the DF. The estimated biodegradability percentage of the tested formula is then calculated.

2.3. OECD 301 F guidelines Used in the *in vitro* Biodegradability Studies

2.3.1. Principle

The biodegradability test according to OECD 301F method is based on the measurement of the oxygen consumed by bacteria to mineralize organic substances, *i.e.*, the biochemical oxygen demand (BOD). N-allylthiourea (ATU) is used to inhibit nitrification and prevent oxygen uptake resulting from ammonium oxidation. The net BOD is obtained by subtracting the BOD due to the endogenous activity of the bacteria (inoculum control) from the gross BOD obtained for the test sample. The biodegradability rate (in percent) is calculating through the ratio of the net BOD to the theoretical oxygen demand (ThOD) provided by test sample. The ThOD corresponds to the theoretical quantity of oxygen needed for the complete mineralization of organic substances. ThOD is obtained by calculation based on the elemental composition of the sample or by

the determination of the chemical oxygen demand (COD) of an aqueous preparation of the sample.

2.3.2. Chemicals

All chemicals were analytical grade and purchased from Merck group or Thermo Fisher Scientific. The analytical grade gases were provided by Air Liquid or Linde. The ready-to-use tube reagents for the determination of the chemical oxygen demand were supplied by Hach. High purity water complying with the grade 3 of EN-ISO 3696 and commercialized by Carlo Erba was used.

2.3.3. ThOD Determination

ThOD was systematically obtained by calculation based on elemental analysis results. In one case, ThOD was also obtained through the COD determination of an aqueous preparation of the sample.

CHNSO analysis was performed by an ISO 17025 accredited laboratory (SGS France) or by SCANAE. Mass percentage of elements were determined using combustion followed by gas chromatographic separation and thermal conductivity or infrared detection for CHNS while pyrolysis followed by gas chromatographic separation and thermal conductivity or infrared detection was used for O. Helium was used as carrier gas. Methionine and aspartic acid were used as calibration standards, respectively, for CHNS and O. Based on the CHNSO composition, the ThOD (in mgO_2/g of sample) was obtained by calculation using the equation proposed by the OECD 301 guidelines.

An aqueous preparation containing a known concentration of the sample is made. The COD of this preparation is measured according to the ISO 15705 standard using a LT200 thermoreactor and a DR3900 spectrophotometer from Hach. The ThOD is obtained by calculating the ratio of the COD (in mgO_2/L) to the mass concentration of the sample (in g/L) in the aqueous preparation.

2.3.4. Biodegradability Tests Protocole

The biodegradability tests were carried out according to the OECD 301F method (manometric respirometry) using the OxiTop system from WTW. A known mass of the test sample was directly introduced into the test vial and mixed with the mineral medium, N-Allylthiourea (ATU) and the bacterial inoculum. In addition, an inoculum control (mineral medium, ATU and inoculum) and a procedure control (sodium acetate, mineral medium, ATU and inoculum) were carried out in parallel. The mass of sample or sodium acetate added to the test vial was set to achieve an initial ThOD of 50 mgO_2/L. Test samples and controls were analysed in triplicate. The inoculum was obtained from urban wastewater supplying the sewage treatment plant of Montpellier metropolitan area (Hérault, France). This sewage treatment plant (500,000 people-equivalent) mainly treats domestic wastewater. Wastewater was collected within 24 hours prior to analysis and decanted before the supernatant was used for inoculation. The inoculation rate did not exceed 100 mL/L of mineral medium and the suspended solids supplied to the test vial by the inoculum were below 30 mg/L dry weight. The tests

took place at 20˚C ± 1˚C, under continuous magnetic stirring, for 28 d. The BOD was measured daily.

Concerning the validity criteria, the biodegradability rate of sodium acetate was above 60% after 14 d and was close to 100% after 28 d. The BOD of the inoculum control was in the range of 5 mgO_2/L after 28 d. The maximum difference between replicates never exceeded 20% biodegradability.

2.4. *In vitro* Ecotoxicology Studies Concerning Phaeodactylum Tricornutum

2.4.1. Purpose

Evaluate the ecotoxicity of a cosmetic formula on a marine algae specie (*Phaeodactylum tricornutum*) by assessing algal growth inhibition after 72 hours.

2.4.2. Normative References

ISO 10253 (2016) - Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*.

2.4.3. Principle

Algae from exponentially growing cultures are exposed to various concentrations or loading rates of the tested formula, and to a control medium (synthetic sea water). After 72 h of exposure without changing the medium (static assay), the concentration or loading rate that induces a 50% reduction of algal growth is determined.

2.4.4. Analytical Series Presentation

Number of flasks

An analytical series includes:

- 6 replicates with synthetic sea water (negative control);
- 3 replicates per tested concentrations or loading rates.

The assay includes at least 5 concentrations or loading rates, in a geometric series: 100 mg/l, 56 mg/l, 32 mg/l, 18 mg/l, 10 mg/l, 5.6 mg/L, 3.2 mg/l

Reference substance

3,5-dichlorophénol (3.5-DCP) is used as a reference substance to check the tested organism's sensitivity during the test.

2.4.5. Test Procedure

Preparation of the tested sample

For water-soluble formula:

A given quantity of the tested formula corresponding to the desired concentration to be tested is placed directly in the test medium containing the organisms.

For non-water-soluble formula

A Water Accommodated Faction (WAF) is prepared by putting in contact a quantity of the cosmetic formula to be tested with the test medium (growth medium) at a given loading rate, in a closed flask, under orbital agitation for 24 hours at approximately 110 revolutions per minute, at 20˚C ± 2˚C. The aqueous fraction of each loading rate (WAF) is then recuperated. The WAF are used raw

and immediately for the test and experimental results are expressed in loading rates.

Pre-culture

Algae are placed in growth medium (synthetic sea water and nutrients) 3 to 4 days before testing in order to have algae in exponential growth phase.

2.4.6. Inoculation and Incubation

The algae from the pre-culture are placed in glass vessels (20 mL per replicate) in order to get an initial cellular density of 104 cells/ml. Each test condition (corresponding to each concentration or loading rate to be tested) is prepared and the vessels are incubated at 20˚C ± 2˚C under continuous illumination during 72 h.

2.4.7. Observations and Data Treatment

After 72 h of incubation at 20˚C ± 2˚C, the cell density of each test condition is determined by microscopic observation using Malassez cells. Growth inhibition percentages are then calculated relative to the control. The EC50-72h is calculated from a logistic model based on Hill equation and the NOEC-72h is determined by the Bonferroni statistic model.

2.4.8. Validity Criteria

- The mean growth rate of the controls at 72h is above 0.9 day^{-1}.
- In the controls, the variation coefficient of growth rate is below 7%.
- In the controls, the variation of pH during the test is below 1.0.

2.4.9. Glossary

- Cell density: number of cells per unit volume of medium.
- Growth rate: logarithmic increase of algal cell density per unit of time.
- EC50-72h: concentration or loading rate resulting in a 50% reduction of growth rate compared to the control after 72 hours. The smaller the EC50-72h, the more toxic the tested sample.
- WAF: "Water Accommodated Fraction"; fraction that forms a homogeneous suspension in water, obtained from a loading rate in mg/L of the tested formula.
- Loading rate: quantity of tested formula added in the test medium, in mg/L, to prepare a WAF solution. The loading rate is equivalent to the initial nominal concentration.
- NOEC: "No Observed Effect Concentration"; the highest concentration causing no significant effects on test organisms.

2.5. *In vitro* Ecotoxicology Studies Concerning Acartia Tonsa

2.5.1. Purpose

Evaluate the ecotoxicity of a cosmetic formula on a marine crustacean specie *Acartia tonsa* by assessing its mortality after 48 hours.

2.5.2. Normative References

ISO 14669 (1999) - Water quality - Determination of acute lethal toxicity to marine copepods.

2.5.3. Principle

Crustaceans Acartia tonsa are placed in different concentrations or loading rates of the tested formula, and in a control medium (synthetic sea water). After 24 h and 48 h, the concentration or loading rate that induces 50% of crustacean's mortality is determined.

2.5.4. Analytical Series Presentation

Number of flasks

An analytical series includes:

- 4 replicates with synthetic sea water (negative control) ;
- 4 replicates per tested concentrations or loading rates.

The assay includes at least 5 concentrations or loading rates, in a geometric series: 100 mg/l, 56 mgl, 32 mg/l, 18 mg/l, 10 mg/l, 5.6 mg/l, 3.2 mg/l.

Reference substance

3,5-dichlorophénol (3.5-DCP) is used as a reference substance to check the tested organism's sensitivity during the test.

2.5.5. Test Procedure

Preparation of the tested sample

For water-soluble formula

A given quantity of the tested formula corresponding to the desired concentration to be tested is placed directly in the test medium containing the organisms.

For non-water-soluble formula

A Water Accommodated Faction (WAF) is prepared by putting in contact a quantity of the cosmetic formula to be tested with the test medium at a given loading rate, in a closed flask, under orbital agitation for 24 hours at approximately 110 revolutions per minute, at 20˚C ± 2˚C. The aqueous fraction of each loading rate (WAF) is then recuperated. The WAF are used raw and immediately for the test and experimental results are expressed in loading rates.

2.5.6. Inoculation and Incubation

Copepods are placed in the test media (synthetic sea water) in such a way that the density does not exceed one copepod per 5 mL of solution, or 5 organisms per 25 mL. The flasks are then incubated in a non-vibrating chamber at 20˚C ± 2˚C and under a day/night cycle of 16 h/8h.

2.5.7. Observations and Data Treatment

After 24 h and 48 h, the number of surviving copepods is counted in each flask. Animals that do not swim or whose appendix is motionless for 10 seconds are considered dead. Any abnormal behavior or appearance of organisms should be noted. The percentage of lethality after 24 h and 48 h are calculated from the data of repeated tests for each concentration or loading rate and in relation to the total number of used copepods. The LC50-24h and LC-50-48h are calculated from the Log-Probit statistic model and the NOEC-48h is determined by the Bonferroni statistic model.

2.5.8. Validity Criteria

- The dissolved oxygen concentration at the end of the test in the controls is greater than or equal to 4 mg/L.
- The lethality percentage of the negative controls equals or is less than 10%.
- The mortality percentage after 48 hours of a 1 mg/L solution of 3.5-Dichlorophenol is between 20% and 80%.

2.6. Results Interpretation of *in vitro* Ecotoxicology Studies Concerning Phaeodactylum Tricornutum and Acartia Tonsa

In the absence of specific regulations for the ecotoxicological assessment of cosmetic products, particularly sunscreen products, we have established an internal classification (Table 1) based on Part 4 entitled "Environmental Hazards" of Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 9th edition (2021).

Bibliographic data indicate that the concentrations of the main UV filters that composed sunscreen products found in the coastal waters, and other aqueous media, are around µg/L or ng/L [4] [5]. These data relate only to the UV filters but give an indication of the order of magnitude of concentrations at which we find sunscreen products in the environment.

The maximum concentration of 100 mg/L chosen for the test described in this study is classically used in the OECD guidelines for ecotoxicological testing (Daphnia, algae) for the CLP classification. This concentration is much higher than those found in the natural environment for the chemicals in contact with organisms. A lack of effect or a small effect met in the conditions of the study, conducted in "extreme" conditions compared to the natural environment, would argue the lack of danger of the product on the tested organisms and studied parameters.

With this in mind, we estimate that a low toxic effect refers to an ecotoxicological descriptor (ED) between 50 and 100 mg/L. The value of the ED observed for most sunscreen products tested by our lab are greater than 50 mg/L. It should be noted that it is less common to find values between 30 and 50 mg/L, which could lead to a more toxic effect compared to most of sunscreen products. Below 30 mg/L, we consider that the observed effect is significant.

Thus, in order to classify products with a low toxic effect linked to their low

Table 1. Internal classification of ecotoxicological effect of cosmetic products based on Part 4 entitled "Environmental Hazards" of Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 9th edition (2021).

ED	Acute toxicity category	Classification
ED ≤ 1 mg/ml	1	Very ecotoxic
1 < ED ≤ 10 mg/ml	2	Ecotoxic
10 < ED ≤ 30 mg/ml	3	Ecoharmful
30 < ED ≤ 50 mg/ml	3	Relatively not ecotoxic
ED > 50 mg/ml	3 or without category	Not ecotoxic

concentrations in the natural environment, we decided to subdivide Category 3 of acute toxicity and create three arbitrary categories with an ED between 10 and 30 mg/L for "Eco- harmful" products, between 30 and 50 mg/L for "Relatively not ecotoxic" and greater than 50 mg/L for "Not ecotoxic" products.

Glossary

- WAF: "Water Accommodated Fraction"; fraction that forms a homogeneous suspension in water, obtained from a loading rate in mg/L of the tested formula.
- LC50-t: concentration or loading rate resulting in a 50% lethality in the crustacean's population compared to the control after a time t. The smaller the LC 50-t, the more toxic the tested sample.
- Loading rate: quantity of tested formula added in the test medium, in mg/L, to prepare a WAF solution. The loading rate is equivalent to the initial nominal concentration.
- NOEC: "No Observed Effect Concentration"; the highest concentration causing no significant effects on tested organisms.

3. Results and Discussion

As shown in Table 2, our *in silico* study predicted that the 2 formulas containing chemical UV-filters that were tested display a high biodegradability rate (superior to the threshold value of 60% given by the OECD 301 F guidelines): 89% and 85% for the formulas 1 and 2 containing 15% and 19% of chemical UV-filters, respectively.

As we can see, the concentration of chemical UV-filters seems to have a very limited influence on the biodegradability rate of the evaluated formulas.

In the second part of our studies, we chose to test the validity of the results obtained through *in silico* studies by evaluating 8 formulas containing UV-filters through *in vitro* studies calling for the OECD 301-F guidelines. As shown in Table 3, the obtained estimated biodegradability percentages are slightly inferior to those which could be expected after the realization of our *in silico* screening.

In a very interesting way, we also note that the "biodegradability" rates of the

Table 2. *In silico* determination of the estimated biodegradability (%) of 2 formulas containing chemical UV-filters (representative experiments of a more global screening study).

	Formula 1	Formula 2
Estimated biodegradability (%)	89	85

Table 3. Average Biodegradability Rate after 28 days (=Regular OECD 301-F test) of 8 formulas containing mineral or chemical UV-filters.

Formula N°	3	4	5	6	7	8	9	10
Mineral (M) or Chemical (C) UV-filters	C	M	C	C	C	C	C	M
% of Biodegradability	45 ± 2	70 ± 3	59 ± 3	57 ± 1	54 ± 2	59 ± 1	59 ± 3	73 ± 6

mineral UV-filters are superior to the biodegradability rates of the chemical ones and superior to the threshold of 60% of the OECD 301-F guidelines indicating that these products are easily "biodegradable". Additionally, we can observe that the biodegradability rates of the formulas containing chemical UV-filters are not very far from the threshold of 60%. In these conditions, we can reasonably think that these products take a longer time to disappear from the medium containing them but that it remains possible that they do not display dramatic ecotoxicological effects. In order to test this hypothesis, we have so realized ecotoxicological studies on a zoo- and a phytoplankton, *i.e. Acartia tonsa* and *Phaeodactylum tricornutum*, respectively.

As shown in Table 4 and Table 5, the 7 tested formulas (as several tested formulas have approximatively the same composition we tested only 7 formulas among the 8 mentioned in the previous experiments) did not display any cytotoxic effect on *Acartia tonsa* or on *Phaeodactylum tricornutum*. As a consequence, all the tested products can be considered as "non ecotoxic" for these zoo- and phytoplanktons.

Table 4. Ecotoxic effect of chemical UV-filters containing formulas on *Phaeodactylum tricornutum* following the ISO 10253 guidelines.

Formula N°	2	5	6	7	8	9	11
EC50-72h (mg/ml)	>100	>100	>100	>100	>100	>100	>100
Ranking	NE	NE	NE	NE	NE	NE	NE

NE: Non Ecotoxic; RNE: Relatively Non Ecotoxic.

Table 5. Ecotoxic effect of chemical UV-filters containing formulas on *Acartia tonsa* following the ISO 14669 guidelines.

Formula N°	2	5	6	7	8	9	11
LC50-48h (mg/ml)	>100	>100	>100	>100	>100	>100	>100
Ranking	NE	NE	NE	NE	NE	NE	NE

4. Conclusion

We here report that mineral UV-filters are readily "biodegradable" and do not display any cytotoxic effect on marine fauna and flora. Concerning the chemical UV-filters incorporated in our "sun formulas", we can conclude that, even if their biodegradability is slightly inferior to the one of the mineral filters, they do not display any significant cytotoxic effects on *Acartia tonsa* and *Phaeodactylum tricornutum*, a zoo- and a phytoplankton of a great importance for the marine fauna and flora sustainability. These results are in line with our work published in 2019 on UV-filters and corals and confirm that cosmetic sunscreen products can be daily used without displaying any significant noxious effects on coastal waters ecosystems.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] De Jager, T., Cockrell, A. and Du Plessis, S. (2017) Ultraviolet Light Induced Generation of Reactive Oxygen Species. In: Ahmad, S. Ed., *Ultraviolet Light in Human Health*, *Diseases and Environment*, *Advances in Experimental Medicine and Biology*, Vol. 996, Springer, Cham, 15-23. https://doi.org/10.1007/978-3-319-56017-5_2

[2] Elwood, J. and Jopson, J. (1997) Melanoma and Sun Exposure: An Overview of Published Studies. *International Journal of Cancer*, **73**, 198-203. https://doi.org/10.1002/(SICI)1097-0215(19971009)73:2<198::AID-IJC6>3.0.CO;2-R

[3] Jesus, A., Sousa, E., Cruz, M., Cidade, H., Sousa Lobo, J. and Almeida, J. (2022) UV-Filters: Challenges and Prospects. *Pharmaceuticals*, **15**, Article 263. https://doi.org/10.3390/ph15030263

[4] Sanchez Quiles, D. and Tovar-Sanchez, A. (2015) Are Sunscreens a New Environmental Risk Associated with Coastal Tourism? *Environment International*, **83**, 158-170. https://doi.org/10.1016/j.envint.2015.06.007

[5] Rainieri, S., Barranco, A., Primec, M. and Langerholc, T. (2017) Occurrence and Toxicity of Musks and UV-Filters in the Marine Environment. *Food and Chemical Toxicology*, **104**, 57-68. https://doi.org/10.1016/j.fct.2016.11.012

[6] Ionescu, M., Le Breton, C., Hubaud, J., Branka, J. and Lefeuvre, L. (2019) Ecotoxicological Effects of Cosmetic Products Containing Chemical and Mineral UV-Filters on Seriat*opora hystrix* Fragments. *Journal of Cosmetics, Dermatological Sciences and Applications*, **9**, 57-68. https://doi.org/10.4236/jcdsa.2019.93020

[7] Jensen, K. (2010) NOBANIS. Invasive Alien Species Fact Sheet—Acartia tonsa—From: Identification Key to Marine Invasive Species in Nordic Waters. http://www.nobanis.org/

[8] Tanaka, A., De Martino, A., Amato, A., Montsant, A., Mathieu, B., Rostaing, P., Tirichine, L. and Bowler, C. (2015) Ultrastructure and Membrane Traffic during Cell Division in the Marine Pennate Diatom *Phaeodactylum tricornutum*. *Protists*, **166**, 506-521. https://doi.org/10.1016/j.protis.2015.07.005