Sunscreen ecoproducts

Product claims, potential effects and environmental risks of applied UV filters

Authors: DME Slijkerman and M Keur

Wageningen University &
Research rapport C056/18

WAGENINGEN
UNIVERSITY & RESEARCH

Sunscreen ecoproducts

Product claims, potential effects and environmental risks of applied UV filters

Author(s): DME Slijkerman and M Keur

Publication date: October 2018

Wageningen Marine Research
Den Helder, October 2018

CONFIDENTIAL No

Wageningen Marine Research report C056/18

WAGENINGEN
UNIVERSITY & RESEARCH

DME Slijkerman and M Keur, Sunscreen ecoproducts ; Product claims, potential effects and environmental risks of applied UV filters. Wageningen, Wageningen Marine Research (University & Research centre), Wageningen Marine Research report C056/18. 75 pp.

Keywords: UV filters, Sunscreen, eco labels

Bioassays and part of the literature reviews were performed during the traineeship of Martijn Keur, currently employee of WMR. .

Name	Martijn Keur
Programme	Aquatic ecotechnology
Department	HZ university of applied sciences
Supervisors	Dr. Diana Slijkerman
Place	Wageningen Marine Research – Den Helder
Date	June 10th, 2018

the reef
Sunscreen Awareness Bonaire

Client: Wereld Natuur Fonds- Nederland
Attn.: Dhr. A. De Groene
Postbus 7
3700 AA Zeist

This report can be downloaded for free from https:// doi.org/10.18174/457209
Wageningen Marine Research provides no printed copies of reports

Wageningen Marine Research is ISO 9001:2008 certified.

Contents

Executive summary

Problem definition

Coastal tourism is one of the most rapidly growing fields worldwide and people spend more time outdoors. The awareness of the importance of skin protection against long time exposure to UV-radiation increases, resulting in increased use of sunscreen products. As a consequence, the emissions of sunscreen products into the environment also intensify.
Sunscreens typically contain UV filters that protect the skin from UV radiation. UV filters can enter the marine environment in direct (swimming & bathing) and indirect ways (wastewater discharges). Various studies describe adverse effects of UV filters on the marine environment and marine organisms, including mortality, growth inhibition, reproduction failure due to endocrine disruption, coral bleaching and accumulation in food webs. The impacts depend on the exposure levels, and thus emissions and resulting field concentrations are relevant.
The adverse effects are mainly ascribed to organic UV filters, especially the compound oxybenzone. Consequently, alternative/eco-friendly sunscreen products have been developed and introduced. Manufacturers claim that these alternative products are "reef safe" and "eco-friendly". These products mainly use UV filters based on minerals, such as zinc or titanium, although also some new organic compounds have been applied. Question is whether these alternative products are really safe and do not pose a risk to the environment.

Aim

The aim of this study was to contribute to the knowledge base within the current sunscreen debate, with a focus on so-called eco-friendly sunscreens, using alternative UV filters . To achieve this objective the following main research question was formulated:
Can eco-friendly sunscreen products have adverse effects on the marine environment?
To support the main question, several sub questions were formulated:

- What are eco-friendly sunscreen products?
- What are the known effects of "alternative" UV filters for the marine environment?
- What are the effect concentrations?
- Do toxicity effects occur in a variety of laboratory tests?
- Are these effects field relevant?
- What are the emissions if everyone changes to using eco-friendly sunscreen products and does this emission lead to environmental risk?

In this study, literature was reviewed. During the review, eco-labels and claims were studied, and UV filters used in eco-products were listed. Toxicity effects, and effect levels of mineral UV filters and new generation UV filters were assessed.
In addition, three bioassays were performed with a selection of 7 sunscreen products (eco and non-eco) to assess effect levels of the whole sunscreen product. Algal growth inhibition, bacteria luminescence, and rotifer mortality were assessed. Based on both the retrieved literature toxicity data and bio-assay results, risk assessments were performed according to international standards (ECHA). Doing so, environmental risk of whole sunscreen products and individual UV filters was evaluated for the situation at Lac Bay Bonaire. Effects of UV filters on humans are not included in this study.

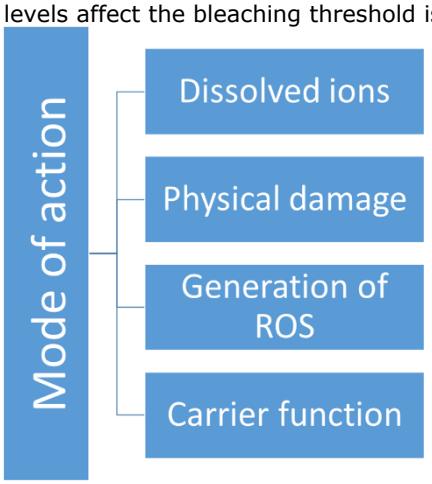
Results

Eco products

Product screening revealed that among the 98 screened products claiming to be eco/reef safe, or in any other way claiming to be environmental friendly, about 9% of the screened products still contain oxybenzone. Another 20% of the products contain organic UV filters. If producers refer to scientific studies, they refer to the study of Downs *et al.* (2015) and the (accompanying) research communication on this matter to argument the use of mineral UV filters instead of organic filters. Some products have conducted environmental effects studies themselves, reporting the results on their websites.
The ecofriendly-sunscreens mainly contain zinc and/or titanium minerals, in both nano and non-nano form. Mineral UV filters protect against UVR via scattering and reflecting the radiation. The actual form is not always reported on the product, depending on country's legislation. Particles can be coated with aluminium (in case of titanium), but this is usually not mentioned on the ingredient list.

Toxicity reported in literature

The literature review revealed that many studies focussed on nano-zinc and nano-titanium. Few studies describe effects of the non-nano forms.
It should be noted that UV filter characteristics probably differ from the compounds tested in reports found. Zinc and titanium particles can differ in size, shape and applied coating, and thus the potential behaviour and adverse effects can differ too.
In general, titanium dioxide (TiO_2) and zinc oxide (ZnO) in both nano and non-nano form, can affect organisms via four pathways (mode of action- see figure below).
An organism can be affected via the released free ions (mainly reported for zinc), physical damage (particles stick to vital organs), the generation of Reactive Oxygen Species (ROS, affecting organism cells, mainly reported for TiO_2), and via a carrier function (absorbing and transporting other toxicants into the organism (reported for TiO_2). Corals can be sensitive to free zinc ions (depending on the concentration), and ROS is related to the thermal bleaching threshold. Wether Titanium affected ROS levels affect the bleaching threshold is not reported.



Besides mineral UV filters, so-called new generation organic UV filters are applied too. These are applied in EU products, not yet in US products. One product that claims to be reef safe uses these new organic filters.
Based on effect concentrations (EC_{50}s and NOEC's) found in literature, no generic conclusive answer can be given on the comparability of toxicity of the compounds groups.
Traditional organic filters can induce endocrine effects, whereas for the mineral filters this is not likely to occur. Effects reported for new generation organic filters occur at very high concentrations, meaning that they are much less toxic than zinc, titanium and the traditional organic UV filters. However, endocrine studies are not yet reported and potential endocrine effects cannot be evaluated in this study.

The fate and effects of mineral UV filters on the environment depend on too many factors to predict a realistic environmental risk at the moment. Moreover, effects described for both ZnO and TiO_2 do not occur at field relevant concentrations (yet). The difficulty in assessing the risk and potential effect of mineral UV filters, is the lack of proper analytical methods. Field levels of nano-ZnO and nano (hereafter nZnO and $nTiO_2$) cannot yet be established in a good way. Analytical developments are expected to make this possible in the near future.

The smaller the particle, the more likely effects will occur due to the release of free ions.

- In contrast to titanium, zinc is an essential element for organisms to grow and survive. In the coastal areas, and in case of zinc limitation, zinc addition could in turn promote algal growth. Based on the effect levels found, zinc is in general more toxic than titanium, and titanium is considered to be more eco-compatible.
-
- No standards have yet been established for environmental levels of nanoparticles. Instead, we report the threshold levels available for the free metal ions. Effects of zinc on coral can occur at levels starting from 10 µg/L. But effects are highly species, treatment and endpoint dependent. To protect the marine environment at a 99% species protection level, the reported threshold levels for zinc are 3.1 µg/L. No threshold levels for titanium are available.

Bio assays

A selection of seven products was tested using three types of bio-assays. Products varied in UV filter composition, and in having an eco-label or not. Traditional organic (including oxybenzone), mineral and new generation organic products were included in the selection. Based on the effect levels observed, products were ranked in order to indicate their relative effect level compared to the other products. In general, products with the organic UV filter oxybenzone were ranked highest. In contrast, eco-products containing titanium did not show any effect at the maximum concentrations tested. The nZnO product showed stronger effects to algae than the non-nano products, indicating an effect of particle size as suggested in literature.

Also, a new generation organic products with an eco-label did show hardly any effects, as was expected from data in literature. Another product with similar new generation filters, but without an eco-label, did show clear effects. This illustrates that the formulation of the whole product is important to consider.

When test solutions were tested after 4 days instead of directly after preparation, the observed toxicity was different. Most likely, the availability of the compounds in the test solutions changed, affecting their bio-availability. Organic compounds were less available (sticking onto the glass), and fewer effects were observed. In contrast, zinc products showed increased effects at solutions that were aged. Ionic release by (n)ZnO most probably explains the increased toxicity to algae. Hence, environmental effects would probably not appear directly after emission of ZnO particles, but sometime later.

Risk assessment

Two risk assessments were performed, one on toxicity data of individual UV filters (non-nano zinc, nano zinc and nano titanium) according to ECHA guidelines . The second risk assessment was done on the bioassay results, which are based on the effects of the whole sunscreen product. Risk assessments resulted in so called risk quotients (RQs). A RQ larger than 1 indicates that environmental risk cannot be excluded.

The assessment based on literature data included an estimation of the future emissions, and the related field concentrations in different zones in Lac Bay Bonaire. These future field concentrations were divided by reported effect levels. Titanium RQs were far below 1, and thus no risk is to be expected from titanium emissions. However, in the direct bathing zone, potential zinc emissions lead to RQs higher than 1. It should be noted that data availability is low, and uncertainty is large, and also the uncertainty factor applied to the toxicity values is large due to data limitation. However, based on this rough estimation, future environmental risk of zinc cannot be excluded.

Risk assessments for the new generation organic filters could not be done due to data limitation. Additional toxicity testing with new organic and ZnO UV filters lowers the applied uncertainty factor, and consequently most probably will decrease the estimated environmental risk. .

In contrast, when conducting a risk assessment on the whole product, using the bio assays results, none of the eco products with zinc and titanium pose a risk at levels to be expected in Lac Bay. Even when using an equally large uncertainty factor as was used in the other assessment. Based on the bioassay results, environmental risks of products containing organic UV filters cannot be excluded.

Final conclusions

Based on the various studies, a conclusive answer to the main question cannot yet be provided. In summary:

The literature review, bioassay results and the theoretical risk assessment showed that toxicity of titanium only occurs at levels that are far above field relevance. A risk of titanium UV filters is currently

not apparent, nor is it expected in the future. Hence, products containing only titanium UV filters can be considered "eco-friendly".
For zinc the conclusion is less clear. The bioassays did not show effects, but literature reports and the risk assessment are not conclusive. Field sampling and additional toxicity testing should provide more information.
Based on the limited information available and the bio-assay results, new generation organic UV filters are not expected to cause environmental effects, but some formulas may. Therefore mineral products are not the only "safe" eco-products to consider as an alternative to "traditional" sunscreens. Endocrine effect testing should, however, still be done for the new generation organic filters.

In general, products without an eco-claim induced much more effects in the tests, at much lower tested concentrations. Eco products tested in this project do not pose a risk to the environment. Products containing traditional organic UV filters showed the highest overall risk to the environment. The assessment factors are applied to toxicity values in order to address uncertainties related to inter-species sensitivity, combination toxicology, etc. Although, all effect concentrations found/produced were above field relevant emissions and concentrations for all sunscreens, risks of non-eco products towards the environment cannot be excluded, due to the uncertainty factors applied in the risk assessment.

It is recommended to proceed with toxicological testing, that include the various UV filters described, and sensitive but highly relevant endpoints such as bleaching thresholds, and endocrine processes.

List of abbreviations

In this report many abbreviations and technical terms are used, which are clarified below.

Bioassays	Assessment of the biological impact of a substance by its effect on living cells or organisms.
Broad spectrum UV filters	UV filters that protect against both UVA and UVB
Coral bleaching	Occurs when coral polyps expel algae that live inside their tissues. Bleached corals continue to live but begin to starve after bleaching. Recovery is possible.
Dissolved organic matter	Defined as the organic matter fraction in solution that can pass through a 0.45 µm filter. It influences a spectrum of biogeochemical processes in the aquatic environment.
EC_{50}	Calculated concentration causing 50% effect (e.g. mortality, growth inhibition) in a bioassay.
Endocrine system	The chemical messenger system consisting of hormones.
Estrogenic disruption	The interference of chemicals with the endocrine system (hormone system).
Fluorescence	Emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a specific form of luminescence.
Lipophilicity	The ability of a compound to dissolve in fats, oils and lipids.
LOEC	Lowest Observed Effect Concentration. The lowest concentration used in a bioassay that showed a significant effect on the test organism.
Luminescence	Emission of light by a substance not resulting from heat, which can be caused by chemical reactions, electrical energy or stress of a crystal.
Lytic cycle	One of the two cycles of viral reproduction. Results in the destruction of the infected cell and its membrane.
Nano particles	Particles between 1 and 100 nanometres.
nZnO	nano Zinc oxide
nTiO2	nano titanium dioxide
NOEC	No Observed Effect Concentration. The highest concentration used in a bioassay that did not show a significant effect.
Parabens	Class of preservatives in cosmetic and pharmaceutical products.
Persistent organic pollutants	Organic compounds that are resistant to environmental degradation through both chemical and biological processes.
Photo catalysis	The acceleration of a photoreaction in the presence of a catalyst.
Photo degradation	The alteration of materials by light.
Photo isomerization	Molecular behaviour in which structural change between isomers takes place.
Photo stability	The rate in which a chemical is unchanged by the influence of light.
Surface microlayer	The first 1mm of the ocean surface. It is the boundary layer where all exchange occurs between the atmosphere and the ocean.
UVA	Ultraviolet A; wavelength 420-320 nm.
UVB	Ultraviolet B; wavelength 320-280 nm.

1 Introduction

1.1 Problem definition

Coastal tourism is one of the most rapidly growing fields worldwide and people spend more time outdoors. The awareness and the importance of skin protection against long time exposure to UV radiation grows (Sánchez-Quiles & Tovar-Sánchez, 2015). Consequently, the use of sunscreen products and the emissions into the environment also increased.
Sunscreens typically contain active ingredients that protects the skin from UV radiation, called UV filters (Danovaro *et al.* 2008). UV filters can enter the marine environment in direct (swimming & bathing) and indirect ways (wastewater discharges) (Giokas *et al.* 2007). Furthermore, several scientists describe the adverse effects of UV filters on the marine environment (Corinaldesi, *et al.*, 2017; Danovaro, *et al.*, 2008; Downs, *et al.*, 2015; Fent *et al.*, 2010; Paredes *et al.*, 2014; Zhang, *et al.*, 2016). Examples of reported adverse effects are mortality, growth inhibition, reproduction failure, coral bleaching and bioaccumulation in food webs. It is, therefore, important to consider the effects of sunscreens in the (coastal) marine environment (Tovar-Sánchez *et al.*, 2013).

The adverse effects are mainly ascribed to organic UV filters. Consequently, alternative/eco-friendly sunscreen products are made and introduced to the consumer market. Manufacturers claim that these alternative products are "reef safe" and "eco-friendly". Most of these products do not contain organic UV filters. These products use UV filters based on minerals, such as zinc or titanium.

On Bonaire UV-filters (the active ingredients in sunscreens) were detected in the water of Lac Bay, at levels causing environmental concern (Schaap & Slijkerman, 2018).
The action perspective to reduce this risk appear realistic: Alternative products are available, claiming to be "reef safe" or environmental friendly. These products contain mineral ingredients such as Zinc Oxide and or Titanium Dioxide instead of organic UV filters such as Oxybenzone.
Current brands and research-groups claim that so-called mineral sunscreens are a better alternative and these claims have been taken over in sunscreen awareness programs all over the world.

Thus, many products claim to be reef safe, but the question rises whether eco-friendly sunscreen products are really eco-friendly. What do the claims mean? And what would be the emission of the alternative UV filters, and is there a potential environmental risk if everyone changes to using eco-friendly sunscreen products instead? What is the toxic potential and environmental risk of the alternative UV filters?

WWF-Netherlands aims to contribute to improved water quality at the BES islands in general. More specifically, WWF- NL wants to contribute to the awareness of sunscreen use and its potential risk to the environment. Suitable alternatives need to be communicated in order to change behaviour and thus emissions. WWF aims at the inclusion of a sound knowledge base in this communication.

1.2 Assignment and aim of the study

The aim of this study was to contribute to the knowledge base within the current sunscreen debate. The focus was on eco-friendly sunscreens and their mineral alternatives Zinc and Titanium.

To achieve this objective the following main research question was formulated:
Can eco-friendly sunscreen products have adverse effects on the marine environment?

To support the main question, several sub questions are formulated:

- What are eco-friendly sunscreen products?
- What are the known effects of "alternative" UV filters for the marine environment?
- What are the effect concentrations?
- Do toxicity effects occur in a variety of laboratory tests?
    - Are these effects field relevant?
- What are the emissions if everyone changes to using eco-friendly sunscreen products and does this emission lead to environmental risk?

1.3 How to read this report

The main findings and conclusions of this report can be found in Chapter 7 (Conclusions and Recommendations). As background, a general introduction to sunscreens is given in Chapter 3, followed in Chapter 4 by a literature review on general environmental issues related to sunscreens. This Chapter is concluded with a small summary.
The results of the bioassays with a selection of sunscreens are given in Chapter 5. The risk assessment based upon these results is described in Chapter 6. Both chapters are concluded with a short summary.
In Chapter 2 (Materials and Methods) the procedures followed, as well as the selection of sunscreens for testing, are described in more detail.
Detailed information on sunscreens products and bioassays, as well as toxicity data obtained from public databases, can be found in the Annexes.

2 Materials and Methods

The following approach was followed.

1. Literature review, focussing on:
    a. Eco-friendly sunscreen products, used UV filters, and claims made.
    b. Alternative UV filters and their environmental fate and effects
2. Bioassays/toxicity testing
    a. Product and bioassay selection
    b. Bioassays
        i. Range finding experiments
        ii. Final tests and end results
    c. Comparison of bioassays results with toxicity data in literature
3. Risk assessment
    a. Emission estimations
    b. Risk quotients based on toxicity data

2.1 Literature review

Aim of the literature review was to provide background information on the "eco-products", their applied labels, used UV filters and the claims brands make. In addition, specifications on the fate and toxicity of the UV filters used in eco products were searched for in order to provide an overview of their relative toxicity compared to commonly used organic UV filters. The toxicity of commonly used UV filters is not described extensively, nor are the other ingredients in sunscreen products evaluated in this report.

2.1.1 Eco-friendly sunscreen products and their claims

The literature review on eco-claims focussed on two sub questions:

- which marketing claims exist among environmental friendly products and what is their relevance towards the marine environment?
- Are eco-claims made by products supported by research?

Various known claims were searched for via google and, thereafter, studied. Via drugstore websites (e. g. Amazon, google shopping, drogisterij.net) product searches were done by searching for "eco", "Bio", "reef", "environment" "safe" within the sunscreen product section. The search was not unlimited, but provided a broad overview of brands and products claiming to be eco-friendly and/or reef safe. This resulted in a product list, in which for each product the claims were noted. The UV filters used were marked in this list, including the percentage of the relevant ingredients, which were obtained from brand and/or product websites.

2.1.2 UV filters review

The literature review focussed on specific UV filters used in eco products. The review includes a description of the compounds, fate and behaviour, background levels, mode of action, and eco-toxicity.

Focus was on most recent publications (2014-2018) and on review papers. The recently published document of International Coral Reef Initiative (ICRI) written by Wood (2018) was a useful summarizing document to include. Additional literature searches were performed via Google Scholar, Science Direct and Scopus, using various combinations of key words, such as:

- zinc
- titanium
- nano/non nano zinc oxide/ titanium dioxide
- (non) nano metals/materials

- Tinosorb, Univul, Ensulizole (and their chemical names)
- toxicity/ effects/impact
- marine/aquatic

In this report, nano-zinc oxide and nano titanium dioxide are reported as nZnO and $nTiO_2$.
To obtain toxicity data for each of the UV filters, toxicity and chemical databases were used (EPA, 2018; ECHA, 2018), and literature was screened. Not all literature could, however, be recovered and checked in this study. Therefore, this overview should be considered a first description.

2.2 Toxicity testing

Aim of toxicity testing of whole sunscreen products was to explore potential toxicity of a selection of products, varying in UV filter composition. Focus was on UV filters used in eco-products, and to determine their relative toxicity. Therefore, "generic" products with organic UV filters were added to the selection.
The selection of seven products is by no means a reflection of all products and their formulations available, but provides a first relative comparison of products in terms of toxicity described by a selection of laboratory toxicity tests, covering different taxonomic groups.

2.2.1 Product selection

A total of 7 products was selected based on their difference in UV filters (Table 1), covering 3 different UV filter groups (2x organic/chemical, 2x "new" organic, 3x mineral). Given the UV filter variability in products (number of filters, and amount) and product availability, a clear UV filter "deduction" set up was not possible in this project (e.g. to test the effect of filter 4, product A includes filters 1-2-3-4, Product B includes all, but not). This makes it difficult to deduce and conclude solely on the UV filter composition and individual UV filter toxicity. Also, not all formulations were available in the Netherlands, or could be ordered and shipped from abroad. Substitute products containing similar UV filter combinations are selected instead (e.g. product C).

According to the Environmental Working Group (2018) UV filters in table 1 are given a score based on their overall hazard ranging from green (low hazard), yellow (moderate hazard) and red (high hazard). The products (D, E and G, in upper row in Table 1) claim to be eco-friendly and are coloured green. Product C is a substitute for eco products containing nano-zinc, and therefor coloured light green. A detailed description of the products is presented in Annex 1.

Table 1 Overview of UV filters and the percentages within the selected products. Percentages of products A, B, D and E are presented on the product specifications. Numbers in Italics (products C, F, G) are estimations based on maximum allowed levels (F + G) or the mean of % in similar product types (list in annex 2). Color codes: see main text.

Sunscreen products

Group	UV filter	Product A	Product B	Product C	Product D	Product E	Product F	Product G
Organic	Avobenzone	3%	3%					
	Homosalate	7.5%	7%					
	Octisalate	5%	3%					
	Octocrylene	2.75%	3%					
	Oxybenzone	2%						
Mineral	ZnO				6.4%			
	ZnO (nano)			*10%*				
	TiO2		5%	*5.8%*	6%	8.8%		
New organic	Ensulizole						*4%*	
	Uvinul A plus						*10%*	*10%*
	Uvinul T150						*15%*	*15%*
	Tinosorb M							*10%*
	Tinosorb S						*10%*	

2.2.2 Bioassays

Bioassays (toxicity tests) were selected to cover a wide range of specific species sensitivities and endpoints, and to cover a range of ecological relevance (algae as primary producers, bacteria as detrivores and rotifers as herbivores).

The tests included:

Test	Species	Duration	Endpoint
Algal growth inhibition test	*Skeletonema constatum*	Chronic, 96 hours	Growth inhibition (EC_{50})
Microtox Basic test	*Vibrio fischeri*	Acute, 5, 15 and 30 minutes	Luminescence (EC_{50})
Rotifer acute test	*Brachionus plicatilis*	Acute, 24+48 hours	Mortality (EC_{50})

Test principles are presented in annex 3.

2.2.3 Boundary conditions

Before performing the bioassays, some boundary conditions were checked, in order to meet performance criteria.

Concentration series

To test the different sunscreen products in the bioassays a concentration series was used, which was determined from the range finding experiments performed beforehand. The results of the range finding results are not included in this report. Knowing the relevant concentration ranges, the concentrations used in the bioassays were made based upon log scales in order to increase calculation accuracy when calculating the EC_{50}. The concentration series was obtained via dilution of a stock solution.

Water quality parameters

To make sure the circumstances in the stock solutions of all sunscreen products were within similar range and meet performance criteria, water quality parameters (pH, oxygen, salinity and temperature) were

measured. The suitable ranges for the parameters were based on the conditions stated specific for each of the bioassays.

Quality Assurance
Reference toxicity tests were performed for all bioassays. Phenol was used for the micro toxicity test, and potassium dichromate (K2Cr2O7) for the other tests. If the reference toxicity test was not according to the standards, the test was redone.

2.2.4 Endpoints reported

Results of the bioassays are reported as EC_{50} values, a measure presenting the effect concentration at which 50% of the organisms was affected (mortality, growth inhibition, luminescence inhibition). The concentration is primarily reported as µl sunscreen/L, and thereafter set into µg sunscreen/L. Thereafter, the effect levels per test are also expressed in mg UV filter/L (for each product and each active ingredient). The latter is a rough assessment, based on the % of each of the UV filters in the product, and the levels tested.

In some tests, the observed effect did not reach 100% effect. This hampers the estimation of the EC_{50}. The model then has to extrapolate (estimate) beyond the concentration range tested. This is less accurate, and 95% confidence intervals are included to indicate how accurate the calculated EC_{50} is. The bigger the interval range, the less accurate the calculated EC_{50}.

As concentrations higher than 200 µl/L could not be tested because of maximum solubility of the sunscreen products, for some products and tests, only slight effects were displayed at the highest possible test concentration. For these test the effect was too low to even estimate an EC_{50}. Instead the effect is noted as 'exceeding the highest test concentration', and the highest mortality or inhibition percentage is given.
Products were compared based on their relative toxicity for all bioassays to determine which products show strongest and weakest relative toxicity.

The volume of sunscreen and effect concentrations of individual UV filters within the tests were calculated and compared to toxicity data obtained by literature.

2.3 Risk assessment scenario study Lac Bay

2.3.1 Introduction

An environmental risk assessment for the UV filters Zinc and Titanium was performed for the area Lac Bay on Bonaire.
The scenario study represents a worst-case risk estimate related to the potential future emission of zinc and titanium into the environment, resulting from sunscreen use. Basic assumptions were defined to simulate a worst-case scenario:

- All tourists at Lac Bay change to products solely containing non-nZnO, nZnO or nTiO2
- Lac bay is a closed area with no water exchange, and the maximum daily concentrations will not be diluted.

The risk assessment is performed following the method of ECHA (2008). This method includes two main descriptors, the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC). The PEC is derived from estimates on emissions of zinc and titanium in Lac Bay, resulting in an estimated field concentration. The PNEC is derived from toxicity endpoints found in literature, including the application of an uncertainty factor to derive a predicted no effect concentration. By dividing these two factors (PEC/PNEC), an environmental risk quotient (RQ) can be determined.
A risk quotient (RQ) above 1 indicates a potential risk from the UV filter towards the environment, because the environmental concentration (PEC) is higher than the no effect concentration (PNEC). Ratios lower than 1 indicate no potential risk towards the marine environment, because the environmental concentration (PEC) is lower than the no effect concentration (PNEC).

2.3.2 Environmental concentration: PEC

For the estimation of the environmental concentration, PEC, first the daily potential release of UV filters is calculated. For this multiple factors are taken into account according to the method of (Sharifan *et al.*, 2016a, 2016b; Schaap & Slijkerman, 2018). Namely:

- The yearly amount of bathers is estimated from peak and non-peak days, based upon 100 cruise days and 200 normal beach days. This results in an estimated yearly amount of 39000 bathers, which is equivalent to an average of 325 bathers per day.
- A sunscreen application rate of 1.5 times/day.
- Sunscreen applied to a body surface area of 2.1 m^2 for males and 1.7 m^2 for females, assuming a body coverage of 87%.
- A sunscreen wash-off rate of 25% when entering the water.
- The average content of UV filters in eco-friendly sunscreens, based upon the list of products in annex 2). The average percentages applied in the estimations were 15% for non-nZnO, 10% for nZnO and 4% for nTiO2. The particulate zinc and titanium forms (nano and non-nano) is taken into account, not the free ionic form that will also be in the field after particles are dissolved.

The PEC is calculated by dividing the daily potential release of UV filters through the rounded volume of three different zones in Lac Bay: the bathing zone (16000 m^3), inner reef zone (826200 m^3) and the mangrove zone (5497300 m^3) (based on Schaap & Slijkerman, 2018).

Figure 1 Three zones in Lac Bay. Bathing zone (red), inner reef zone (green) and mangrove zone (yellow)

2.3.3 Predicted No Effect Concentration: PNEC

The PNEC was calculated based on the criteria set by ECHA (2008). Toxicity data are the basis of the PNEC derivation. A long list of all available data was made, and the lowest toxicity endpoint selected. The number and type of all available data (EC_{50} values, or NOECs) determines the level of assessment factor (AF) to be applied on the lowest available toxicity endpoint to derive the PNEC.

The assessment factor (AF) is a kind of uncertainty factor, that compensates for unknown interspecies or interindividual differences in toxicokinetics and toxicodynamics and multiple stress situations in the real environment.

2.3.4 Risk assessment bioassay results

Based on similar principles as described above, bioassay results were used to assess environmental risk in order to estimate the field relevance of the obtained data.

3 Background information on sunscreen products

3.1 Composition of sunscreen

Sunscreen products are a complex mixture of different functional ingredients and can be defined as "any cosmetic product containing UV filters in its formulation in order to protect the skin from UV radiation, avoiding or minimizing the damage that this radiation might cause on human health" (Sánchez-Quiles & Tovar-Sánchez, 2015).

UV filters are considered as one of the most important ingredients of sunscreen products. Generally speaking, UV filters are colourless or yellowish substances and have nearly no ability to absorb visible radiation in the form of light, but do significantly absorb radiation of ultraviolet A (UVA), which ranges between wavelengths of 320-420 nanometre (nm), and ultraviolet B (UVB), ranging from 280-320 nm. UV filters can be classified in different groups (Sánchez-Quiles & Tovar-Sánchez, 2015).;

- organic/chemical, divided into the so called "traditional" organics and "new" organics
- inorganic/mineral based.

Besides UV filters, two other main components of sunscreens are emollients and emulsifiers. Emollients have properties that cause better solubilizing and photo stabilizing of the sunscreen (Osterwalder *et al.*, 2014). Emulsifiers are, since sunscreen contains 60 to 80% water, important to create the preferred type of emulsion of sunscreen. Apart from these main ingredients, sunscreens consist of many other ingredients, see for example Figure 1 (Osterwalder *et al.*, 2014; Sánchez-Quiles & Tovar-Sánchez, 2015).

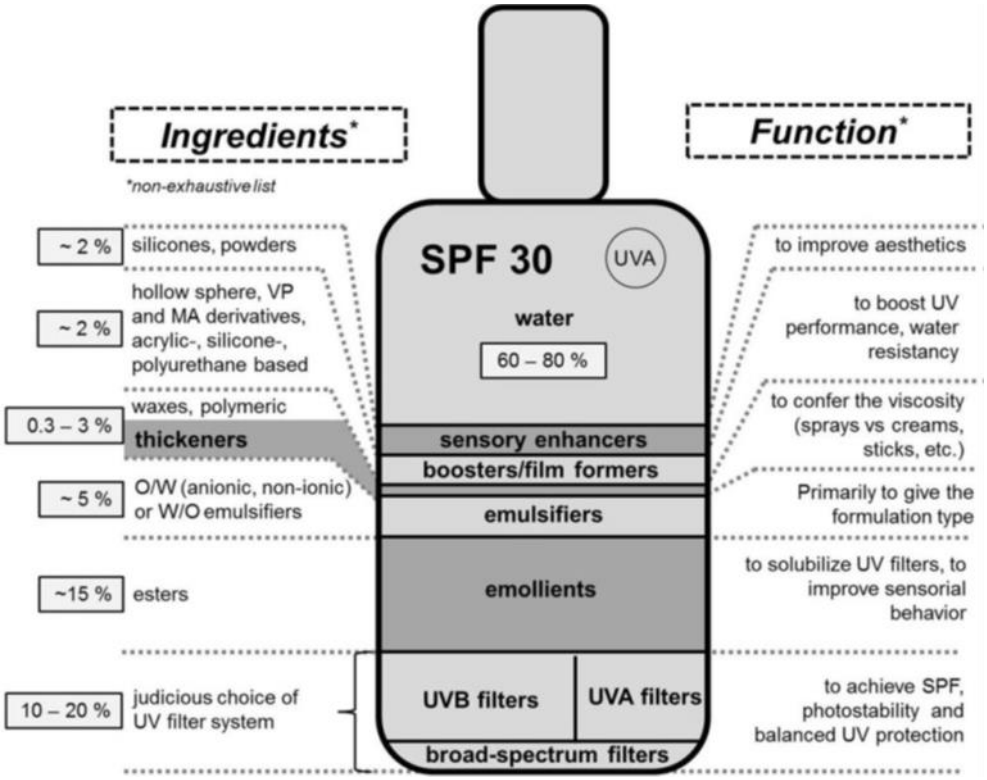


Figure 2 Sunscreen ingredients and their functions (Osterwalder et al., 2014)

3.2 UV filters

As mentioned, two main groups of UV filters exists, organic (also called chemical) and mineral (also called physical or inorganic) based. Their mode of action towards protection against UV radiation is based on absorption and reflection respectively (Figure 3).

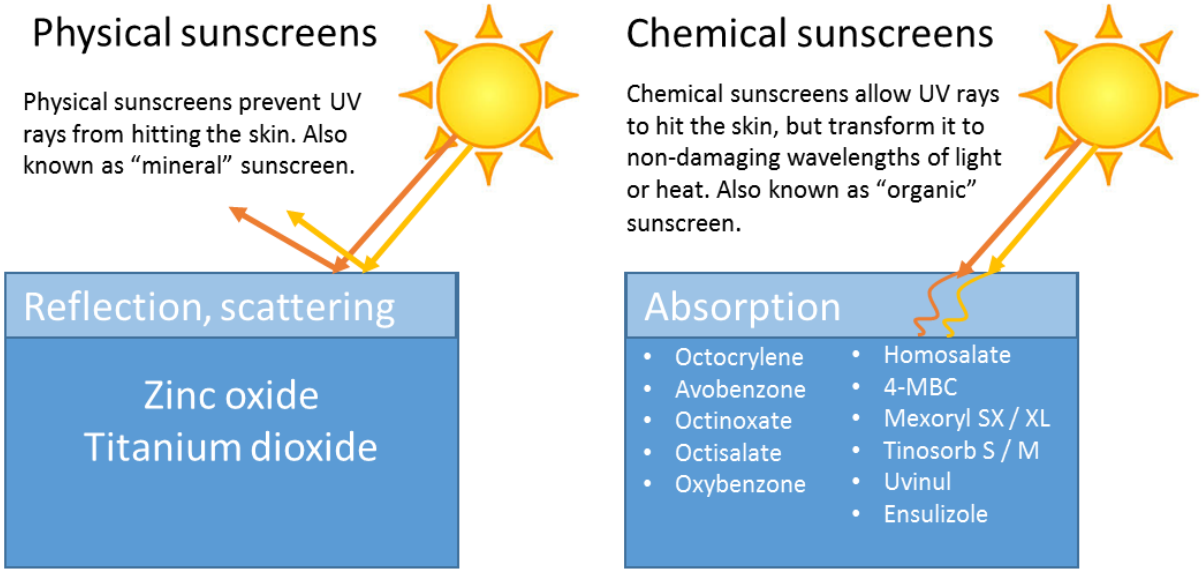


Figure 3 Representation of mode of action of UV filter types.

A standard maximum of concentration levels in sun care products does not exist. Due to varying opinions on toxicity thresholds and adequate skin protection, the percentages of the varying components differ among countries (Table 2). Differences in permitted compounds and corresponding levels largely depend on whether a country states sunscreen as a "drug" (Australia and US), or a cosmetic product (EU) (Osterwalder *et al.*, 2014). As not all filters provide broad spectrum protection towards UV radiation (Figure 4), combinations of UV filters are used in most sunscreen formulations.

Table 2 Common UV filters approved in Australia (AUS), Europe (EU), Japan (JP), and United States (USA) (Osterwalder et al., 2014)

	INCI (International Nomenclature of Cosmetic ingredients)	COLIPA (Cosmetics Europe)	USAN (United States Adopted Names)	Trademark	INCI abbreviation	Form	Concentration limits in sunscreen (%) AUS	EU	JP	USA
Broad-Spectrum and UVAI (340–400 nm)	Bis-ethylhexyloxyphenol methoxyphenyl triazine	S 81	Bemotrizinol	Tinosorb® S	BEMT	p	10	10	3	*
	Butyl methoxydibenzoylmethane	S 66	Avobenzone	Parsol® 1789	BMBM	p	5	5	10	3
	Diethylamino hydroxybenzoyl hexyl benzoate	S 83	–	Uvinul® A Plus	DHHB	p	10	10	10	–
	Disodium phenyl dibenzimidazole tetrasulfonate	S 80	Bisdisulizole Disodium	Neo Heliopan® AP	DPDT	p	10	10	–	–
	Drometrizole trisiloxane	S 73	Drometrizole Trisiloxane	Mexoryl® XL	DTS	p	15	15	–	–
	Menthyl anthranilate	–	Meradimate	–	MA	p	5	–	–	5
	Methylene bis-benzotriazolyl tetramethylbutylphenol	S 79	Bisoctrizole	Tinosorb® M (active)	MBBT	d	10	10	10	*
	Terephthalylidene dicamphor sulfonic acid	S 71	Ecamsule	Mexoryl® SX	TDSA	p	10	10	10	*,†
	Zinc oxide	S 76	Zinc Oxide	Z-Cote® HP1	ZnO	p, d	no limit	‡	no limit	25
UVB (290–320 nm) and UVAII (320–340 nm)	4-Methylbenzylidene camphor	S 60	Enzacamene	Eusolex® 6300	MBC	p	4	4	–	*
	Benzophenone-3	S 38	Oxybenzone	–	BP3	p	10	10	5	6
	Benzophenone-4	S 40	Sulisobenzone	Uvinul® MS40	BP4	p	10	5	10	10
	Polysilicone-15	S 74	–	Parsol® SLX	PS15	l	10	10	10	–
	Diethylhexyl butamido triazone	S 78	Iscotrizinol	Uvasorb® HEB	DBT	p	–	10	–	*
	Ethylhexyl dimethyl PABA	S 08	Padimate O	Eusolex® 6007	EHDP	l	8	8§	10	8
	Ethylhexyl methoxycinnamate	S 28	Octinoxate	Uvinul® MC 80	EHMC	l	10	10	20	7.5
	Ethylhexyl salicylate	S 13	Octisalate	Neo Heliopan® OS	EHS	l	5	5	10	5
	Ethylhexyl triazone	S 69	Octyltriazone	Uvinul® T150	EHT	p	5	5	3	*
	Homomenthyl salicylate	S 12	Homosalate	Eusolex® HMS	HMS	l	15	10	10	15
	Isoamyl p-methoxycinnamate	S 27	Amiloxate	Neo Heliopan® E1000	IMC	l	10	10	–	*
	Octocrylene	S 32	Octocrylene	Uvinul® N539 T	OCR	l	10	10	10	10
	Phenylbenzimidazole sulfonic acid	S 45	Ensulizole	Eusolex® 232	PBSA	p	4	8	3	4
	Titanium dioxide	S 75	Titanium Dioxide	Eusolex® T2000	TiO_2	p, d	25	25	no limit	25
	Tris biphenyl triazine	S 84	–	Tinosorb® A2B	TBPT	d	¶	¶	¶	¶

*Time and Extent Application (TEA), Proposed Rule on FDA approval expected not before 2014.
†Approved in certain formulations up to 3% via New Drug Application (NDA) Route.
‡Not yet approved in EU, positive opinion by Scientific Committee on Consumer Safety (SCCS).
§Not being supported in the EU and may be delisted.
¶Not yet approved in EU or anywhere else (but positive Safety Opinion on 1,3,5-Triazine, 2,4,6-tris[1,1′-biphenyl]-4-yl-, SCCS Sept/Dec. 2011).
Cosmetics Europe (formerly COLIPA): http://www.cosmeticseurope.eu/, order number shows chronology of UV filter development.
Trademarks: Tinosorb®, trademark of BASF SE, Ludwigshafen Germany; Parsol®, trademark of DSM, Kaiseraugst, Switzerland; Uvasorb®, trademark of 3V Sigma, Bergamo, Italy; Uvinul®, trademark of BASF SE, Ludwigshafen Germany; Neo Heliopan®, trademark of Symrise AG, Holzminden Germany; Mexoryl®, trademark of L'Oréal, Paris France; Z-Cote®, trademark of BASF SE, Ludwigshafen Germany; Eusolex®, trademark of Merck, Darmstadt Germany.
p, powder; l, liquid; d, dispersion.

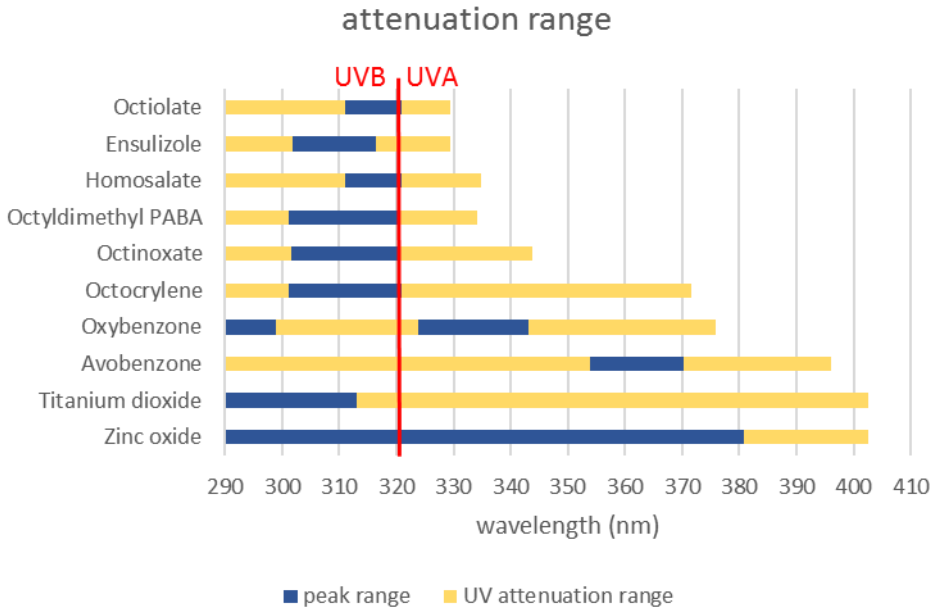


Figure 4 UV filters and their radiation attenuation range (best translated as UV extinction range).

3.2.1 Organic (chemical) UV filters

Organic UV filters work through scattering, reflecting and/or absorbing the UV radiation. Common examples of organic UV filters are salicylates, benzophenones, avobenzone, homosalate, octocrylene and cinnamates. Benzophenones are frequently used in organic sunscreen products and one of the most widely used in US-products is oxybenzone (Antoniou *et al.*, 2008). In the EU, commonly used organic UV filters are octocrylene, homosalate and avobenzone. Organic UV filters are criticised for their potential environmental effects (see introduction chapter).

3.2.2 New organic UV filters

In recent years, products have been introduced on the EU market containing so called 'new organic' UV filters. The main reason for their development was that these UV filters have a lower chance of skin irritation and skin allergies (Dr. Jetske Ultee, 2018). Examples of new organic UV filters are Tinosorb S/M, Uvinul A plus/T150, Ensulizole and Mexoryl SX/XL. Their mode of action is mainly absorption. Most of these compounds are not yet approved by the FDA, and are, therefore, not yet applied in US products as main UV filter (Skinacea, 2012).

3.2.3 Inorganic (mineral based) UV filters

Zinc oxide (ZnO) and titanium dioxide (TiO^2) are mineral-based UV filters (Manaia *et al.*, 2013), for which the particle size ranges from nano to non-nano sizes. ZnO and TiO^2 sufficiently reflect UV radiation to prevent sunburn (Osterwalder *et al.*, 2014).
Zinc oxide is the metal zinc that has been oxidized. The powder is insoluble in water. Zinc oxide in sunscreens is a fine powdered mineral that sits on top of the skin, scattering and reflecting UVA & UVB. Larger particles (200-400 nm) reflect and scatter sunlight and, therefore, the sunscreen appears white. A smaller average particle size of ZnO of 40~100 nm, absorbs most visible light (still scatters UV rays), making it transparent.

nTiO2 and nZnO in sun care products have received criticism for their possible adverse effects on the aquatic environment (see elaboration in chapter 4). Consequently, non-nano forms with nanoparticles larger than 100 nm become more popular for sunscreen formulations produced by eco-conscious sunscreen companies (Maipas and Nicolopoulou, 2015).

In the EU, a nanomaterial is defined as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1nm – 100nm." In the US, no clarification on nano and non-nano is made.

- The definition is further explained in that "materials where for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1nm – 100nm are classified as nanomaterials."
- This means that if < 50% of the total particles in the distribution, including aggregates and agglomerates, are within the 1-100nm range in any dimension the particles would be considered non-nano.

3.3 Levels in the environment

A variety of studies was reviewed by ICRI (2018) providing an overview of environmental levels of UV filters. This overview shows that levels found are highly variable. Sunscreen filters are generally found at barely detectable levels of a few parts per trillion (ng/L) (ICRI, 2018), but also concentrations of over 1 mg/L have been reported by Downs *et al.* (2015) at a monitoring site on the US Virgin Islands.

Although for many commonly used UV filters data become more and more available (see Figure 5, based on a study by Tsui in 2014), reported levels of zinc and titanium remain scarce (see also chapter 4). The highest concentrations reported in the unfiltered fraction of the surface microlayer at beach sites on Mallorca were for titanium: 38 µg/L and for zinc: 10.8 µg/L (Tovar-Sánchez *et al.*, 2013).

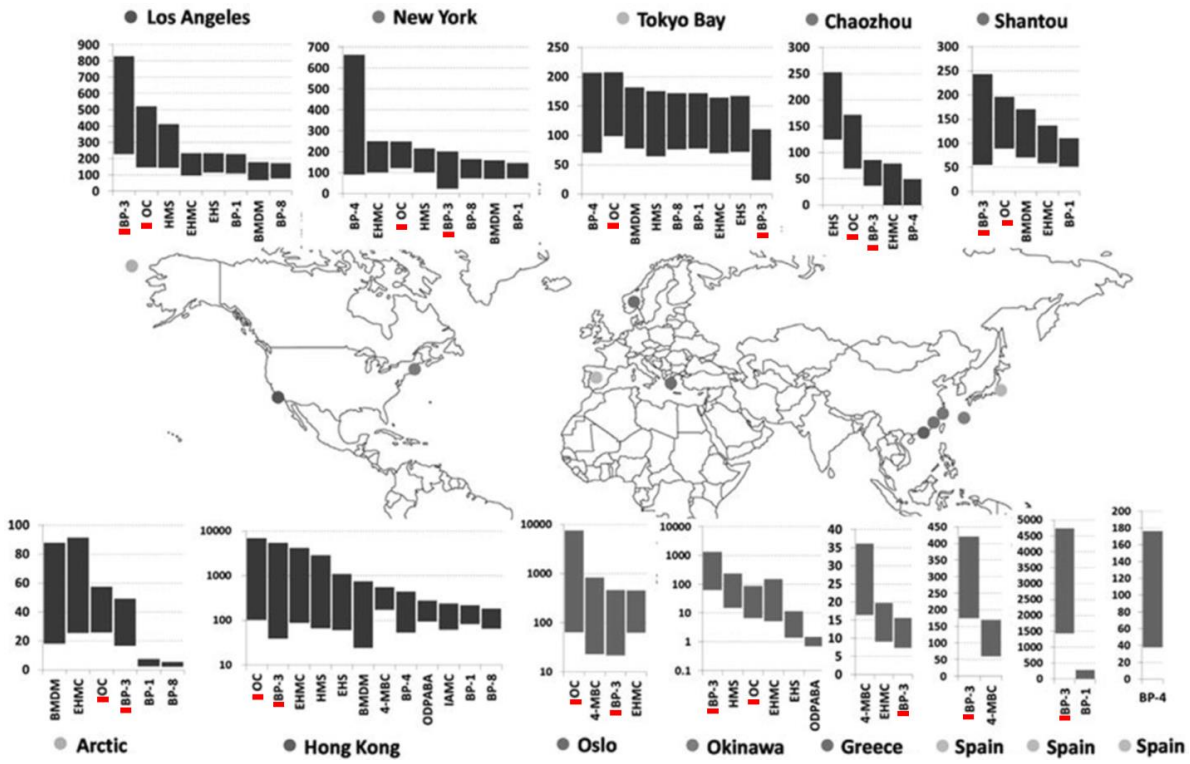


Figure 5 Various concentrations of different UV filters worldwide (ng/L) (Tsui et al., 2014). BP-3 = oxybenzone, OC = octocrylene. Other organic filters are not reviewed in this report.

4 Ecoproducts: literature review

Many alternative sunscreen products are available. To get a better understanding of all the types of "eco"-products available, including their active ingredients, a detailed overview list is made (annex 2) This list is made by researching online drugstores in the US and the Netherlands (April 2018).
In total 98 different sunscreen products could be found which claim to be safe for the environment.

As much as 71.4% of the total number of products evaluated contain mineral UV filters, being (n)ZnO, (n)TiO2, or a combination. Most products contain non- nZnO.
For some products no specific particle size was reported so it could not be determined whether nano or non-nano particles are used. These are reported in the table as ZnO and TiO2.
Moreover, 29% of the total products contain one or more organic UV filters, and 9% of the total products contain oxybenzone.
The products used various labels and claims, for which the exact meaning was unclear. In the following section more information is provided.

Table 3 Overview of products found in online drugstores, with a claim of being eco friendly in some way (see for claims 4.1.2), including UV filters used.

UV filter	Number	Mean concentration	Min	Max
Mineral	**70**			
Non-nZnO	50	15	6	25
Non-nTiO_2	17	6	2	11
(n)ZnO	19	14.5	4	22.5
(n)TiO_2	14	4	2	7.5
Organic	**28**			
Avobenzone	17	3	2	3
Homosalate	15	10	4	15
Octocrylene	19	7.25	1.4	10
Octyl Salicylate	25	4.5	2	5
Octinoxate	14	7.3	6	7.5
Padimate O	2	7	7	7
Oxybenzone	8	5	2	6

4.1 Ecoproducts- claims and labels

Many brands claim to be "eco-friendly", but currently regulations that enforce the integrity of sunscreen advertisement claims do not exist. Over 460 eco labels exist (based on ecolabelindex.com, status July 2018), of which some are applied regularly to sunscreen products. Some labels are not listed on the index, but seem to be applied informally. Labels identified on products are highlighted below.

4.1.1 Eco labels

Ecocert

The Ecocert label was the first certification label for cosmetica products that are completely eco- and biological. Criteria to apply the Ecocert label are that the ingredients need to be made from renewable sources without using nano particles, parabens and other substances. Secondly 95% of the ingredients need to be of natural origin (Ecocert eco label, 2018). This label does not include criteria on environmental effect studies.

SCS Biodegradable certification

SCS Biodegradable Certification applies to liquid products such as cleaners, detergents, and soaps. The certificate is designed to verify the safe and efficient degradability under worst-case circumstances and prevents chemicals from entering the environment at a rate causing harmful concentrations before degradation can occur. To be certified 'biodegradable', a product must degrade within a 10-day timeframe, meeting standards set by the Organization for Economic Cooperation and Development (OECD) for "Ready Biodegradability." In addition, the product must be non-toxic to aquatic life and must not contribute to the growth of oxygen-depleting plant life.

EWG verified

EWG (Environmental Working Group) is an organization that performs research in personal care products to assess whether they are safe for human health and the environment. EWG presents an online database which lists and scores individual ingredients used in personal care products based on being not healthy, toxic or dangerous. To obtain the EWG verified label, products must be free of EWG's ingredients of concern, fully disclose all ingredients, and follow good manufacturing practices (Environmental Working Group, 2018).

Besides the label, EWG promotes "Skin Deep ratings". This is a list on the EWG website which provides information on ingredients in personal care product including scientific literature, and data available from companies and governments. The ratings indicate the relative level of concern for the ingredients present in the product for human exposure and the marine environment compared to other products (Environmental Working Group, 2018).

B certified corporations (B corps)

The so called B-corps certification meets certain social and environmental standards. Certified manufacturers are given a score in multiple factors, among which is the environment (B corperation, 2018). This label isn't directly referring to products being safe for the marine environment.

4.1.2 Products claims

Claims that are often used in "eco-friendly" sunscreen products are: non-nano, mineral based, reef safe, eco-friendly, sea-safe, marine-friendly, oxybenzone free, non-toxic, natural, biodegradable and chemical free. Other applied claims are for example vegan, bait safe, "Hawaiian", PABA/parabene free, cruelty free. The most frequently applied and relevant claims are described in more detail:

Mineral based
Almost all brands offering mineral based sunscreen products use UV filters that are either made up of ZnO or TiO_2, in the forms of nano and non-nano. In general, the claim mineral based refers to the UV filters being more safe and more effective against UV rays (human health). Only few products also claim mineral based being better for the environment compared to chemical UV filters (thus not containing chemical/organic filters, and thereby promoting the use of mineral UV filters in an indirect manner). Study summaries of product/brand Stream2sea, using titanium dioxide, describe no effects on the tested organisms (fish, corals). Studies were performed by Eckhard college students, and reviewed by an assistant Professor in Biology& Marine Science. However, test design and boundary conditions were not included in the summaries, and the reliability of these tests cannot be evaluated for this report.

Non-nano vs nano
The claim "non-nano" is stated on many products that use mineral based UV filters. A reasoning behind the products being non-nano is given by Badger:
"The controversy about nano particles is that they form a potential health risk because they can enter the human body. Additionally there are studies showing that very small nanoparticles (<35nm) of uncoated ZnO and TiO_2 can be harmful to the environment by being toxic to marine life. The extremely small size of these particles generates oxidative stress under UV light, potentially causing cellular damage to sensitive organisms such as coral or juvenile fish and invertebrates. This is the main reason why badger doesn't use nano particles" (Badger healthy body care, 2018).

However, many mineral sunscreens do use nano-ZnO because it is less whitening and, therefore, more aesthetically appealing than larger particle non-nano ZnO. Differences in nano, non-nano and clear zinc particles is visualised in Figure 6. Clear zinc oxide is composed of large aggregates ranging between 500 and 9000nm[1]. Non-nano zinc oxide contains only few particles smaller than 100nm, most particles appear in the range of 100-500nm. Nano zinc-oxide consists of a variety of smaller particle sizes (<100 nm) (Badger healthy body care, 2018).

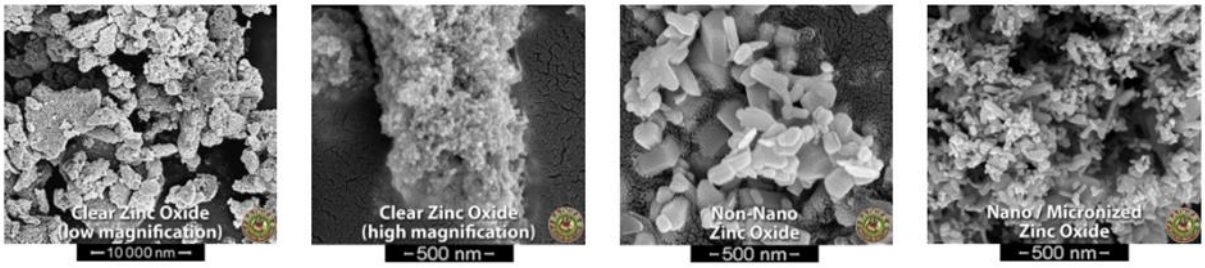


Figure 6 Different zinc oxide particles (raw zinc oxide to nano form) at microscopic level (Badger healthy body care, 2018).

Reef safe/coral reef friendly
The claims 'reef safe' or 'coral reef friendly' are the most stated claims used for alternative products. Many brands include example emission statements, saying 14,000 tons of sunscreen is washed off by swimmers, scuba divers and snorkelers into coral reef environments each year. Several brands (Alba

[1] Uncoated "Clear" Zinc Oxide acts like a non-nano particle in that it stays on the surface of your skin. However, it is actually made up of smaller nano-sized particles that are fused together into larger clumps so that no detectable nanoparticles exist in the product. The clumps are porous, like sea sponges, and their structure allows them to appear clear on the skin (Badger, 2018).

Botanica, Badger , Beyond coastal, Raw elements) relate these emissions and their potential effects to the research of Downs, *et al.* (2015).
Some brands apply criteria to meet "reef safe" claims (Figure 7). These criteria are said to relate to research of Downs *et al.* (2015) also. However, not all these aspects are studied nor reported by Downs in his research papers. Brands must thus refer to other media on which statements of Downs are reported (various media such as his institutes website).

Some products have actually tested their product in toxicity tests (bio-assays), and are transparent in providing the scientific test results in reports or summaries. Examples of these products/brands are Stream2sea (https://stream2sea.com/product-testing) and Tropical Seas. Study summaries of Stream2sea, using titanium dioxide, describe no effects on the tested organisms (fish, corals). As already mentioned above the reliability of these tests cannot be evaluated for this report.
"Reef safe suncare" is a sunscreen product by Tropical Seas that in some of their formulations apply oxybenzone. The manufacturer established and reported scientific data to support and authenticate that the products are biodegradable and are non-toxic to sea and marine life, including corals[2]. The test results report no effects on corals, based on the exposure to sunscreen levels to a maximum of 965 µg/L in the test aquaria. The set up of the test can be evaluated to be solid, with reasonable design and number of replicates to result in enough power in their statistical testing. Even the actual levels of oxybenzone were chemically analysed, instead of predicted from calculations. Given this information, there is no reason to question the outcome. However, Dr. Downs was asked to review this work and he was doubting the credibility of the executing laboratory and scientists involved, given their track record and expertise.

Figure 7 Reef friendly criteria (AllGoodproducts, 2018)

[2] https://www.tropicalseas.com/pages/real-science

Eco-friendly
Many products claim to be eco-friendly without proper explanation. The claim eco-friendly may be used for products and industries being green in production, manufacturing, carbon footprint reduction, etc., without the product directly contributing to the safety of the marine environment (Emergin C, 2018; Anytime , 2018; Lurelux, 2018; Smartshield, 2018)

Biodegradable
The claim biodegradable is stated in product descriptions many times, often without any further explanation. Usually, it means that the formula is tested by an accredited third party laboratory in accordance with industry standard methodology for biodegradability claims. These formulations meet the required standards necessary for "Biodegradable Certification". This means the formulation is designed to break down in nature with minimal impact on the earth. However, the actual environmental fate thereafter is not taken into account.

No oxybenzone and/or octinoxate
Multiple products state that their products doesn't contain the UV filters oxybenzone and octinoxate. Especially oxybenzone is known to have effects on marine organisms. For example, Alba Botanica (2018) claims that their sunscreens are free of oxybenzone and other active ingredients that may harm coral reefs, referring to the Haereticus Environmental Lab of C. Downs.
Chemical free is another statement made on products, but some of these products contain organic UV filters as was found in the product search.

In summary, labels and claims provide rough and generic reference to the objectives of the product/brand. Intentions towards environment might be positive, but are not (yet) proven by scientific sound test reports.
In general, products that do name a reference for their claims, redirect to the coral toxicity and bleaching research of the Haereticus Environmental Lab of Dr. C. Downs, or the scientific reports on oxybenzone and coral bleaching of Danovaro (Danovaro & Corinaldesi, 2003; Danovaro, *et al.*, 2008). The fact that a product excludes oxybenzone and other organic UV filters from their formulations is used as indirect "proof" that the product is safe/ eco/ etc.
But not all claims are true. Chemical free is not true by principle, as all compounds are in fact chemicals. Also, even when "chemical free" actually means "organic UV filter free", the claim is not true as some of the products do contain organic UV filters.

4.2 Fate and toxicity of UV filters

After a small introduction on the "traditional" organic UV filters, focus will be on zinc, titanium and the new generation organic filters as main ingredients used in eco products.

4.2.1 Organic UV filters

Organic UV filters can be hazardous to the marine aquatic environment and can result in several types of impacts at various trophic levels and in varying concentrations. Several studies estimated the negative adverse effects induced by organic UV filters on fish, coral, planulae, algae, flatworms, viruses, plankton, crustaceans and sea urchins (Danovaro, *et al.*, 2008; Paredes, *et al.*, 2014, Downs, *et al.*, 2015, Bachelot, *et al.*, 2012, Fent *et al.*, 2008). Oxybenzone and octocrylene are studied most. Toxicity data for oxybenzone and octocrylene are included in Annex 4.

The following effects have been described for organic UV filters:

- Genotoxicity; DNA damage to coral species by oxybenzone (Downs, *et al.*, 2015).
- Endocrine toxicity; alteration in the endocrine system, causing estrogenic disruption by oxybenzone, octocrylene and homosalate (Fent *et al.*, 2008).
- Decreased reproduction success of the fish japanese medaka by oxybenzone at 26 µg/L (Kim *et al.*, 2014).
- Developmental toxicity effects in zebra fish embryos by oxybenzone and octocrylene (Balázs, *et al.*, 2016; Blüthgen *et al.*, 2014).
- Phototoxicity by photo degradation of UV filters resulting in lipid, proteins and DNA damage by oxybenzone and octocrylene (Sánchez-Quiles & Tovar-Sánchez, 2015).
- Toxicity to corals; organic UV filters induce coral bleaching in several ways, e.g. promoting viral infections, inducing the lytic cycle in hard corals or directly harm zooxanthellae by photo degradation (Danovaro, *et al.*, 2008; Downs, *et al.*, 2015).

-
- In summary, and in comparison to the other UV filters, their lowest EC_{50}'s are presented in Table 5.

4.2.2 Mineral UV filters

4.2.2.1 (Non-)nano metals in general

Searching for information on nano and non-nano zinc and titanium effects, it became apparent that most studies focus on the nano form of these metals (hereafter nM, or nZnO or nTiO2). Studies or general information on the non-nano form is limited.
Although not much specific studies on non-nano metals were found the main difference is the relative size. Processes as described below might occur in similar ways but with different speed and intensity.

Unlike traditional metal pollutants, nM have different surface properties and compositions, which may modify their impact on aquatic environments as well as their bioavailability to aquatic organisms (Wang and Wang 2014). Figure 8 provides an example of the complex interactions of factors involved in the fate of a nano-metal particle, in this case illustrated with Zinc-oxide in the marine environment (Yung *et al.*, 2015). After release into the marine environment, the particles are expected to undergo various transitions. In general, the nanoparticles can stay in suspension as individual particles, dissolve, aggregate and form larger particles that are subsequently deposited on sediment. Furthermore, they may adsorb onto various components in marine waters (e.g., dissolved organic matter, DOM), and transform chemically based on reduction-oxidation (redox) reactions, or transform biologically in the presence of biota (e.g., microorganisms) in the marine environment (Tiede *et al.*, 2009).

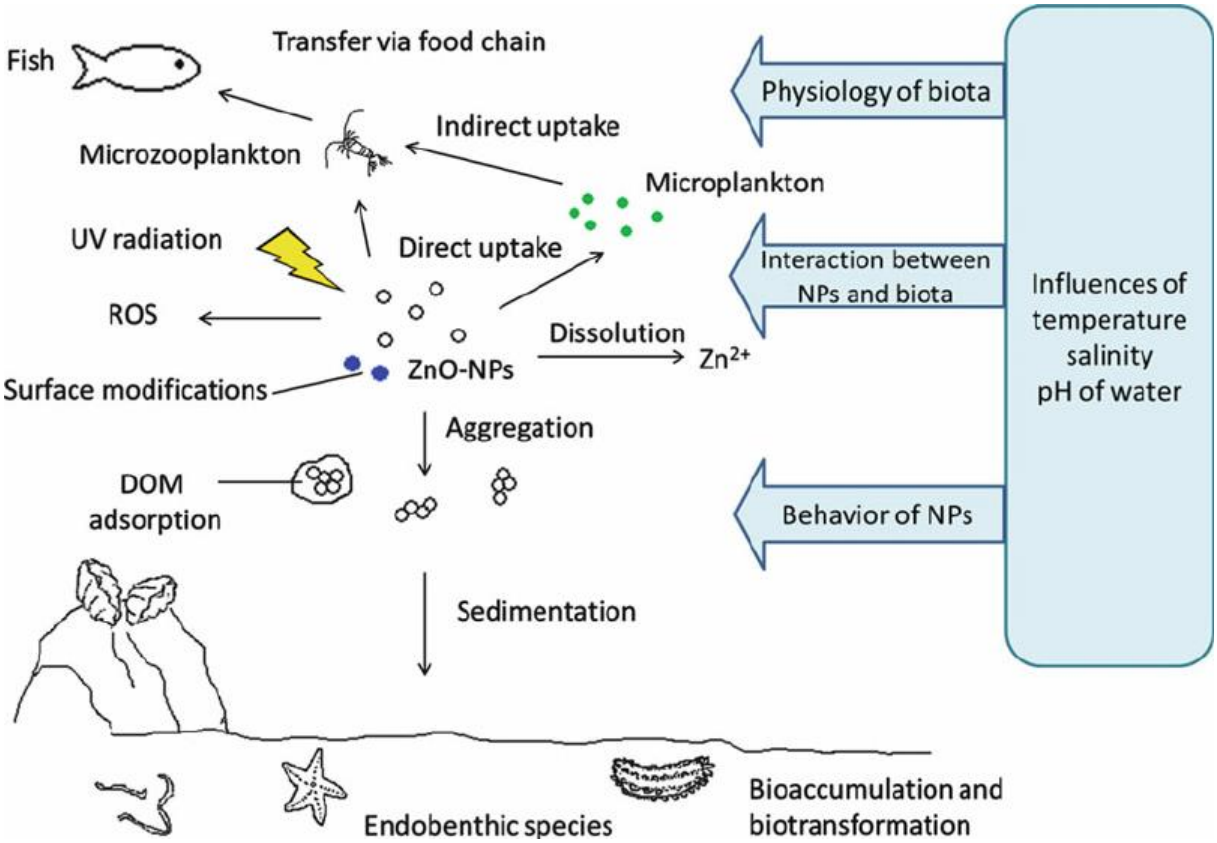


Figure 8 Schematic illustration of the behaviour and transport of ZnO-NPs (nano particles) in the marine environment (Yung et al., 2015).

The environmental fate and behaviour of nM are thus controlled by **physical, chemical and biological** transformations of nM (Lowry *et al.*, 2012). **Physical factors** include for example degradation of surface coating, advection, dispersion, aggregation, disaggregation, deposition and resuspension (Peijnenburg *et al.*, 2015). Nanoparticles can stay in suspension as individual particles, dissolve in the seawater, aggregate and form larger particles and subsequently deposit on sediment, adsorb onto various elements in marine waters (e.g., dissolved organic matter, DOM). Aggregation results in increased particle size, and subsequently decreased reactivity, bioavailability and toxicity (Amde *et al.*, 2017). Hence, the larger the particle(s), the less toxicity.
Aggregates of nanoparticles usually have a higher chance to deposit on sediments. The reduction of nano-particle aggregates from the water column through deposition or sedimentation lowers the concentration of bioavailable fraction for pelagic species such as fish. Although these aggregates become less mobile, uptake by sediment-dwelling organisms or filter feeders is possible (Yung *et al.*, 2015), transferring potential effects to other ecosystem compartments.
Nonetheless, other processes could complicate the deposition action of aggregated nanoparticles. Changes in aquatic chemistry of the surrounding environment and the activity of aquatic organisms complicate this process due to dissociation and resuspension.

Biological factors include for instance phase transformations. Although biodegradation of nano-metals is considered an important process determining fate, behaviour and impact, there is little information available on the extent and rates of NM biodegradation in environmental and biological systems (Peijnenburg *et al.*, 2015). Depending on the surface chemistry, nMs can interact with different biological entities, which may have a substantial effect on the distribution and excretion of an nM within an organism (Dobrovolskaia, 2007). In addition, Lynch and Dawson (2008) suggest that nMs become coated with certain biomolecules, such as proteins, which can redirect the nM to specific locations in an organisms body.

Chemical factors affect their ultimate distribution, persistence, and toxicity. Physicochemical characteristics of a nanoparticle (*e.g.* ionic strengths), together with the conditions of the surrounding

environment (*e.g.* temperature, oxygen level and natural organic matter), determine the behaviour and transport of nanoparticles in the environment (Peijnenburg *et al.*, 2015).

All the processes affecting nano-metals described above, are important in determining their bioavailability and subsequent toxicity to aquatic organisms (Wang & Wang 2014).
Our literature research revealed four different generic pathways nM can undergo, that affect an organism (Figure 9). The toxicity of nM have been suggested to be due to their physical effects, the amount of dissolved free ions, and production of Reactive Oxygen Species (ROS, based on the review by Yung *et al.*, 2015). More recent studies show a fourth pathway, namely a carrier function to transfer other toxicants (Ren *et al*., 2017).

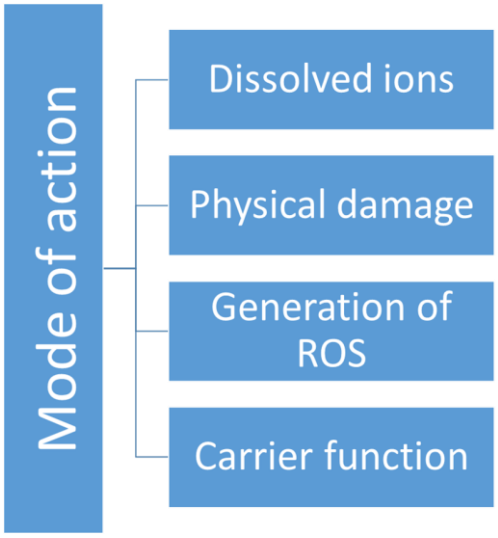


Figure 9 Four main modes of actions of metal nanoparticles

Dissolved ions
Both nano and non-nano metals undergo redox reactions[3] with their environment. As a result, metal-ions dissolve leading to increased ionic metal concentrations. A metal's speciation and bioavailability (and thus potential toxicity) is determined by complex interactions with the environment and is strongly dependent on the characteristics of the environment.
The factor time also steers the redox process, and thus the amount of free ions. Attention should be paid to environmentally relevant low concentrations of nano-metals. At low levels of the nano-form, its actual bioavailability in the original form (nano particle) may decrease. But, the dissolved ion concentration will in turn increase (Zhao & Wang, 2012), leading to an elevated accumulation of ions towards organisms (Wang & Wang, 2014). Toxicity of nZnO is mostly related to the dissolved ions (Ma *et al.*, 2013; Reidy *et al.*, 2013).

Physical damage
Physical damage includes the effects from the particle itself via interaction of the particle and the organism. Depending on size and form, the effects and pathways can differ. For example, particles attached onto the exoskeleton of the *T. japonicus* nauplii hampered their movement (Wong *et al.*, 2010). Gills and digestive gland tissues of marine oysters accumulated nZnO particles (Trevisan *et al.*, 2014), possibly leading to ineffective feeding.
Ates *et al.* (2013) observed that brine shrimp larvae ingested nanoparticles but were unable to excrete these after it formed aggregates inside the gut. Such gut congestions could significantly affect the food uptake and assimilation leading to weight loss and illness (Ates *et al.*, 2013).

[3] Chemical reaction in which there's a simultaneous transfer of electrons from one chemical species to another. It composes two different reactions: *oxidation* (a loss of electrons) and *reduction* (a gain of electrons).

Generation of Reactive Oxygen Species (ROS)
ROS are a group of free radicals -reactive molecules and ions derived from oxygen- that can be harmful to organisms at high concentrations because they can induce 'oxidative stress' in cells. Oxidative stress can damage health and ultimately cause death (Sharma *et al.*, 2012). In the environment, ROS are formed as a natural byproduct of normal metabolism. However, environmental conditions such as UV radiation, heat exposure, or presence of pollutants can increase ROS levels.
Photo excitation from inorganic UV particles (such as TiO2 and ZnO) under solar radiation produces hydrogen peroxide (H_2O_2), a typical ROS. This has been shown to induce oxidative stress to marine phytoplankton and negatively affect their growth rate (Sánchez-Quiles & Tovar-Sánchez, 2014).
Some types of nTiO2 produce reactive oxygen species (ROS) under UV illumination (Lewicka *et al.*, 2013). Toxicity of nTiO2 is mainly associated with their oxidative stress (Sharma, 2009).
In addition, Trevisan *et al.*, (2014) state that adsorbed or the ingested nZnO could release zinc ions and/or ROS, causing oxidative stresses, or biotransformation of the nanoparticles inside the organism.

Titanium dioxide nanoparticles used in sunscreen are often coated with silica, magnesium, or aluminium to eliminate their reactivity to UV radiation. It has been shown that, in the coated form, ROS production is lower (Lewicka *et al.*, 2013).

Carrier function
Interactions between organic toxicants and nano-particles in the aquatic environment may modify toxicant bioavailability and consequently the toxicant's fate and toxicity (Ren *et al.*, 2017). Recent studies report that the presence of nTio2 can result in either a decrease, or an increase in the availability of co-occurring pollutants in the environment. For example, Farkas *et al.* (2015) observed a reduction of accumulation of the hydrocarbon ***B(a)P*** in mussels. Due to its hydrophobicity, B(a)P absorbed to $nTiO_2$. Consequently, B(a)P was removed from the water column becoming less bioavailable to the mussels.
In contrast, Ren *et al.* (2017) reported higher exposure of an organophosphate tri-ester in zebrafish in co-occurrence with $nTiO_2$. $nTiO_2$ absorbed the compound tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), which was in turn taken up into zebrafish. The enhanced bio-concentration of TDCIPP in the presence of $nTiO_2$ led to adverse reproductive outcomes in zebrafish such as malformations in the F1 larvae.

4.2.2.2 (n)ZnO effects
Zinc is an essential element for all biological systems (Depledge & Rainbow, 1990), whereas titanium is not. Zinc can also be more abundant in the environment than other trace metals, and consequently organisms have developed mechanisms for bioregulation (Depledge & Rainbow, 1990; Morrison *et al.*, 1989). Zinc plays an important role in multiple physiological processes of algae and diatoms (Rueter & Morel, 1981, Morel *et al.*, 1994, Shaked *et al.*, 2006). In addition, studies looking into the role of zinc and plankton growth, showed that coastal planktonic species could be zinc limited (*e.g.* Anderson *et al.*, 1978; Brand *et al.*, 1983; Sunda & Huntsman, 1992). These studies strongly suggested that zinc limitation in the ocean could be a strong regulator on phytoplankton productivity and nutrient cycles (Croot *et al.*, 2011). This also means that under zinc limiting circumstances, any addition of zinc may accelerate growth of primary producers such as algae.

Toxicological data indicate that fish are relatively resistant to zinc (Mance, 1987), while molluscs, crustaceans and bivalves tend to be more sensitive. Depending on the natural background levels of zinc, species will have adapted their vulnerability to zinc exposure to fit their natural habitat.
More specifically, with regards to the particulate forms (nano and non nano), the effects result from either ROS, the free ions or the particulates. Redox reactions on nZnO surface may lead to the production of ROS, which are able to oxidize organic compounds and lead to oxidative stress. nZnO particles may also dissolve, releasing free Zn ions (Zn^{2-}) which may induce toxic effects (Franklin *et al.*, 2007). The smaller the size, the faster the dilution (Bian *et al.*, 2011). These Zn ions in turn can be part of various chemical reactions leading to increased, or decreased bio-availability.

Algal effect studies summarized by Yung *et al.* (2015) suggested that dissolved zinc ions released from nZnO, rather than the nanoparticles themselves, are the only cause of toxicity. However, others (e.g. Peng *et al.*, 2011) argued that also morphology and aggregates of the nanoparticles are important. No difference in toxicity between nZnO and bulk ZnO to diatoms was found (Wong *et al.*, 2010).

Exposure of zooplankton to nZnO has been associated with a number of harmful effects, which are often linked to the physicochemical properties of the particles, such as the particle size, concentration of free zinc ions, and the aggregates of nZnO. Effect assessments for nZnO particles on marine crustaceans show differences in species vulnerability. Copepods and amphipods seem to be more sensitive to nZnO than brine shrimps. Some studies reported that toxic effects of nZnO may occur at environmentally realistic concentrations (Jarvis *et al.*, 2013). Due to the aggregates formed by nZnO, benthic invertebrates, especially filter feeders and suspension feeders, face a higher exposure to nZnO (Keller, *et al.*, 2010). Latter studies focus on mussels for example, but corals are filter feeding organisms too, and can thus be exposed to aggregates.
Most effects are, however, reported at relatively high levels. Exposure to oyster *Crassostrea gigas* caused an accumulation of zinc in gills and later in their digestive system. Mitochondrial damage and oxidative stress was observed, eventually resulting in mortality and an LC_{50} of 37.2 mg/l (Trevisan, *et al.*, 2014). Oxidative stress is also observed for *Artemia salina* crustaceans resulting in some mortality (LC_{50} >100 mg/l) (Ates *et al.*, 2013).

Laboratory studies by Schiavo *et al.* (2018) showed that nZnO toxicity was strictly dependent on the exposure time. They also reported different sensitivities between test species. nZnO particles affected algae more (EC_{50} 2.2 mg Zn/L) compared to bacteria (EC_{50} 17 mg Zn/L) and crustaceans (EC_{50} 58 mg Zn/L). nZnO toxicity was related to both zinc ions and to interactions of particle/ aggregates with target organisms. The nano particle behaviour in the testing matrix thus played an important role too, in addition to the different testing time exposures (Schiavo *et al.*, 2018).

Coral sensitivity
Although heavy metals may induce a variety of negative effects, studies examining metal pollution in corals have received minimal attention worldwide, particularly in the wider Caribbean region (Hudspit *et al.*, 2017).

Coral sensitivity to zinc is reported by a limited number of studies. Effects of zinc addition seem to be dependent on coral species, treatment and endpoint chosen.

Fel *et al.* (unpublished data- referred in ICRI, 2018) exposed coral nubbins (Stylophora pistillata) for 5 weeks to the mineral UV filter zinc oxide. Zinc oxide form was not reported. They found that the treatment had no effect at 10 µ/L, but altered the photosynthetic efficiency at 100 µg/L and induced coral bleaching at 1000 µg/L.

Tijssen *et al.* (2017) tested the effects of zinc sulfide additions (0, 1, 10 and 100 µg/L) on the health, growth, NDVI (a proxy for chlorophyll *a*) and overall coloration of the stony coral *Stylophora pistillata*. Hence, free Zn^{2+} was tested, not the metallic form as used in sunscreens. After two weeks, no signs of necrosis were observed in any of the treatments. However, at 100 µg/l, a ~62% growth reduction was observed, compared to zinc levels of 0 to 10 µg/l. In addition, NDVI was significantly reduced by ~36% at 100 µg/L zinc, indicating loss of chlorophyll *a*. In conclusion, the No Observed Effect Concentration (NOEC) after a two–week zinc exposure was 10 µg/l for *S. pistillata (*Tijssen *et al., 2017)*.

- Studies performed by Reichelt-Brushett (1999) showed that fertilization success of gametes from the coral *Goniastrea aspera* was not affected by zinc-sulfide concentrations up to 500 µg/l. In contrast, in a study done with the coral *Acropora tenuis*, the mean fertilization success was significantly reduced at (free ionic) zinc concentrations as low as 10 µg/L Reichelt-Brushett 2005).
- Heyward (1988) found that up to 60% fertilization occurred in coral *P. ryukyuensis* gametes at 500 µg/L zinc exposure, and up to 30% fertilization at 1000 µg/L. Heyward (1988) also tested the effects of zinc on the coral *F. chinensis* and found minimal fertilization at 1000 µg/L. These levels are similar to the fertilization rate found by Reichelt-Brushett in 2005. Based on these results, Reichelt-Brushett (2005) concluded that trace metal inputs into reef waters should be limited and controlled to avoid potential interference with sexual reproductive processes of reef corals.

Recently, Corinaldesi *et al.*, (2018) demonstrated that uncoated ZnO UV filters induced a severe and fast coral bleaching of *Acropora* sp., whereas coated Titanium UV filter types did not cause bleaching. Corinaldesi concluded that coated titanium filters were more eco-compatible than ZnO. The level at which

ZnO posed effects on the corals in the study was 6.3 mg/l, which is far above expected and reported environmental concentrations (chapter 3.3 and 4.2.2.4).

4.2.2.3 (n)TiO_2 toxicity

One of the crucial issues of toxicity testing with nTiO2 is the limited knowledge about the real exposure levels. Most often, the determination of the nTiO2 exposure levels within toxicity tests using saltwater organisms are not established yet.

According to effect studies performed, as summarized by Minetto *et al.* (2014), no effects of $nTiO_2$ have to be expected at field concentrations. Marine bacteria (*V. fischeri*) showed no effect towards $nTiO_2$ within the concentration range tested (from >100 to >20,000 mg/L) (Müller & Nowack, 2008; Tiede *et al.*, 2009). Although the responses were species specific, algae did not show effects up to levels of 1 mg/L (based on studies by Hu *et al.,* 2018; Miller *et al.,* 2010 and Clément *et al.,* 2013). EC_{50}'s ranged between 5.37 and 14.3 mg/L, levels not being field relevant. Also for rotifers (28 days chronic test) effects levels were in the same ranges as for algae (Clement et al, 2013). Even higher effect levels were reported for crustaceans, with EC_{50}'s starting at 17 mg/L (Minetto, 2012).
Fish embryo's (Japanese Medeka) appear to be more sensitive to $nTiO_2$. Effects included reduction of hatching time, altered swimming activity and malformations at concentrations starting at 0.03 mg/l. Unhatched embryos exposed to levels of 7 and 14 mg/L were fully encapsulated in TiO_2 material by study completion, possibly resulting in oxygen stress (Paterson *et al.*, 2010).
Zebrafish larvae exposed to assumed environmentally relevant concentrations (1–10 mg/L) of $nTiO_2$ showed neurologic adverse symptoms, such as locomotor alteration, and alterations in mRNA. The authors observed TiO_2 accumulation in brain and oxidative stress, with cell death in the hypothalamus (Hu *et al.*, 2017).

The relative absence of toxicity, as well as the absence of indirect effects might be a result of the low solubility. $nTiO_2$ dispersed in saltwater is unstable and tends to form agglomerates within few hours after water contact time, sinking to the bottom of the container (Keller *et al.*, 2010). In general, $nTiO_2$ is capable of producing highly reactive oxygen species (ROS) leading to oxidative stress, and related effects.

Coral sensitivity

In general, dissolved ion contributes partially to toxicity of nZnO, while the toxicity of $nTiO_2$ is mainly due to the generated reactive oxygen species (ROS) (Wang and Wang, 2014).

The photo-inhibition of photosynthetic electron transport, the consequent photo-damage to photosystem II (PSII), and the resultant excess production of damaging reactive oxygen species (ROS) are characteristic signs of the thermal bleaching response (e.g. Lesser, 1996; Jones and Hoegh-Guldberg, 2001). However, it is yet to be determined whether this detrimental cellular cascade within the photosynthetic "light reactions" is the initial trigger, or just a secondary consequence of other processes (reviewed by Smith et al., 2005).

Elevated external ROS levels might affect the coral bleaching threshold levels, but yet it remains unsure whether this does play a role. And if so, the question remains what the contribution of elevated titanium levels to the natural ROS production might be, and the bleaching stress thereafter.

4.2.2.4 Concentrations of (n)ZnO and (n)TiO2 in the environment

It is generally assumed that (non-)nano particles will enter the aquatic environment (Canesi *et al.*, 2009). Application of sunscreens containing $nTIO_2$ and nZnO is an example of potential direct release by swimming recreants. It may be expected that released nM have the aquatic environment as their final sink (Buffet *et al.*, 2011).

Information on background levels of $nTiO_2$ and nZnO in the environment is hardly available, mostly due to the technical difficulties of sufficiently measuring these compounds at low levels. Near future advances in analytical techniques will hopefully allow quantification and accurate characterization of nanoparticles in the environment. Then, the establishment of potentially impacted areas, monitoring of levels and effects on biota from those sites will become possible.

Some predictive models are currently available (*e.g.* Gottschalk *et al.*, 2009; Müller and Nowack, 2008; Tiede *et al.*, 2009) providing estimates of environmental concentrations. Various studies report $nTiO_2$ levels in the environment ranging between 0.7-24.5 µg/l (surface water) (Müller & Nowack, 2008; Tiede *et al.,* 2009). According to Gottschalk *et al.* (2009), the predicted annual increase of $nTiO_2$ is 0.55 µg/L for surface water.
The theoretically predicted average environmental concentration of nZnO in European surface waters is 0.09 µg/l (with 85% confidence intervals: 0.05–0.29) (Sun *et al.*, 2014).

Besides the nano and non-nano forms of the minerals, the resulting free ionic levels are also relevant. Reported concentrations of zinc in seawater vary widely, probably due both to variability in concentrations actually present and to sample contamination (Nef, 2002). Typical concentrations of zinc in marine surface waters reported vary from 0.4 ng/L (based on studies by Elwood 2004 in the Tasman Sea) to 0.131 µg/L in the North East Atlantic (Nolting *et al.*, 2000). No Caribbean background levels for zinc were found. While concentrations of dissolved zinc are often very low in surface waters (ca. 0.1 nmol/L (Martin *et al,.* 1989; Bruland, 1989) they can be up to 50 times higher in some reef areas, such as in the Gulf of Aqaba (5.5 nmol/L) (Ruiz-Pino *et al.,* 1991). Tijssen *et al.* (2017) report that pristine seawaters can have a zinc content of 0.01-0.36 µg/L. However, zinc concentrations in surface waters tend to decrease with increasing distance from shore. Zinc concentrations in estuaries and coastal waters frequently are much higher than those in the ocean, with concentrations often as high as 4 µg/L and occasionally as high as 25 µg/L (Nef, 2002). A significant fraction of the total zinc in seawater may be adsorbed to particles or complexed with dissolved organic matter (Nef, 2002).

4.2.3 New organic UV filter toxicity

A limited number of studies report toxicity of new generation organic UV filters. The available data originate from classification and regulation reports and only represent freshwater species and endpoints describing reproduction, growth inhibition or mortality. No other scientific reports reporting (toxicity) effects were found for these UV filters. Hence, any effects on endocrine and developmental processes such as reported for oxybenzone are yet unknown.
All the available data show that effects occur at levels exceeding the highest test concentration. No environmental effects are reported for Tinosorb S and Mexoryl X. The highest reported endpoints for other UV filters are presented in Table 4.

Table 4 Overview of lowest reported EC50s for various "new generation" organic UV filters.

UV filter	endoint	concentration	species	reference
Uvinul A plus	EC_{50} reproduction 21 days	>0.01 mg/L	crustacean *D. magna*	European chemicals agency, 2018.
Uvinul T150	EC_{50} growth inhibition 72 hours	≥80 mg/L	Algae (*S. subspicatus*)	Federal Institute for Occupational Safety and Health, 2016).
Tinosorb M	EC_{50} 72 hours	>2 mg/L	Algae (*S. subspicatus*)	Federal Institute for Occupational Safety and Health, 2016).
Mexoryl SX	EC_{50} mortality 96 hours	>100 mg/L	fish *O. mykiss*	European chemical agency, 2018
Ensulizole	NOEC growth inhibition 72 hours	≥100 mg/L	algae *P. subcapitata*	European chemicals agency, 2018

Levels of the new organic UV filters in coastal areas have not been found in this study. It can be assumed that these do not exceed concentrations as found for the traditional UV filters as the new generation organic UV filters are not extensively applied yet.

4.2.4 UV filter toxicity in comparison

To provide a summary of reported effect levels in literature, the lowest EC_{50}'s and NOECs of a selection of UV filters are summarized in Table 5 and Table 6. New generation filters were not included as they show hardly any effects (Table 4) in the test reported. Effects of new generation filters upon developmental processes are not reported and cannot be evaluated.
The data show a range in data availability and the narrow range of overlap of tests performed and reported hamper a quick evaluation of the reported data. Based on the available data, some preliminary generic assumptions can be made.
Algae seem more vulnerable to oxybenzone than to mineral compounds, whereas for crustaceans ZnO filters display effects in the same concentration range as for oxybenzone (LC50) or much at much lower concentrations when taking NOEC values in to account. TiO_2 on the other hand does impact crustaceans (within the tests conducted) at much higher concentrations.
Sea urchin larval development seems more sensitive to nZnO, compared to the organic UV filters.

Based on this comparison, no clear and overall distinction on the toxicity between organic and mineral UV filters can be made. In general for all new generation UV filters (Table 4)- and although tested very limited too- EC_{50}'s are much higher, indicating a much lower toxicity in basic bio-assay testing. Additional comparative testing has to be conducted to be conclusive.

Table 5 Lowest EC50 values in mg/L summarized from all available toxicity data (Annex 4) for 5 different compounds used as UV filter. References to the data are included in annex 4.

Type effect	oxybenzone	octocrylene	nZnO	Non-nZnO	nTiO2	Non-nTiO2
Coral development	0.017 24h	-	-	-	-	-
Algae growth inhibition	0.014 *I. galbana* 72h	-	1.94 *D. tertiolecta (96h)*	2.97 *S. constatum*	10.91 *P. tricornutum* 72 h	-
Crustacean mortality	0.7 *S. armata* 96h	-	0.85 *T. japonicus (96h)*	0.37 *E. rapax*	1.3 *D. magna* (72h)	>20000 *T. platyurus* (24 h- FW)
Rotifer mortality	-	-	-	-	5.37 *B. plicatilis* (48h)	-
Molluscs larval malformation	3.5 *M. galloprovincialis* (48h)	-	-	-	1.23 *M. galloprovincialis* (48h)	-
Mollusc mortality	-	>0.65 *M. galloprovincialis* 96 h	37 *C. gigas* 96 h	-	-	-
Sea urchin larval development	3.3 *P. lividus* 48 h	0.74 *P.lividus* 48 h	0.1 *L. pictus* 96h	-	-	-
Bacteria (*Vibrio fischeri*)	-	-	1.9	1.8	651	>20000

Table 6 Lowest NOEC values summarized from all available toxicity data (Annex 4) for 5 different compounds used as UV filter. References to the data are included in annex 4.

Type effect	oxybenzone	octocrylene	ZnO nano	ZnO non nano	Nano TiO2	Non nano TiO2
Coral development	2.28 µg/l Planula mortality 24 h	-	-	-	-	-
Algal growth inhibition	3.7 ug/l I. galbana 96 h EC10	40 µg/l I. galbana 96h	10 µg/l *Th. weissflogii* 72 h	-	-	-
Crustacean mortality	375 µg/l S. armata 96h	-	- 10 µg/l - A. tonsa 72h	50 µg/l *Th. platyurus* 48 h	1000 µg/l D. magna 48 h	>20000 mg/l Th. platyurus 48 h
Molluscs	30 µg/l M. galloprovincialis 48 h	20 µg/l M. galloprovincialis 96 h	-	-	2 mg/l H. diversicolor supertexta (10h)	-

4.2.5 Water quality standards

- No standards have yet been established for permissible levels of nanoparticles in the environment. Instead, we report the threshold levels available for the free metal ions.

- Various water quality standards for zinc in marine waters are installed by different countries (Table 7). It should be noted that these can differ due to differences in applied derivation method. Standards can also differ, because these have been validated/corrected for the bio-availability of zinc related to regional specific water characteristics (e.g., pH, DOC3 and water hardness (Biotic Ligand Model)). The ANZECC and ARMCANZ (2000) trigger value for zinc to protect 99% of species in marine waters was set 7.0 µg/L zinc in 2010, but was updated in 2016 and lowered to 3.1 by Gadd & Hickey (2016). From the table it can be deduced that the quality standard also depends on the protection level (as done by Gadd & Hickey in New Zealand). The water quality standard by Gadd & Hickey for New Zealand of 3.1 µg/L is the most recent, and conservative (99% protection, based on chronic effect data). In the latest dataset, effects on fertility on coral was included also, resulting in a protection level for coral reef ecosystems too (personal communication dr. Gadd).

Based an initial literature review the RIVM (2013) stated that the Dutch water quality standard (MAC) of 20 µg/L is sufficiently protective for short-term exposure of aquatic organisms. This also holds for the nano-form. It is, however, not known, whether this is also the case for chronic effects, since almost no studies are available in which aquatic organisms have been exposed for longer periods of time (RIVM, 2013). RIVM also states that $nTiO_2$ has a stronger effect on aquatic organisms than the traditional form. Since the status of nano-titanium in Dutch surface waters is unknown, it is not clear whether laboratory observations are relevant for the field situation. Derivation of water quality standards for nano-titanium dioxide should be considered if the presence of nano-particles is confirmed. A standard protocol for such an analysis is, however, not yet available. No other water quality standards for titanium were found in this study.

Table 7 Overview of some Environmental quality standards in various countries based on chronic effect studies and data.

	Name of standard	**µg/L**	**year of establishment**	**Reference**
US	EPA-Water quality criteria	81	1995	EPA (2004)
New Zealand + Australia	Default guideline value (DGV)		2016	Gadd & Hickey (2016)
99% species protection		3.1		
95% species protection		6.5		
90% species protection		9.8		
80% species protection		16		
Netherlands	Maximum Acceptable Concertation – Quality Standard (MAC-QS)	4 (acute data)	2007	Bodar (2007)
UK	Environmental quality standard (EQS)	10	1997	Grimwood & Dixon (1997)

4.3 Summary literature review

- Eco labels are not necessarily based on environmental toxicity testing procedures of cosmetic products. More often they reflect business principles.
- Based on toxicity data reported in literature comparison, no clear and overall distinction between the toxicity of organic and mineral UV filters can be made.
- New generation UV filters are scarcely tested. Reported data indicate a much lower toxicity but additional comparative testing (e.g. on developmental and endocrine disruptive processes) has to be conducted to be conclusive.
- Zn is in general more toxic then Ti, and Ti is considered to be more eco-compatible
- The smaller the particle, the more likely effects will occur due to the release of free ions (Zinc). Hence, nZnO products might show more effects in the bio-assays than ZnO products.
- Effects of bio available zinc towards coral can occur at levels starting from 10 µg/l, but is also coral species, treatment and endpoint dependent. To protect the marine environment at a 99% species protection level, threshold levels for zinc (ions) are set at 3.1 µg/l.
- Field levels of nZnO and $nTiO_2$ cannot yet be established in a proper way.
- Fate and effects of mineral UV filters depend on too many factors to predict a realistic risk at the moment. Moreover, effects described for both ZnO and TiO_2 do not occur in field relevant concentrations (yet).
- In case of zinc limitation, zinc could stimulate algal growth.

5 Results bioassays with sunscreens

In this chapter, a summary of the results of the various laboratory tests is presented by providing overview tables of effect concentrations (expressed as EC_{50}) per product. In addition, the ranking of relative observed effect concentrations is presented, as well a translation to the contribution of each UV filter to the observed effect.

5.1 General performance of the bioassays and products

Based on the reference toxicant applied, all bioassays performed within their range. Quality parameters met the criteria in all bioassays. Generic observations were:

- Dissolving sunscreen in stock solutions. Some solutions were not completely homogenous due to their formulation (fatty substances, not easy to dissolve without solvents). Especially at higher concentrations undissolved particles remain in the stock solution.
- Microtox test.
    - For none of the products, the 50% effect level was reached, even in the highest possible concentrations tested. Mathematically, the EC_{50} is based on extrapolated data giving an estimation, instead of a more precise calculation. This explains large confidence intervals.
    - Samples were also centrifuged to get rid of particles and to test whether this had an effect on the observations. Centrifuged samples do not differ from not-centrifuged samples, which indicates that the smaller undissolved particles did not interfere with the measurements.
- Rotifer acute tests. Rotifers that die during the test usually sink to the bottom of the test-well. In some of the tested products, rotifers died, but instead of laying at the bottom, they floated at the surface. The organisms were trapped in the fatty substance floating at the surface, not able to escape from this layer, leading to their death. This mechanism is translated as being a so-called physical effect, rather than a direct effect from dissolved substances in the water. Even though this is another type of effect, this is still field relevant. Fatty substances could end up in the surface microlayer and could cause similar effects in the environment. This effect was most apparent at product B.

5.2 Relative toxicity of the 7 products

5.2.1 Product ranking

Table 8 shows the combined results of all the sunscreen products (rows) for all the conducted bioassays (columns). If data allowed, an exact EC_{50} in (µl/L) is calculated. When data did not allow because e.g. 50% mortality was not reached, the EC_{50} is noted as exceeding the highest tested concentration. The 95% confidence interval is presented between brackets. For the microtox results, the actual intervals aren't reported. These were high (see 5.1). The range of the centrifuged and non-centrifuged results are provided instead.
EC_{50}s for the rotifer test could be estimated for only two out of the seven products, being the organic products. The other products did not affect rotifer survival at the maximum possible concentrations tested. Algae tests showed varying level of effects in four products. Mineral products did not affect microtox test at all, organic products however showed some effects in this test, with product F showing largest effect of all products. Algae tests were affected by both organic and mineral products. A clear difference in effect between 1 and 4 day old stock solutions was detected and discussed in more detail in the following paragraph.

*Table 8 EC50 of 7 sunscreen products in µl/L (95% CI). *microtox data are combined centrifuged and non-centrifuged data.*

Product	Microtox *	Rotifer acute	Algae 1day	Algae 4days
Product A	107-138	38.06 *(31.4-46.1)*	16.1 *(10.9-23.6)*	27.5 *(16.6-45.5)*
Product B	90-180	3.93 *(>>>)*	69.9 *(54.9-88.9)*	>100
Product C	>90	>150	21.5 *(18.0-25.6)*	18.9 *(16.7-21.3)*
Product D	>90	>150	127 *(111.9-144.1)*	94.6 *(74.1-120.7)*
Product E	>90	>150	>200	>200
Product F	23-44	>150	>125	>125
Product G	235-242	>150	>200	>200

In Table 9, a summary is presented in order to indicate the relative effect of each product by comparison. Four categories of relative scores were applied in order to rank the products in their relative response. No effect = blank, score 0. Weakest effect = pale blue (score 1), middle effect = middle blue (score 2); highest effect = dark blue (score 4). Per product, all scores were averaged into an overall score. This overall score was set onto the maximum score possible (4), resulting in a relative score between 0-1 for each of the products.

Table 9. Summary table bio-assay response. No effect = blank, score 0. Weakest effect = pale blue (score 1), middle effect = middle blue (score 2); dark blue = highest effect (score 4).

Product:	Microtox	Rotifer acute	Algae 1day stock	Algae 4day stock	Score
Product A					0.88
Product B					0.44
Product C					0.50
Product D					0.13
Product E					0.00
Product F					0.38
Product G					0.06

The difference in relative effect-scores of the eco products and traditional products is substantial. The ranking shows that eco-friendly products D, E and G have lowest overall scores (and thus effects). Products D and E include mineral UV filters in the non-nano form, product G includes organic "new generation" UV filters. This means that not only mineral based products respond well, but also products with new generation UV filters. Product C and D differ in containing respectively nano and non-nano ZnO. It contains a nano mineral UV filter, which might have steered the relative higher effect-score compared to the other non-nano mineral products. This indicates the relevance of particle size as was also observed in the literature review.
Product A is ranked highest, followed by products C, B and F. Products A and B include "traditional" organic UV filters, and affect almost all organisms used in the tests. Product A is the only product containing oxybenzone (2%). Product C, containing nZnO, affected algae only.
Product F, also scores relatively high. This product contains new generation organic UV filters, some similar to those in product G. Product F does not claim to be "eco". From the information on the label no specific harmful compounds were apparent.

Product E scores overall low. This can be explained by the fact that product E only contains non-nano TiO_2 as UV filter, which is reported to affect organisms only at very high levels (see chapter 4), which were not reached in these tests.

5.2.2 Algal growth in aged test solutions

Toxicity of metal particles is probably mainly caused by free ions (chapter 4). Time is a major factor in this process, as ions have to be released by redox reactions. Based on this information, our hypotheses was that the observed effects of sunscreen solutions in tests might change when stock solutions applied have different "aging" periods. In addition to testing with sunscreen solutions of 1 days old (prepared on the day the test starts), another test was performed with stock solutions of 4 days old (leaving it in the dark at room temperature after preparation).
Algae growth inhibition tests were performed with these two type of solutions for all sunscreen products.

The results showed clear differences in effect of stock aging. This is illustrated in Figure 10, in which for two products the growth inhibition over time is presented for stock solutions differing in age.
Product B, an organic product showed less growth inhibition when the test material was 4 days old (at the start of the test). Product A, also organic, showed a similar response. An explanation could be that the UV filters in the 4 day-old stock solution were stuck onto the glass before making the test series, and that the actual concentration of UV filters and other ingredients in the water was lower compared to the 1 day old test material. Another explanation might be that degradation of the compounds occurred, thereby changing their inhibiting potential.
Products F and G with new organic UV filters did not show any effects with stocks of both ages.

In contrast, mineral products containing ZnO showed a higher growth inhibition in the test with 4 day old solutions. The strongest effect is presented in Figure 10 (right figure), in which the effect of product D is shown. Product D contains non-nano zinc and non-nano titanium. Product C, containing nZnO and TiO_2, showed a similar increase of the growth inhibition with the 4 day-old solution. Product (E) with solely TiO_2 did not show this effect, indicating that it might be steered by ZnO and its released ions. The overall growth inhibition (both with 1 and 4 day old stock) by product C was much stronger than with product D, indicating a stronger effect of the nZnO compared to non-nZnO.

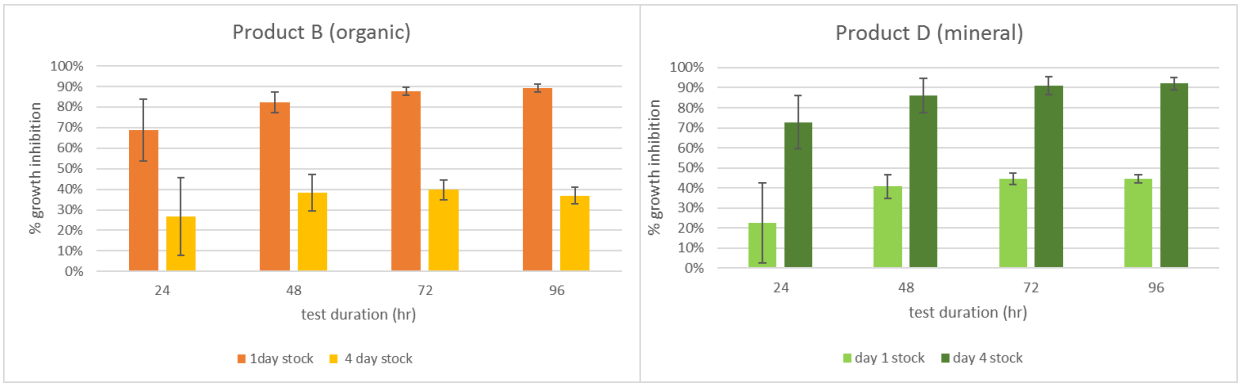


Figure 10 Effect of solution aging on algal growth inhibition for 2 types of products (organic and mineral).

5.3 EC_{50} expressed per UV filter content

Calculated EC_{50}'s in µl product/L, are translated to the concentration of each individual UV filter in order to derive an EC_{50} in mg UV filter/l. These concentrations are estimations, based on the known presence (%) in the product formulations given by the producer or based on legislation.
These estimated levels indicate the EC_{50} per UV filter, assumed the effect was solely caused by the single substance. This is only an estimation, in order to indicate the field relevance of the effect levels observed in the performed tests.

Table 10 shows the overview of the calculated concentrations of each of the active ingredients for all tested products. The % substance is derived from the percentages on the products, in the case of product A, B, D and E. Percentage of product C is derived from the average of all found products in appendix 1, which contains nZnO and non-$nTiO_2$. Percentages of product F and G are derived from maximum approved concentrations by European and USA regulations (Skinacea, 2012).

Table 10 Per sunscreen EC_{50} expressed in mg/l per active ingredient per test

Product	Active ingredients	% substance	Microtox*	Rotifer	Algae
A	avobenzone	*3*	3.1	1.10	0.5-0.8
	homosalate	*7.5*	7.7	2.75	1.2-1.99
	octyl salicate	*5*	5.2	1.84	0.8-1.33
	octocrylene	*2.75*	2.8	1.01	0.4-0.7
	oxybenzone	*2*	2.1	0.73	0.3-0.5
B	homosalate	*7*	12.4	0.27	4.8
	octocrylene	*3*	5.3	0.12	2.1
	avobenzone	*3*	5.3	0.12	2.1
	octyl salicate	*3*	5.33	0.12	2.1
	TiO2 (nano)	*5*	8.9	0.19	3.5
C	ZnO (nano)	*10*			1.96-2.2
	TiO2	*5.8*			1.1-1.3
D	TiO2	*6.4*			6.3-8.4
	ZnO	*6*			5.9-7.9
E	TiO2	*8.8*			
F	Uvinul A plus	*10*	2.2		
	Uvinul T 150	*15*	3.3		
	Tinosorb S	*10*	2.2		
	Ensulizole	*4*	0.9		
G	Tinosorb M	*10*	23.5		
	Uvinul T 150	*5*	11.7		
	Uvinul A plus	*10*	23.5		

Toxicity effect levels in the bioassays differ from the toxicity data found in literature (table 4). Overall, effect levels in the tests in this study are higher (thus toxicity of tested sunscreens was lower).

For example, for oxybenzone, the lowest EC_{50} of Haptophyte algal species is 0.013 mg/L (table 4) while the lowest effect level for *S. constatum* algae in the bioassays performed was 0.31 mg/L. This can be explained by variance in species sensitivity. The rotifer toxicity data obtained in our bio-assays for oxybenzone are in the same range as found in literature for a crustacean (0.7 mg/L for *S. armata*).

When comparing effect levels of non-nZnO in literature, a lowest EC_{50} of 2.97 mg/L for *S. constatum* algae is reported. The bioassays showed slightly higher EC_{50}'s of 5.88 mg/L, indicating that non-nZnO showed less toxicity in the bioassays compared to data in literature. A possible explanation for these differences is that the mineral UV filters in sunscreen formulations are less bioavailable while the individual chemical tested is more available to the organism and results in stronger toxicity effects. Question remains whether this difference is significant.
Comparing toxicity observed for nZnO with literature toxicity data, the observation is similar. The lowest EC_{50} for *S. constatum* in literature is 2.36 mg/L (table 4). Bioassays resulted in estimated EC_{50}'s of 1.96 to 2.23 mg/L related to nZnO, which are the same levels as reported in literature.
Overall, comparing nano and non-nano zinc products indicates that algae were more vulnerable for nano zinc product than for the non-nano zinc product in these tests.

Octocrylene effect levels in literature are scarce, and no comparison on EC_{50} values could be made. A reported NOEC (algae) for octocrylene is 0.040 mg/L, being the same concentration as the EC_{50} found with product A in our study. This might indicate a stronger observed effect of octocrylene when using a formulation. However, mixture toxicity could account for the effects too.

TiO_2 toxicity was not observed in the bioassays. This observation is in line by the high reported EC_{50} values in literature (table 4).

5.4 Summary of bio-assay results

- Eco-products showed no or much fewer effects in the bio-assays compared to traditional sunscreen products.
- The TiO_2 product showed the least effects, the oxybenzone product the most
- Both the new generation organic UV filter eco-products, as well as the mineral eco-products show no/very limited effects on the organisms tested.
- The nZnO product showed stronger effects to algae than the non-nano products.
- Ionic release by (n)ZnO most probably explains the increased toxicity towards algae. Hence, environmental effects would probably not appear directly after emission of ZnO particles, but sometime later.
- Traditional products containing organic UV filters such as oxybenzone and octocrylene showed most severe effects in the tests performed.

6 Risk assessment

Based on both the retrieved literature toxicity data and bio-assay results, risk assessments were performed according to international standards (ECHA). Doing so, environmental risk of whole sunscreen products and individual UV filters was evaluated for the situation at Lac Bay Bonaire.

6.1 Worst case scenario study Lac Bay, literature based.

A risk assessment was performed for the release of UV filters non-nZnO, nZnO and $nTiO_2$. For new generation organic UV filters no, or too little toxicity data was present to enable a risk assessment according to ECHA criteria.

Field concentrations of zinc and titanium (particulate and ionic) are currently not available, nor is it known what levels in the future might be. Instead, a rough estimation on future levels of the particulate minerals was made, in order to evaluate a worst-case risk assessment. No assessment on the free ionic elements was performed.

6.1.1 PEC estimation

Following the method described in Chapter 2, the estimated released amounts are presented in Table 11 for the three UV filters in kg/day and gram/day. Non-nZnO has the highest potential release, because the average content is the highest (15%).

Table 11 Daily estimated release of UV filters in kg/day and gram/day

UV filter	Kg/day	Gram/day
Non-nZnO	0.20	201.3
nZnO	0.13	134
nTiO2	0.05	54

PEC's for each of the three zones (bathing zone, inner reef zone and mangrove zone) are presented in Table 12. PEC's in the bathing zone are higher than in the other zones, because of the small volume of this area.

Table 12 PEC's (µg/l) for three UV filters for three zones at Lac Bay

UV filter	Bathing zone	Inner-reef zone	Mangrove- zone
Non-nZnO	12.58	0.24	0.05
nZnO	8.39	0.16	0.04
nTiO2	3.36	0.06	0.01

6.1.2 PNEC estimation

The PNEC's per UV filter are presented in Table 13. From the toxicity database the corresponding ECHA criteria are given, resulting in a certain assessment factor (AF). The PNEC is derived by dividing the lowest LOEC/NOEC, as given in the next column including the species and type of effect, by the assessment factor. For the PNEC estimation both freshwater and marine data were used, as marine toxicity data was limiting.

Table 13 PNEC's (µg/l) derived according to (ECHA, 2008), based on corresponding AF's. Lowest test concentration and species is reported

UV filter	Data coverage criteria	AF	Lowest test concentration + species	PNEC
Non-nZnO	Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish)	500	Crustaceans (FW): *Thamnocephalus platyurus* NOEC (immobilisation) 50µg/l (Heinlaan, *et al.*, 2008)	0.10
nZnO	Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) + one long-term result from an additional marine taxonomic group (e.g. echinoderms, molluscs)	50	Algae: *Thalassiosira weissflogii* NOEC (growth inhibition) 10µg/l (Jarvis *et al.*, 2013)	0.20
$nTiO_2$	Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) + one long-term result from an additional marine taxonomic group (e.g. echinoderms, molluscs)	50	Crustaceans (FW): *Daphnia magna* NOEC (mortality) 1000µg/l (Lovern & Klapper, 2006)	20

6.1.3 Risk Quotients

RQ's based on PEC/PNEC's are presented in Table 14.

RQ's for non-nZnO are 126 in the bathing zone and 2.4 in the inner reef zone. This suggests that when everyone shifts to using sunscreen products containing non-nZnO, these two zones in Lac Bay could face a potential environmental risk. No risk for non-nZnO was apparent in the mangrove zone.

RQ's for nZnO are 42 in the bathing zone. This suggests that when everyone shifts to using sunscreen products containing nZnO, the bathing zone in Lac Bay faces a potential environmental risk. nZnO does not pose a risk to the other zones in Lac Bay.

Comparing the RQ's of these two UV filters, non-nZnO potentially poses a higher risk than nZnO, even though the toxicity database and literature suggest that nZnO is more toxic. This can be explained by the low number of toxicity data available for non-nZnO, resulting in the application of a much higher AF (500) for non-nZnO, whereas for nZnO much more data are available, resulting in an AF of 50. Addition of toxicity data and measuring the actual field concentrations to derive a realistic PEC would largely improve the overall risk assessment.
Also, the risk assessment is fully based on particulate zinc, while the ionic form of zinc (probably causing much of the nano zinc toxicity) is considered the more toxic form.

RQ's for $nTiO_2$ are all <1, which suggests that Lac Bay doesn't face a potential environmental risk by the use of sunscreens based in TiO_2. This can be explained by the average low amount of TiO_2 (4%) used in sunscreen products, resulting in lower potential release and thus lower PEC's. But also the lower toxicity of $nTiO_2$ compared to both types of ZnO compounds, resulting in a higher PNEC.

Table 14 Risk quotients per UV filter, derived from PEC (Table 5) and PNEC (Table 6) at three locations at Lac Bay

UV filter	Bathing zone	Inner-reef zone	Mangrove- zone
Non-nZnO	126	2.4	0.5
nZnO	42	0.8	0.2
$nTiO_2$	0.2	0	0

6.1.4 Risks compared: Zinc vs Oxybenzone

Schaap & Slijkerman (2018) reported RQ's of 2.4-4.2 for oxybenzone, based on measured concentrations in the bathing zone, which is much lower than the estimated RQs for particulate Zinc (nano and non-nano). The measured concentrations do, however, not yet imply worst case scenario as probably not all bathers used oxybenzone-products. The latter was an assumption for zinc-product application and corresponding emissions of zinc.
On the other hand, the risk factor for oxybenzone within risk assessment was 50, and 500 for Zinc due to the limited data availability.

Based on these assessments, zinc would pose a higher risk in Lac than oxybenzone, given the lowest reported effects on algae growth (oxybenzone) and crustacean mortality (Zinc). Even when the risk factor can be reduced by gathering more toxicity data for zinc, the RQ will not easily become lower than oxybenzone. Alternative "eco friendly" sunscreen based on zinc application in Lac would thus not be a sustainable alternative given this rough and worst case assessment.

With respect to the vulnerability of corals at nearby reefs, the significance of other effect types such as hormonal disruption should be considered in additional studies. Reported hormone disruption and effects on development in critical metamorphoses stages of various organisms (coral, sea urchins, fish) are most likely to occur when exposed to organic UV filters (e.g. Downs *et al.*, 2015), and probably unlikely to occur at corresponding levels of zinc.

Additional toxicity testing with the "new generation organics" and traditional organic UV filters such as octocrylene in comparison to oxybenzone and zinc, focussing on their in vitro hormone disruptive potential with respect to coral development would be most valuable.

6.2 Bio-assay based risk assessment

The concentrations series tested per product were a result of the so-called range finding tests, in which the most suitable concentration range is established to be able to calculate reliable EC_{50} values. The question is how the tested concentrations relate to field relevant levels and pose a risk to the environment. The environmental risk was assessed using the method described in chapter 2 (ECHA, 2008).

Based on sunscreen emission and field concentration estimates done by Schaap & Slijkerman (2018), the total sunscreen concentration was estimated. The calculations followed the same principles as is explained in chapter 2, which are described in detail in chapter 6.1.

A mid-result in this calculation, is the total amount of sunscreen used by bathers, resulting in an estimated field concentration when a wash off factor of 25 % is applied.

The bathing zone (worst case, higher concentration possible) might contain a highest concentration of 0.08 mg/L sunscreen product. A mg/L equals µl/L, which is the notation in Table 8 in which the effect concentrations are presented.

The observed effect levels (EC_{50}) of the bio-assays are all much higher than the estimated field levels of sunscreen, indicating that effects as observed in the tests are not likely to occur.

When applying an uncertainty factor to account for variance in species sensitivity of 500 (equal to the most conservative factor applied in chapter 6), the effect levels would, however, be much lower. Table 15 presents estimated risk quotients based on the bio-assays results. This assessment shows that ecoproducts D, E and G do not pose a risk to the environment of Lac Bay, but that whole (non-eco) products A, B, C and F might.

Note also that this risk assessment is based on EC_{50}-values, that cause 50% effect. When NOEC values are used, the PNEC will be much lower and consequently the RQ higher. On the other hand, when sufficient NOEC-values are available, the uncertainty factor will be lower.

Table 15 PNECs and risk quotients derived from bioassay results on the whole sunscreen product.

Product	**Lowest EC50**	**test**	**"PNEC" with AF 500**	**RQ**
Product A	16.1	Algae	0.03	2.5
Product B	3.93	rotifer	0.01	10.2
Product C	18.9	Algae	0.04	2.1
Product D	94.6	Algae	0.19	0.42
Product E	-	-	-	-
Product F	23	Microtox	0.05	1.70
Product G	235	Microtox	0.47	0.2

6.3 Summary risk assessment

- Bio-assay effect levels do not pose a direct risk when compared to estimated field levels of sunscreen. When an uncertainty factor is applied to account for variance in species sensitivity and other variables, risk cannot be excluded for some of the products. Eco-products do not pose an environmental risk.
- A worst-case risk assessment based on literature data, however, shows potential environmental risk of zinc oxide UV-filters in the bathing zone of Lac Bay. These higher risk levels are partly resulting from the limited data availability and a high uncertainty factor.
- Additional toxicity testing with new organic and ZnO UV filters would most probably decrease the estimated environmental risk.

7 Conclusions and recommendations

7.1 Can eco-friendly sunscreen products have adverse effects on the marine environment?

The aim of this study was to contribute to the knowledge base within the current sunscreen debate, with a focus on so-called eco-friendly sunscreens and the alternative UV filters based on zinc and titanium, and on new organic UV filters.

To achieve this objective, the following main research question was formulated:
Can eco-friendly sunscreen products have adverse effects on the marine environment?

To support the main question, several sub questions are formulated:

- What are eco-friendly sunscreen products?
- What are the known effects of "alternative" UV filters for the marine environment?
- What are the effect concentrations?
- Do toxicity effects occur in a variety of laboratory tests?
- Are these effects field relevant?
- What are the emissions if everyone changes to using eco-friendly sunscreen products and does this emission lead to environmental risk?

Based on the current study, a conclusive answer to the main question cannot be provided.

Literature review, bio- assay results, and the theoretical risk assessment showed that toxicity of titanium only occurs at levels that much higher than field relevant concentrations. A direct risk of titanium based UV filters is not expected, nor will it be in future. The consequences of additional ROS production related to titanium exposure should however be looked into, in context with natural occurring levels. Hence, based on the current knowledge and the bioassays performed in this study, products containing only titanium-based UV filters seem to be without harm.

In contrast, effects of zinc are more likely to occur, depending on the type of zinc UV filter, the levels in the field. Effects of zinc on corals can occur at levels starting from 10 µg/L. To protect the marine environment, the threshold levels for (free) zinc are set at 3.1 µg/L. Estimated risk quotients for zinc illustrated that risk of future nano-zinc concentrations cannot be excluded in the bathing zone of lac Bay. This is in the hypothetical situation when all bathers change to zinc products. Information on field concentrations is currently lacking, and fate and effects of mineral UV filters depend on too many factors to predict a realistic actual risk. Moreover, the effects described for both ZnO and TiO_2 do not occur in current field relevant concentrations. Additional screening on the wash-off fraction of zinc, zinc nanoparticles and the actual emissions and information on field concentrations are a very important issue in order to assess the actual risk of zinc towards the environment.

Based on the limited information available and the bio-assay results, new generation organic UV filters in products claiming to be eco compatible do not cause environmental effects. Hormonal and developmental processes are however not yet included in toxicity testing and could not be evaluated. Based on the available information, mineral products might thus not be the only "safe" eco-products to consider as an alternative to "traditional" sunscreens.

The bio-assays used, do not indicate that any of the eco-products poses a risk to the environment:

- Eco-products did not induce (severe) effects on the selected organisms. These include both mineral and new organic UV filter eco products
- No environmental risk is to be expected of eco products at sunscreen levels at Lac Bay.
- Products without an eco-claim induced much more effects, at much lower tested concentrations. Organic products showed overall highest risk to the environment.

- All tested effect concentrations were far above field relevant emissions and concentrations for all sunscreens evaluated.
- When applying an **uncertainty factor**, the risk of non-eco products towards the environment can, however, not be excluded.

Based on current knowledge, when making a choice between suncare products containing UV filters, taking into account environmental risk only, the following order of UV filters is considered.:

1. Titanium,
2. New generation organic UV filters (when tested on hormonal/developmental endpoints, and without effects)
3. ZnO
4. nZnO
5. Organic (Oxybezone, octocrylene, homosalate, ..)

7.2 Recommendations

For all sunscreens, including zinc and titanium and new generation UV filters, there is an urgent need to carry out additional bioassays and thorough environmental assessments on field levels and potential effects. As field levels of nZnO and $nTiO_2$ cannot yet be established in a proper way, free zinc and titanium and ROS levels could be assessed as a proxy. Natural variability should be taken into account in such field research.

Ecotoxicological tests should include *in vitro* endocrine bio-assays with corals, as well as with relevant metamorphosis and endocrine disruption processes in other organisms, to be able to compare the described effects of oxybenzone to corals observed by Downs *et al.*, (2015). Furthermore, actual field effects should be looked into. Doing so, regulators obtain the information they need to assess the actual and local risk and discuss to what extent it is needed to mitigate risks associated with widespread use of these and other chemical ingredients in personal care products.

8 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2008 certified quality management system (certificate number: 187378-2015-AQ-NLD-RvA). This certificate is valid until 15 September 2018. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V.

References

Alba Botanica. (2018, March 8). *Infomation about earth friendly statements*. Retrieved from Alba botanica website: https://www.albabotanica.com/en/earth-friendly/

AllGoodproducts. (2018, March 8). *Reef friendly criteria of AllGoodproducts*. Retrieved from AllGoodproducts: https://allgoodproducts.com/reef-friendly/criteria

Amde, M., Liu, J.–F., Tan, Z.–Q., Bekana, D., "Transformation and Bioavailability of Metal Oxide Nanoparticles in Aquatic and Terrestrial Environments: A Review", Environmental Pollution, 2017, 250–267.

Anderson, M.A. F.M.M. Morel, R.R.L. Guillard (1978) Growth limitation of a coastal diatom by low zinc ion activity. Nature, 276 (5683) (1978), p. 70

Antoniou, C., Kosmadaki, M., Stratigos, A., & Katsambas, A. (2008). Sunscreens-what's important to know. *Journal of the European academy of dermatology and venereology , 22*(9), 1110-1119. doi:10.1111/j.1468-3083.2007.02580.x

Ates, M., Daniels, J., Arslan, Z., Farah, I. O., & Rivera, H. F. (2013). Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (Artemia salina) larvae: effects of particle size and solubility on toxicity. *Environmental sciences, 15*, 225-233. doi:10.1039/c2em30540b

Australian and New Zealand Environment and Conservation Council (ANZECC) and Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) (2000) Australian and New Zealand water quality guidelines for fresh and marine waters. Environment Australia

Azur Environmental. (1998). *Microtox Acute Toxicity Test.* Retrieved from http://www.coastalbio.com/images/Acute_Overview.pdf

B corperation. (2018, March 8). *What are B corps*. Retrieved from Certified B corporations: https://www.bcorporation.net/what-are-b-corps

Bachelot, M., Li, Z., Munaron, D., Le Gall, P., Casellas, C., Fenet, H., & Gomez, E. (2012). Organic UV filters concentrations in marine mussels from French coastal regions. *Science of the Total Environment, 420*, 273-279. doi:10.1016/j.scitotenv.2011.12.051

Badger healthy body care. (2018, March 8). *Information about the ingredients used in the products*. Retrieved from Badger sunscreen products: https://www.badgerbalm.com/s-29-about-badger-organic-sunscreen.aspx

Balázs, A., Krifaton, C., Orosz, I., Szoboszlay, S., Kovács, R., Csenki, Z., Urbányi, B. (2016). Hormonal activity, cytotoxicity and developmental toxicity of UV filters. *Exotoxicology and environmental safety, 131*, 45-53.

Beyond coastal. (2018, March 9). *Information about ingredients used*. Retrieved from Beyond coastal sunscreen products: https://www.beyondcoastal.com/ingredients

Bian S-W, Mudunkotuwa IA, Rupasinghe T, Grassian VH. (2011). Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir. 2011;27:6059–6068. doi:10.1021/ la200570n

Blüthgen, N., Meili, N., Chew, G., Odermatt, A., & Fent, K. (2014). Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish. *Science of the Total Environment, 476*, 207-217.

Bodar C (2007). Environmental Risk Limits for Zinc. RIVM letter report 11235/2007

Brand L.E., W.G. Sunda, R.R.L. Guillard (1983). Limitation of marine phytoplankton reproductive rates by zinc, manganese and iron. Limnology and Oceanography, 28 (1983), pp. 1182-1198

Bruland, K.W (1989) Complexation of zinc by natural organic ligands in the Central North Pacific. Limnology and Oceanography, 34 (1989), pp. 269-285

Buffet, P.E., Amiard-Triquet, C., Dybowska, A., Faverney, C.R., Guibbolini, M., Valsami-Jones, E., Mouneyrac, C.: Fate of isotopically labeled zinc oxide nanoparticles in sediment and effects on two endobenthic species, the clam Scrobicularia plana and the ragworm Hediste diversicolor. Ecotoxicol. Environ. Saf. 84, 191–198 (2011)

Canesi L, Ciacci C, Balbi T. (2015) Interactive effects of nanoparticles with other contaminants in aquatic organisms: friend or foe? Mar Environ Res. 2015;111:128–134. doi: 10.1016/j.marenvres.2015.03.010.

Clement, L., Hurel, C., & Marmier, N. (2013). Toxicity of TiO(2) nanoparticles to cladocerans, algae, rotifers and plants - effects of size and crystalline structure. *Chemosphere, 90*(3), 1083-1090. doi:10.1016/j.chemosphere.2012.09.013

Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F., & Danovaro, R. (2017). Sunscreen products impair the early developmental stages of sea urchins. *Scientific reports, 7*(1), 7815. doi:10.1038/s41598-017-08013-x

Croot, P.L., O. Baars, P. Streu, (2011). The distribution of dissolved zinc in the Atlantic sector of the Southern Ocean, Deep Sea Research Part II: Topical Studies in Oceanography, Volume 58, Issues 25–26, 2011, p 2707-2719, DOI 10.1016/j.dsr2.2010.10.041.

Danovaro, R., & Corinaldesi, C. (2003). Sunscreen products increase virus production through prophage induction in marine bacterioplankton. *Microbial ecology, 45*(2), 109-118.

Danovaro, R., Bongiorni, L., Corinaldesi, C., Giovannelli, D., Damiani, E., Astolfi, P., Pusceddu, A. (2008). Sunscreens Cause Coral Bleaching by Promoting Viral Infections. *Enivornmental health perspectives, 116*(4), 441. doi:10.1289/ehp.10966

Depledge M.H., and P.S. Rainbow., (1990) Models of regulation and accumulations of trace metals in marine invertebrates: a mini-review. Comp. Biochem. Physiol., 97C (1990), pp. 1-7

Dobrovolskaia, M. (2007). Immunological properties of engineered nanomaterials. Nature Nanotech. 2, 469–478.

Farkas *et al.* (2015)

Downs, C., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Loya, Y. (2015). Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. *Archives of environmental contamination and toxicology, 70*(2), 265-288. doi:10.1007/s00244-015-0227-7

Dr. Jetske Ultee. (2018, April 3). *Information about Ultee sunscreen*. Retrieved from Webiste of Dr. Jetske Ultee: https://www.uncover-skincare.nl/producten/zonnebrandcremes-dr-jetske-ultee/suncover-spf30-250ml.html

ECHA. (2008). *Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment.* © European Chemicals Agency.

Ecocert eco label. (2018, March 7). *Information about the rules of Ecocert eco label*. Retrieved from Website of Ecocert: http://www.ecocert.com/cosmetique-ecologique-et-biologique

Ellwood M.J., (2004) Zinc and cadmium speciation in subantarctic waters east of New Zealand. Mar. Chem., 87 (2004), pp. 37-58

Emergin C. (2018, March 14). *Environmentally friendly practices*. Retrieved from Green initiatives of Emergin C: https://www.emerginc.com/green-initiatives.html

Emilie C. Johnsen. 2018. *Toxicological Effects of Commercial Sunscreens on Coral Reef Ecosystems: New Protocols for Coral Restoration.* Capstone. Nova Southeastern University. Retrieved from NSUWorks, . (335) https://nsuworks.nova.edu/cnso_stucap/335.

Environmental Working Group. (2018, March 8). *Info about EWG verified label*. Retrieved from EWG: https://www.ewg.org/ewgverified/

EPA (2004) National Recommended Water Quality Criteria. United States Office of Water. Environmental Protection Office of Science and Technology Agency (4304T). retreived on 25062018 from: https://www.epa.gov/sites/production/files/2015-06/documents/nrwqc-2004.pdf

EPA. (2018, April 16). *Toxicity database*. Retrieved from United States Environmental Protection Agency: https://www.epa.gov/

EU-RAR (2006) Zinc and zinc compounds (EC Regulation 793/93). Draft version of June 2006.

European chemical agency. (2018, May 22). *Ecotoxicological data for Mexoryl SX*. Retrieved from ECHA: https://echa.europa.eu/registration-dossier/-/registered-dossier/9621

European chemicals agency. (2018, May 16). *Ecotoxicological data ensulizole*. Retrieved from ECHA: https://echa.europa.eu/registration-dossier/-/registered-dossier/5464/6/1

European chemicals agency. (2018, May 16). *Ecotoxicological summary of Uvinul A plus*. Retrieved from ECHA: https://echa.europa.eu/registration-dossier/-/registered-dossier/4877/6/1

Federal Institute for Occupational Safety and Health. (2016). *CLH report Proposal for Harmonised Classification and Labelling for Uvinul T 150.* Dortmund: Federal Institute for Occupational Safety and Health. Retrieved from CLH report.

Federal Institute for Occupational Safety and Health. (2016). *CLH report Proposal for Harmonised Classification and Labelling of Tinosorb M.* Dortmund: Federal Institute for Occupational Safety and Health.

Fel J-P, Béraud E, Bensetra A, Lacherez C, Mezzache S, Léonard M, Allemand D and Ferrier-Pages C, 2017. Predictive laboratory methodology to assess coral bleaching. Application to UV filters. European Coral Reef Symposium, December 2017, Oxford. Cited in ICRI document " Impacts of sunscreens on corals - ICRI briefing - Feb 2018"

Fent, K., Kunz, P. Y., Zenker, A., & Rapp, M. (2010). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Marine environmental research, 69*, S4-S6.

Fent, K., Kunz, P., & Gomez, E. (2008). UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. *CHIMIA international journal for chemistry, 62*(5), 368-375. doi:10.2533/chimia.2008.368

Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS. (2007). Comparative toxicity of nanoparticulate ZnO, Bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): the importance of particle solubility. Environmental Science and Technology. 2007;41:8484–8490. doi:10.1021/es071445r

Gadd, J., Hickey, C.W. (2016). Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Zinc – Marine. Australian and New Zealand guidelines for fresh and marine water quality. Draft September 2016. Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia.

Giokas, D. L., Salvador, A., & Chisvert, A. (2007). UV filters : From sunscreens to human body and the environment. *TrAC. Trends in analytical chemistry, 26*(5), 360-374.

Grimwood, M.J. & Dixon, E. 1997. Assessment of risks posed by List II metals to Sensitive Marine Areas (SMAs) and adequacy of existing environmental quality standards (EQSs) for SMA protection. Report to English Nature.

Haereticus Environmental Laboratory. (2018, June 5). *Haereticus Environmental Laboratory, scientific organisation*. Retrieved from Scientific laboratory that investigates toxicants in the marine environment: http://www.haereticus-lab.org/

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.-C., & Kahry, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. *Chemosphere, 71*(7), 1308-1316. doi:10.1016/j.chemosphere.2007.11.047

Heyward AJ (1988) Inhibitory effects of copper and zinc sulphates on fertilization in corals. Proc 6th Int Coral Reef Symp 2:299– 303

Hoegh-Guldberg, O. (2001). Population dynamics of symbiotic zooxanthellaein the coral Pocillopora damicornis exposed to elevated ammonium [(NH4)2SO4] concentrations, Pac. Sci., 48, 263– 272

Hu, J., Wang, J., Liu, S., Zhang, Z., Zhang, H., Cai, X., . . . Liu, J. (2018). Effect of TiO2 nanoparticle aggregation on marine microalgae Isochrysis galbana. *Journal of environmental sciences, 66*, 208-215. doi:doi.org/10.1016/j.jes.2017.05.026

Hudspith, M. A. Reichelt-Brushett, P.L. Harrison (2017) Factors affecting the toxicity of trace metals to fertilization success in broadcast spawning marine invertebrates: a review Aquat. Toxicol., 184 (2017), pp. 1-13, 10.1016/j.aquatox.2016.12.019

Jarvis, T., Miller, R., Lenihan, H., & Bielmyer, G. (2013). Toxicity of ZnO nanoparticles to the copepod Acartia tonsa, exposed through a phytoplankton diet. *Environmental toxicology and chemistry, 32*(6), 1264-1269. doi:10.1002/etc.2180

Keller, A., Wang, H., Zhou, D., Lenihan, H., Cherr, G., Cardinale, B., . . . Ji, Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental science & technology, 44*(6), 1962-1967. doi:10.1021/es902987d

Kim, S., Jung, D., Kho, Y., & Choi, K. (2014). Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka. *Aquatic toxiclogy, 155*, 244-252.

Lab&process. (2018, April 3). *Information about the microtox analyser model 500*. Retrieved from Website of Lab&process: https://www.labandprocess.com/en/products/microtox-model-500

Lesser, M. P. (1996). Exposure of symbiotic dinoflagellates to elevated temperatures and ultraviolet radiation causes oxidative stress and inhibits photosynthesis, Limnol. Oceanogr., 41, 271–283, 1996.

Lewicka, Zuzanna & Yu, William & L. Oliva, Brittany & Contreras, Elizabeth & L. Colvin, Vicki. (2013). Photochemical behavior of nanoscale TiO2 and ZnO sunscreen ingredients. Journal of Photochemistry and Photobiology A: Chemistry. 263. 24–33. DOI 10.1016/j.jphotochem.2013.04.019.

Lovern, S. B., & Klapper, R. (2006). Daphnia magna mortality when exposed to TiO2 and fullerene (C60) nanoparticles. *Environmental toxicology and chemistry, 25*(4), 1132-1137.

Lowry, G. V., Gregory, K. B., Apte, S. C., and Lead, J. R. (2012). Transformations of nanomaterials in the environment. Environ. Sci. Technol. 46, 6893–6899.

Lynch, I., and Dawson, K. A. (2008). Protein-nanomaterial interactions. Nano Today 3, 40–47.

Ma, R., Levard, C., Marinakos, S. M., Cheng, Y., Liu, J., Michel, F. M., Brown, G. E., and Lowry, G. V. (2012). Size-controlled dissolution of organic-coated silver nanomaterials. Environ. Sci. Tech. 46, 752–759.

Maipas, S. and P. Nicolopoulou-Stamati. (2015) "Sun lotion chemicals as endocrine disruptors." Hormones 14 1 (2015): 32-46.

Manaia, E. B., Kaminski, C. K., Corrêa, M. A., & Chiavacci, L. A. (2013). Inorganic UV filters. *Brazilian journal of pharmaceutical sciences, 49*(2), 201-209.

Mance G (1987) Pollution threat of heavy metals in aquatic environments. Elsevier Applied Science Publishers, London

Martin J.H, R.M Gordon, S Fitzwater, W.W Broenkow (1989) VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Research Part A—Oceanographic Research Papers, 36 (1989), pp. 649-680

MicroBioTests Inc. (2018). *ROTOX M acute toxicity test.* Gent: MicroBioTests Inc. Retrieved from http://www.microbiotests.be/

Miller, R., Lenihan, H., Muller, E., Tseng, N., Hanna, S., & Keller, A. (2010). Impacts of metal oxide nanoparticles on marine phytoplankton. *Environmental science & technology, 44*(19), 7329-7334. doi:10.1021/es100247x

Minetto, D., Libralato, G., & Ghirardini, A. (2014). Ecotoxicity of engineered TiO2 nanoparticles to saltwater organisms:. *Environment international*(66), 18-27. doi:10.1016/j.envint.2014.01.012

Morel F.M.M., J.R. Reinfelder, S.B. Roberts, C.P. Chamberlain, J.G. Lee, D. Yee (1994). Zinc and carbon co-limitation of marine phytoplankton. Nature, 369 (1994), pp. 740-742

Morrison G P, Batley GE, Florence TM (1989) Metal speciation and toxicity. Chem Britain 791–796

Müller N.C., B. Nowack (2008) Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol, 42 (12) (2008), pp. 4447-4453

National Nanotechnology Initiative. (2018a, June 8). *What's so special about the nanoscale*. Retrieved from Nanotech 101: https://www.nano.gov/nanotech-101/special

Neff, J. (2002). Zinc in the Ocean. 175-189. Chapter in: Bioaccumulation in Marine Organisms. DOI, 10.1016/B978-008043716-3/50011-7.

Nolting RF, Heijne M, de Jong JT, Timmermans KR, de Baar HJ (2000) The determination and distribution of Zn in surface water samples collected in the northeast Atlantic Ocean. J Environ Monit. 2000 Oct;2(5):534-8.

Osterwalder, U., Sohn, M., & Herzog, B. (2014). Global state of sunscreens:. *Photodermatology, Photoimmunology & Photomedicine, 30*(2-3), 62-80.

Paredes, E., Perez, S., Rodil, R., Quintana, J., & Beiras, R. (2014). Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. *Chemosphere, 104*, 44-50. doi:10.1016/j.chemosphere.2013.10.053

Paterson, G., Ataria, J., Hoque, M., Burns, D., & Metcalfe, C. (2010). The toxicity of titanium dioxide nanopowder to early life stages of the Japanese medaka (Oryzias latipes). *Chemosphere, 82*(7), 1002-1009. doi:10.1016/j.chemosphere.2010.10.068

Peijnenburg W J. G. M., M Baalousha, J Chen, Q Chaudry, F Von der kammer, T A. J. Kuhlbusch, Jamie Lead, C Nickel, J T. K. Quik, M Renker, Z Wang & A A. Koelmans (2015). A Review of the Properties and Processes Determining the Fate of Engineered Nanomaterials in the Aquatic Environment, Critical Reviews in Environmental Science and Technology, 45:19, 2084-2134, DOI: 10.1080/10643389.2015.1010430

Peng, Xiaohui, Shelagh Palma, Nicholas S Fisher, and Stanislaus S Wong. "Effect of Morphology of ZnO Nanostructures on Their Toxicity to Marine Algae." Aquatic Toxicology 102, no. 3 (2011): 186-96.

Raw elements. (2018, March 15). *information about reef safe sunscreen products*. Retrieved from Raw elements eco sunscreen products: https://www.rawelementsusa.com/pages/environmental-impact

Reichelt-Brushett AJ, Harrison PL (1999) The effect of copper, zinc and cadmium on fertilization success of the scleractinian coral Goniastrea aspera. Mar Pollut Bull 38:182–187

Reichelt-Brushett AJ, Harrison PL (2005) The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs 24:524-534

Reidy, BC., A. Haase, A. Luch, K. Dawson, I. Lynch, Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials 2013 , 6 , 2295

Ren *et al.*, (2018) Enhanced bio-concentration of tris(1,3-dichloro-2-propyl) phosphate in the presence of nano-TiO2 can lead to adverse reproductive outcomes in zebrafish. Environmental Pollution 233 (2018) 612e622

Rueter J.G., F.M.M. Morel (1981). The interaction between zinc deficiency and copper toxicity as it effects the silicic acid uptake mechanisms in Thalassiosira pseudonana. Limnology and Oceanography, 26 (1981), pp. 67-73

Ruiz-Pino, D, Erica Nicolas, Jean Pierre Bethoux, C.E Lambert (1991) Zinc budget in the Mediterranean Sea: a hypothesis for non-steady-state behavior. Marine Chemistry 33(1-2):145-169. DOI: 10.1016/0304-4203(91)90063-3

Sánchez-Quiles, D., & Tovar-Sánchez, A. (2015). Are sunscreens a new environmental risk associated with coastal tourism? *Environment international, 83*, 158-170. doi:10.1016/j.envint.2015.06.007

Schaap, I., & Slijkerman, D. M. (2018). *An environmental risk assessment of three organic UV filters at Lac Bay, Bonaire, Southern Carribean.* Marine Pollution Bulletin (in press) October 2018

Schiavo *et al.*, (2018) Testing ZnO nanoparticle ecotoxicity: linking time variable exposure to effects on different marine model organisms. Environmental Science and Pollution Research (2018) 25:4871–4880. https://doi.org/10.1007/s11356-017-0815-3

Shaked, Y. Y. Xu, K. Leblanc, F.M.M. Morel (2006). Zinc availability and alkaline phosphatase activity in Emiliania huxleyi: implications for Zn–P co-limitation in the ocean. Limnology and Oceanography, 51 (1) (2006), pp. 299-309

Sharifan, H., Klein, D., & Morse, A. (2016). Environmental occurence and ecological risk assessment of organic UV filters in marine organisms from Hong Kong coastal waters. *Environmental research, 148*, 273-276.

Sharifan, H., Klein, D., & Morse, A. (2016). UV filters are an environmental threat in the Gulf of Mexico: a case study of Texas coastal zones. *Oceanologia, 58*(4), 327-335.

Sharma VK (2009) Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment--a review. J Environ Sci Health A Tox Hazard Subst Environ Eng. 44(14):1485-95. doi: 10.1080/10934520903263231.

Sharma, P., Ambuj Bhushan Jha, Rama Shanker Dubey, and Mohammad Pessarakli, (2012) "Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions," Journal of Botany, vol. 2012, Article ID 217037, 26 pages, 2012. https://doi.org/10.1155/2012/217037.

Skinacea. (2012, September 13). *UV filters chart: sunscreen active ingredients*. Retrieved from Website of Skinacea : http://www.skinacea.com/sunscreen/uv-filters-chart.html#.WvkxfICFPcv

Smartshield. (2018, March 16). *Information about marine friendly sunscreen products*. Retrieved from Smartshield sunscreen products: http://www.smartshield.com/your-skin-a-the-sun/marine-friendly

Smith, D. J., Suggett, D. J., and Baker, N. (2005). Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals?, Global Change Biol., 11, 1–11, 2005.

Sun T.Y., Gottschalk F., Hungerbuhler K., Nowack B. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environ. Pollut. 2014;185:69–76.

Sunda W.G., S.A. Huntsman (1992). Feedback interactions between zinc and phytoplankton in seawater. Limnology and Oceanography, 37 (1992), pp. 25-40

Tiede K., M. Hasselov, E. Breitbarth, Q. Chaudhry, A.B..A. Boxhall (2009) Considerations for environmental fate and ecotoxicity testing to support environmental risk assessments for engineered nanoparticles. J Chromatogr A, 1216 (3) (2009), pp. 503-509

Tijssen J, Wijgerde T, Leal MC, Osinga R (2017) Effects of zinc supplementation on growth and colouration of the scleractinian coral Stylophora pistillata. PeerJ Preprints 5:e2858v1 https://doi.org/10.7287/peerj.preprints.2858v1

Tovar-Sánchez, A., Sánchez-Quiles, D., Basterretxea, G., Benedé, J. L., Chisvert, A., Salvador, A., . . . Blasco, J. (2013). Sunscreen Products as Emerging Pollutants to Coastal Waters. *PLoS One, 8*(6). doi:10.1371/journal.pone.0065451

Trevisan, R., Delapedra, G., Mello, D., Arl, M., Schmidt, E., Meder, F., Dafre, A. (2014). Gills are an initial target of zinc oxide nanoparticles in oysters Crassostrea gigas, leading to mitochondrial disruption and oxidative stress. *Aquatic toxicology, 153*, 27-38. doi:10.1016/j.aquatox.2014.03.018

Wang, J.; Wang, W.X. Significance of physicochemical and uptake kinetics in controlling the toxicity of metallic nanomaterials to aquatic organisms. J. Zhejiang Univ. Sci. A 2014, 15, 573–592.

Wood, E., (2018) Impacts of sunscreens on coral reefs. Report by the International Coral Reef Initiative (ICRI).

Wong, S., Leung, P., Djurisic, A., & Leung, K. (2010). Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. *Analytical and bioanalytical chemistry, 396*(2), 609-618.

Wong, S.W.Y., Leung, K.M.Y. (2014) Temperature-dependent toxicities of nano zinc oxide to marine diatom, amphipod and fish in relation to its aggregation size and ion dissolution. Nanotoxicology 8(51), 24–35 (2014)

Wooldridge S.A. (2013) Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences, 10, 1647–1658, 2013. DOI:10.5194/bg-10-1647-2013

Yung, M., Mouneyrac, C., & Leung, K. M. (2015). Ecotoxicity of Zinc oxide nanoparticles in the marine environment. *ResearchGate*, 0-17. doi:10.1007/978-94-007-6178-0_100970-1

Yung, Mana MN, Stella WY Wong, Kevin WH Kwok, FZ Liu, YH Leung, WT Chan, XY Li, AB Djurišić, and Kenneth MY Leung. (2015). "Salinity-Dependent Toxicities of Zinc Oxide Nanoparticles to the Marine Diatom Thalassiosira pseudonana."Aquatic Toxicology 165 (2015): 31-40.

Zhang, Q. Y., Ma, X. Y., Wang, X. C., & Ngo, H. (2016). Assessment of multiple hormone activities of a UV-filter (octocrylene) in zebrafish (Danio rerio). *Chemosphere, 159*, 433-441. doi:10.1016/j.chemosphere.2016.06.037

Justification

Report C056/18
Project Number: 4315100082

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Dr. Klaas Kaag
Senior reseacher

Signature: 

Date: 4 October 2018

Approved: Drs. Jakob Asjes
Manager Integration

Signature: 

Date: 4 October 2018

Annex 1 Product description

A detailed description of the tested products and why the products are chosen is given below, including the specific substances and claims.

Product A
Active ingredients (UV-filters): avobenzone 3%, homosalate 7,5%, octyl salicate 5%, octocrylene 2,75%, oxybenzone 2%
Claims: paraben and PABA free
Labels: -

Product A is selected because it is an organic sunscreen, containing organic UV filters, including oxybenzone. Furthermore it is selected to serve as an indicator of toxicity, which can be compared to other products.

Product B
Active ingredients (UV-filters): homosalate 7%, octocrylene 3%, avobenzone 3%, octyl salicate 3%, titanium dioxide (nano) 5%
Claims: water resistant, effective UVA/UVB protection
Labels: -

Product B is selected because it is an organic sunscreen, containing ingredients also present in product A, but doesn't contain oxybenzone. Furthermore, titaniumdioxide (nano) is also present in product B. Therefore product B is chosen because it consists of an interesting combination of organic UV filters, excluding oxybenzone, which in theory are known to cause effects for marine organisms.

Product C
Active ingredients (UV-filters): zinc oxide (nano), titanium dioxide
Claims: Paraben free, mineral based, biological
Labels: Ecocert bio label

Product C is a mineral based sunscreen product that contains nZnO. The product claims to be paraben free and biological and has the ecolabel of ecocert. The principles of this label indicate that a product needs to be free of nano particles. However, product C uses nZnO as one of the active ingredients. Furthermore, nano particles are known to cause effects for marine organisms. Product C is chosen to compare the potential toxicity of mineral based nano filters with non-nano filters.

Product D
Active ingredients (UV-filters): 6,4% titanium dioxide (non-nano), 6,0% zinc oxide (non nano)
Claims: reef safe, non nano, biodegradable
Labels: -

Product D is a mineral based sunscreen product using non-nano TiO_2 and non-nano ZnO. Product D claims to be reef safe, with biodegradable ingredients and without nano particles. Moreover, most of the inactive ingredients are certified organic ingredients. All this suggests that the claims of product D result in a sunscreen product that isn't harmful for the marine environment. It is expected that this product will be one of the products that show the least effects.

Product E

Active ingredients (UV-filters): 8,8% titanium dioxide (non-nano)
Claims: mineral based, eco-conscious, reef safe, biodegradable, oxybenzone free, chemical free
Labels: -

Product E is a mineral based sunscreen product using non-nano TiO_2. Product E claims to be free of chemicals, reef safe and their ingredients will biodegrade in the environment. Furthermore, product E claims that their products are laboratory tested and the conclusion was that their products are safe for the marine environment. It is expected that this product will be one of the products that shows the least effects.

Product F

Active ingredients (UV-filters): uvinul A plus, uvinul T150, tinosorb S, ensulizole
Claims: broad spectrum protection
Labels: -

Product F is a sunscreen product that uses new organic organic UV filters, that are comparable with the UV filters of product G. But unlike product G, product F doesn't make any claims on their product being safe for the marine environment. Product F is chosen, because it is interesting to test the relative toxicity of a product that can, on UV filter specifications, be compared with the eco compatible product G.

Product G

Active ingredients (UV-filters): uvinul A plus, uvinul T150, tinosorb M (nano)
Claims: eco compatible (with test results)
Labels: -

Product G is a sunscreen product that uses UV filters that are categorized in the group of new organic UV filters. Product G claims to be eco compatible. They state that their product is laboratory tested for both the individual ingredients as the whole product, however this is not published. Product G claims to have the world's only patented eco compatible formula. Therefore product G is chosen because it is expected that this product will be one of the products which show the least effects. It has to be kept in mind that minimal toxicity data is present for these UV filters.

Annex 2 Overview eco-friendly sunscreen products

Table includes sunscreen products who claim to be reef safe, eco-friendly, etc. The brand and product name, the sun protection factor (SPF), main eco label (e.g. reef safe, eco-friendly), the other relevant claims and the active ingredients are all given. List is based on internet searches (see methods section) between February – June 2018.

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
Alba Botanica, baby mineral sunscreen	50+	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano	17%										
Alba Botanica, cool sport sunscreen	50	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	15.00%	8.00%	5%			
Alba Botanica, hawaiian aloe vera lotion	30	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	7.50%	7.50%	5%			
Alba Botanica, hawaiian clear spray	50	Reef safe	Biodegradable, no parabens, no animal testing, free of oxybenzone and octinoxate					3%	15.00%	8.00%	5%			
Alba Botanica, hawaiian dry oil	15	Reef-safe	Biodegradable, no animal testing, vegan, no parabens					2%		7.50%	5%			
Alba Botanica, hawaiian sunscreen	30	-	Biodegradable, no parabens, Hawaiian					3%	7.50%	7.50%	5%			
Alba Botanica, hawaiian sunscreen	45	Reef-safe	Biodegradable, no parabens, no animal testing, free of oxybenzone and octinoxate					3%	10.00%	10.00%	5%			
Alba Botanica, kids mineral sunscreen	30	Reef-safe	Biodegradable mineral, free of active ingredients, non nano, reducing env. Impact	14.50%		2.00%								
Alba Botanica, kids sunscreen	50	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	15.00%	8.00%	5%			
Alba Botanica, kids sunscreen lotion	45	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	10.00%	10.00%	5%			
Alba Botanica, kids sunscreen spray lotion	40	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	12.00%	7.00%	5%			
Alba Botanica, refreshing mineral sunscreen	35	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano	6%		7%								

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
Alba Botanica, sensitive mineral sunscreen	30	Reef-safe	Biodegradable mineral, free of active ingredients, non nano, reducing env. Impact	14.50%		2.00%								
Alba Botanica, sensitive mineral sunscreen	35	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano	6%		7%								
Alba Botanica, sensitive sunscreen	30	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	7.50%	7.50%	5%			
Alba Botanica, soothing sunscreen lavender	45	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	10.00%	10.00%	5%			
Alba Botanica, sport mineral sunscreen	45	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano	9%		7%								
Alba Botanica, sport sunscreen fragrance free	45	Reef-safe	Biodegradable, no animal testing, vegan, no parabens, free of oxybenzone octinoxate PABA and nano					3%	10.00%	10.00%	5%			
All Good, kids sunscreen lotion	30	Reef-safe	Oxybenzone free, vegan, non nano, biodegradable, coral reef friendly, natural, mineral based	12%										
All Good, kids sunscreen spray	30	Reef-safe	Oxybenzone free, vegan, non nano, biodegradable, coral reef friendly, natural, mineral based	12%										
All Good, sport sunscreen lotion	30	Reef-safe	Oxybenzone free, vegan, non nano, biodegradable, coral reef friendly, natural, mineral based	12%										
All Good, sport sunscreen spray	30	Reef-safe	Oxybenzone free, vegan, non nano, biodegradable, coral reef friendly, natural, mineral based	12%										
Anytime 2in1 combination	30+	Eco-friendly	Family friendly, botanical, non nano, no chemical filters, oxybenzone of parabens	6%										
Babo Botanicals clear zinc sunscreen lotion fragrance free	30	Reef-safe	Botanical, natural mineral, sensitive, vegan, non-nano, no oxybenzone and octinoxate	19.00%										

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
Babo Botanicals clear zinc sunscreen lotion summer scent	30	Reef-safe	Botanical, natural mineral, sensitive, vegan, non-nano, no oxybenzone and octinoxate	19.00%										
Badger baby sunscreen	30	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	18.75%										
Badger bug repellent & sunscreen	34	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	20.00%										
Badger clear zinc sport sunscreen	35	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, clear zinc		22.50%									
Badger clear zinc sunscreen	30	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, clear zinc		18.75%									
Badger kids sunscreen	30	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	18.75%										
Badger Lavender sunscreen	30	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	18.75%										
Badger sport sunscreen	35	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	23%										
Badger unscented sunscreen	15	Reef-safe	planet friendly, no oxybenzone or other chemical activies, biodegradable, non nano	10.00%										
Beyond coastal, natural sunscreen	30	Reef-safe	Oxybenzone free, paraben free, cruelty free, natural		6%		7%							
Block island, natural mineral sunscreen	30	Reef-safe, eco-friendly	Natural, mineral based, organic, non toxic, no chemical UV filters, non nano, no parabens	22%										
Blue Lizard, Australian sunscreen	30	-	Chemical free, paraben free		8%					2%			7.50%	

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
Burn Out, eco sensitive sunscreen	35	Ocean tested, eco-friendly	Ocean tested, non nano, petroleum free, paraben free, biodegradable, eco-sensitive	19%										
Burn Out, kids physical sunscreen	35	Ocean tested, eco-friendly	Ocean tested, non nano, petroleum free, paraben free, biodegradable, eco-sensitive	19%										
Burn Out, ocean tested sunscreen	30	Ocean tested, eco-friendly	Ocean tested, non nano, petroleum free, paraben free, biodegradable	20%										
Caribbean solutions, sol guard sunscreen	15	Reef-safe, eco-friendly	Mineral based, biodegradable, non nano, oxybenzone free, natural	11%										
Caribbean solutions, sol guard sunscreen	30	Reef-safe, eco-friendly	Mineral based, biodegradable, non nano, oxybenzone free, natural	16%										
Caribbean solutions, sol kid care sunscreen	30	Reef-safe, eco-friendly	Mineral based, biodegradable, non nano, oxybenzone free, natural	16%										
Climb on!	30	Reef-safe	Coral reef safe, naural, mineral based, biodegradable, non toxic	20%										
Coconut Joe's organic zinc oxide sunscreen	15	Reef-safe, eco-friendly	Natural, organic, no harmful chemicals, free of oxybenzone, homosalate & octinoxate	x										
Coconut Joe's organic zinc oxide sunscreen	30	Reef-safe, eco-friendly	Natural, organic, no harmful chemicals, free of oxybenzone, homosalate & octinoxate	x										
Coconut Joe's organic zinc oxide sunscreen	50	Reef-safe, eco-friendly	Natural, organic, no harmful chemicals, free of oxybenzone, homosalate & octinoxate	x										
Coral Safe sunscreen	30	Reef-safe	Biodegradable, natural, coral friendly, non nano, no harsh chemicals	6%		6%								
Emergin C	30+	Eco-friendly	Botanical, paraben free, natural, mineral based		6%		7.50%							

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
EQ EVOA organic sunscreen	30	Eco-friendly	Non toxic, organic, mineral based		x		x							
EQ EVOA organic sunscreen	15	Eco-friendly	Non toxic, organic, mineral based				x							
Goddess Garden, kids sport natural sunscreen	30	Reef-safe	Natural, non nano, biodegradable, vegan, free of oxybenzone and parabens	6%		6%								
Goddess Garden, natural sunscreen	30	Reef-safe	Natural, non nano, biodegradable, vegan, free of oxybenzone and parabens	6%		6%								
Goddess Garden, sport natural sunscreen	50	Reef-safe	Natural, non nano, biodegradable, vegan, free of oxybenzone and parabens	11%		11%								
Hampton Sun Mist Sunscreen	70	Eco-friendly						3%	15%	10%	5%	6%		
Innisfree (Korean product)		Eco-friendly			x		x							
Jason	30	Reef-safe	Mineral based, no parabens		14.50%		2%							
Joshua tree sunscreen	30	Reef-safe	100% natural, no harsh chemicals, mineral based		x									
LureLux	25	Reef-safe, eco-friendly	Safe for aquatic environment, biodegradable, no titanium dioxide(Canada considers carcinogenic)	15%										
MyChelle	50	Reef-safe	Mineral based, vegan, biodegradable, no parabens, non nano	17%										
MyChelle, replenishing solar defense	30	Reef safe	Biodegradable, vegan, cruelty free, no parabens	14%										
MyChelle, sun shield coconut	28	Reef-safe	Cruelty free, no parabens, vegan	12%		7%								
MyChelle, sun shield stick	50	Reef safe	Biodegradable, vegan, cruelty free, no parabens	22%										
MyChelle, sun shield unscented	28	Reef-safe	Cruelty free, no parabens, vegan	12%		7%								

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
NexxGen, Doc martin's of Maui	36	Reef-safe						3%		4%		5%	7%	
Peak natural sunscreen	30	Reef-safe, eco-friendly	Mineral based, biodegradable, non toxic, no oxybenzone, no parabens, no harmful chemicals, non nano	6%		6%								
Raw Elements Eco Form Sunscreen	30	Reef-safe	Biodegradable	23%										
Reef Safe	30	Reef-safe, eco-friendly	Biodegradable, bait safe, non toxic						10%		2%	6%	6%	
Safe Sea	50	Eco-friendly	Biodegradable, harmless to aquatic life		5%		2%				5%		7.50%	
Safe Sea	15	Eco-friendly	Biodegradable, harmless to aquatic life		5%		2%				5%		7.50%	
Safe Sea	40	Eco-friendly	Biodegradable, harmless to aquatic life		5%		2%				5%		7.50%	
Secret in a tube	32	Reef-safe	Biodegradable, non nano, vegan, anti aging, botanical	25%										
Smart Stuff	30	Reef-safe, eco-friendly	Natural, biodegradable, no parabens		20%									
SmartShield, kids sunscreen lotion	30	Eco-friendly	Biodegradable					2%		1%	3%		8%	7%
SmartShield, sunscreen lotion	30	Eco-friendly	Biodegradable								2.50%	5%	7.50%	7%
SmartShield, sunscreen lotion	15	Eco-friendly	Biodegradable					2%		1%	3.00%		7.50%	
SPF Rx	30	Reef-safe	Mineral based, natural		7%		3%							
Stream2Sea	20	Reef-safe	Biodegradable, tested and proven reef safe, mineral based, organic			6.60%								
Stream2Sea	30	Reef-safe	Biodegradable, tested and proven reef safe, mineral based, organic			8.80%								

Product	Type (SPF)	Eco label:	Claims:	Zinc-non nano	Zinc oxide	Ti-non nano	Titanium dioxide	Avobenzone	Homosalate	Octocrylene	Octyl Salicylate	Oxybenzone	Octinoxate	Padimate O
Sunblocz sunscreen	50	Reef-safe	100% natural, non nano, no parabens, mineral based	24.50%										
Sunology	50	Reef-safe	Mineral based, oxybenzone free, avobenzone free, paraben free		10%		7.50%							
Tender Sprouts	35	Reef-safe, eco-friendly, wildlife safe	Natural, chemical free, organic, mineral based, non nano	25%										
Tropical sands	30	Reef-safe	Biodegradable mineral, natural, no nanoparticles	6%		6%								
Tropical seas	50	Reef-safe	Biodegradable, oxybenzone free, non toxic to sea life					3.00%		10.00%	5.00%			
Tropical seas, reef safe	15	Reef-safe	Biodegradable, non toxic to sea-life, bait safe								3%	2%	7.00%	
Tropical seas, reef safe	30	Reef-safe	Biodegradable, non toxic to sea-life, bait safe								5%	4%	7.50%	
Tropical seas, reef safe	45	Reef-safe	Biodegradable, non toxic to sea-life, bait safe						5%	8%	5%	6%	7.50%	
Tropical seas, reef safe	36	Reef-safe	Biodegradable, non toxic to sea-life, bait safe						4%		5%	6%	7.50%	
TruKid Sunny days Sport	30	Reef-safe, eco-friendly	100% natural, non nano, no parabens, mineral based	20%										
Vanicream	30	-	Preservative and parabens free, kid friendly		6%		3.40%							
Warrior sunscreens	50	Reef-safe	Mineral based, biodegradable		4%		4.50%							
Waxhead Tinted Sunscreen	35	Reef-safe, eco-friendly	Non toxic, baby safe, biodegradable, no oxybenzone, free of toxins	25%										

Annex 3 Bio-assay principles

Microtox

A micro toxicity test determines the degree of acute toxicity, based on the luminescence inhibition, in the bacteria *V. fischeri*, which is exposed to a concentration series. For this test the used concentration series is 0, 11.2, 22.4, 45.0 and 90.0 µl/l. The test is performed in the micro toxicity analyser which measures the luminescence emission of *V. fischeri* for the 4 concentrations and a blank control in singularity. For more accurate results the test is performed in duplicate for all seven products. This is measured at 5, 15 and 30 minutes after the start of the test. Afterwards, the analyser sends the data to a computer program, which is used to obtain and analyse the results. From these results an EC50 is deducted. This test is according to the standard test protocol of (Azur Environmental, 1998).

Based on the range finding experiment it is predicted that small particles that don't dissolve in the stock solutions can influence the luminescence measured, so can affect the results. So to (partially) take away this effect, the sunscreen products are tested with samples directly coming from the stock solution and samples which are centrifuged beforehand. This way the potential difference in effect is tested between "normal" and centrifuged samples.

Algal growth inhibition test (Skeletonema constatum)

An algae growth inhibition test is performed, according to the standard protocol of NEN-EN-ISO 10253:2006. This test determines the degree of chronic toxicity, based on growth inhibition in the algae *Skeletonema constatum*, by means of a concentration series. S. constatum is exposed to the different sunscreen products, over a period of 96 hours. Based on the range finding experiment it was predicted that the toxicity of sunscreen products can variate over time. Therefore, both stock solutions that are made 1 and 4 days before the start of the experiment are tested.

For this test plates with 96 wells are used. The way the plate is filled is as following; each test concentration has 8 replicates, apart from the blanc which has 16 replicates. Furthermore for each concentration and 16 for the blanc wells are filled with sample but without algae. These wells serve as colour correction of the substances, so that changes of the results due to colour differences cannot occur. Over the period of the test, the fluorescence is measured every 24 hours, by means of the "Biotek microtiter" plate reader and the corresponding Gen5 software. The measured fluorescence and colour correction and compared to the blanc are implemented as raw data in a pre-constructed excel file. This is used to calculate the growth inhibition of each individual test well. This is used to make growth inhibition graphs for each measurement time, of which an EC50 is calculated.

Table 16 Concentration series final test

Product:	Product A	Product B	Product C	Product D	Product E	Product F	Product G
C0 (µl/l)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C1 (µl/l)	4.0	10.0	12.6	31.7	31.7	12.5	31.7
C2 (µl/l)	6.3	17.8	17.7	50.2	50.2	22.2	50.2
C3 (µl/l)	10.0	31.6	25.1	79.6	79.6	39.5	79.6
C4 (µl/l)	15.8	56.2	35.4	126.2	126.2	70.3	126.2
C5 (µl/l)	25.0	100.0	50.0	200.0	200.0	125.0	200.0

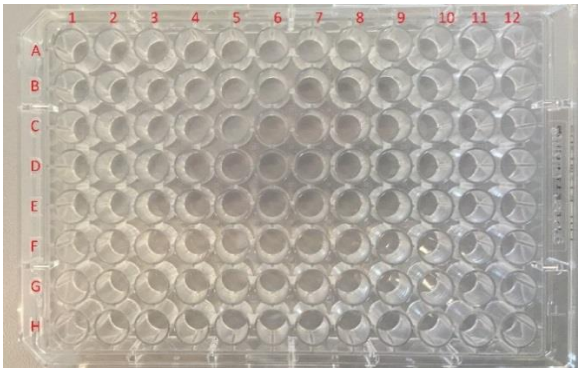


Figure 11 Algae growth inhibition test plate setup

Rotifer acute test with the rotifer Brachionus plicatilis

An acute/mortality bioassay with the marine rotifer *B. plicatilis* is performed, according to the standards protocol of (MicroBioTests Inc., 2018). The concentrations used in both experiments can be seen in Table 8 and Table 9. For the Rotoxkit M acute test only product A, B and F are tested, because the other products showed no toxicity effects in the range finding tests and are therefore excluded in the final tests. The cysts of *B. plicatilis* are stored in tubes in a fridge and for the test the cysts are hatched around 24 hours before the start of the test. The test plates includes 6 rows of 6 test wells and 5 so called rinsing wells, see Figure 17. The rinsing wells are used to transfer and distribute the hatched *B. plicatilis* in the test plates, so no dilution of the test wells can occur. Each test well has 5 *B. plicatilis* and the *B. plicatilis* are exposed to a concentration series of 4 concentrations and a blank control, with 6 replicas. The mortality is measured at 24 and 48 hours, by counting the alive and death organisms in each test well under a binocular. All seven products are tested, plus a reference toxicity test. From the mortality values of each well an EC50 value is deducted for each concentration in an pre constructed excel sheet.

Table 17 Concentration series Rotifer acute

Product:	Product A	Product B	Product F
C0 (µl/l)	0.0	0.0	0.0
C1 (µl/l)	25.1	37.7	37.7
C2 (µl/l)	35.5	53.2	53.2
C3 (µl/l)	50.1	75.2	75.2
C4 (µl/l)	70.8	106.2	106.2
C5 (µl/l)	100.0	150.0	150.0

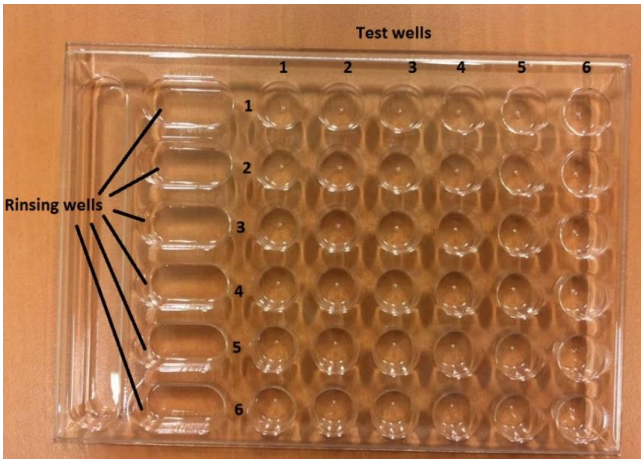


Figure 12 Overview acute rotifer test plate

Annex 4 Toxicity data of selected UV filters

Table 18 Oxybenzone toxicity data

Species scientific name	Species group	Endpoint	Effect measure	Trend	Concentration	Unit	Duration	Reference
Isochryris galbana	Algae	EC50	Growth	Reduction	13,87	µg/L	72 hour	Paredes et al. (2014)
Isochryris galbana	Algae	EC10	Growth	Reduction	3.7	µg/L		Paredes et al. (2014)
Isochryris galbana	Algae	NOEC	Growth	Reduction	30	µg/L		Paredes et al. (2014)
Isochryris galbana	Algae	LOEC	Growth	Reduction	300	µg/L		Paredes et al. (2014)
Montastrea annularis	Coral	LC50	Calicoblast cells mortality	Increase	74	µg/L	4 hour	Downs et al. (2016)
Montastrea annularis	Coral	LC20	Calicoblast cells mortality	Increase	0.562	µg/L	4 hour	Downs et al. (2016)
Montastrea cavernosa	Coral	LC50	Calicoblast cells mortality	Increase	52	µg/L	4 hour	Downs et al. (2016)
Montastrea cavernosa	Coral	LC20	Calicoblast cells mortality	Increase	0.502	µg/L	4 hour	Downs et al. (2016)
Porites astreoides	Coral	LC50	Calicoblast cells mortality	Increase	340	µg/L	4 hour	Downs et al. (2016)
Porites astreoides	Coral	LC20	Calicoblast cells mortality	Increase	8	µg/L	4 hour	Downs et al. (2016)
Porites divaricata	Coral	LC50	Calicoblast cells mortality	Increase	36	µg/L	4 hour	Downs et al. (2016)
Porites divaricata	Coral	LC20	Calicoblast cells mortality	Increase	0.175	µg/L	4 hour	Downs et al. (2016)
Acropora cervicornis	Coral	LC50	Calicoblast cells mortality	Increase	9	µg/L	4 hour	Downs et al. (2016)
Acropora cervicornis	Coral	LC20	Calicoblast cells mortality	Increase	0.063	µg/L	4 hour	Downs et al. (2016)
Pocillopora damicornis	Coral	LC50	Calicoblast cells mortality	Increase	8	µg/L	4 hour	Downs et al. (2016)
Pocillopora damicornis	Coral	LC20	Calicoblast cells mortality	Increase	0.062	µg/L	4 hour	Downs et al. (2016)
Stylophora pistillata	Coral	LC50	Planula mortality	Increase	139	µg/L	24 hour	Downs et al. (2016)
Stylophora pistillata	Coral	NOEC	Planula mortality	Increase	2.28	µg/L	24 hour	Downs et al. (2016)
Stylophora pistillata	Coral	LC50	Calicoblast cells mortality	Increase	42	µg/L	4 hour	Downs et al. (2016)
Stylophora pistillata	Coral	LC20	Calicoblast cells mortality	Increase	2	µg/L	4 hour	Downs et al. (2016)
Stylophora pistillata	Coral	EC50	Planula deformation	Increase	17-49	µg/L	24 hour	Downs et al. (2016)
Stylophora pistillata	Coral	EC20	Planula deformation	Increase	6.5	µg/L	24 hour	Downs et al. (2016)
Siriella armata	Crustaceans	EC50	Mortality	Increase	711	µg/L	96 hour	Paredes et al. (2014)
Siriella armata	Crustaceans	EC10	Mortality	Increase	421	µg/L		Paredes et al. (2014)
Siriella armata	Crustaceans	NOEC	Mortality	Increase	375	µg/L		Paredes et al. (2014)
Siriella armata	Crustaceans	LOEC	Mortality	Increase	500	µg/L		Paredes et al. (2014)
Daphnia magma	Crustaceans; Fres	EC50	Mortality	Increase	2.01	mg/L		Liu et al. (2015)
Danio rerio	Fish; Freshwater	NOEC	Vitellogenin induction	Increase	312	µg/L	14 days	Bluthgen et al. (2012)
Danio rerio	Fish; Freshwater	NOEC	Female gonad maturation	Increase	191	µg/L	12 days	Kinnberg et al. (2015)
Danio rerio	Fish; Freshwater	LOEC	Female gonad maturation	Increase	388	µg/L	12 days	Kinnberg et al. (2015)
Danio rerio	Fish; Freshwater	NOEC	Male gonad maturation	Reduction	388	µg/L	12 days	Kinnberg et al. (2015)
Danio rerio	Fish; Freshwater	LOEC	Male gonad maturation	Reduction	470	µg/L	12 days	Kinnberg et al. (2015)
Danio rerio	Fish; Freshwater	EC50	Mortality	Increase	17.46	mg/L	72 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Mortality	Increase	15.91	mg/L	96 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Mortality	Increase	13.06	mg/L	120 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Swim bladder formation	Reduction	6.73	mg/L	120 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	(Swim) Tail formation	Reduction	9.55	mg/L	72 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Malformation of the somites	Increase	11.99	mg/L	96 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Malformation of the somites	Increase	17.99	mg/L	120 hour	Balazs et al. (2016)
Danio rerio	Fish; Freshwater	EC50	Hatchability	Reduction	12.38	mg/L	96 hour	Balazs et al. (2016)
Pimephales promelas	Fish; Freshwater	NOEC	Vitellogenin induction	Increase	3900	µg/L	21 days	Kunz et al. (2006)
Oncorhynchus mykiss	Fish;Euryhaline	LOEC	Vitellogenin induction	Increase	749	µg/L	14 days	Coronado et al. (2008)
Oncorhynchus mykiss	Fish;Euryhaline	NOEC	Vitellogenin induction	Increase	132	µg/L	14 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	LOEC	Reproduction	Reduction	620	µg/L	7 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	NOEC	Reproduction	Reduction	132	µg/L	7 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	LOEC	Hatchability	Reduction	620	µg/L	21 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	NOEC	Hatchability	Reduction	132	µg/L	21 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	LOEC	Vitellogenin induction	Increase	620	µg/L	21 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	NOEC	Vitellogenin induction	Increase	132	µg/L	21 days	Coronado et al. (2008)
Oryzias latipes	Fish;Euryhaline	NOEC	Growth	Reduction	90	µg/L	14/30 days	Kim,S., D. Jung, Y. Kho, and K. Choi
Oryzias latipes	Fish;Euryhaline	NOEC	Mortality	Increase	90	µg/L	14/21/28 days	Kim,S., D. Jung, Y. Kho, and K. Choi
Oryzias latipes	Fish;Euryhaline	LOEC	Hatchability/reproduction	Reduction	16	µg/L	13/15 days	Coronado et al. (2008)
Paracentrotus lividus	Invertebrates	EC50	Development larvae/growth rate	Reduction	3280	µg/L	48 hour	Paredes et al. (2014)
Paracentrotus lividus	Invertebrates	EC10	Development larvae/growth rate	Reduction	2423	µg/L		Paredes et al. (2014)
Paracentrotus lividus	Invertebrates	NOEC	Development larvae/growth rate	Reduction	1920	µg/L		Paredes et al. (2014)
Paracentrotus lividus	Invertebrates	LOEC	Development larvae/growth rate	Reduction	3840	µg/L		Paredes et al. (2014)
Mytilus galloprovincialis	Mollucs	EC50	Development larvae/growth rate	Reduction	3472	µg/L	48 hour	Paredes et al. (2014)
Mytilus galloprovincialis	Mollucs	EC10	Development larvae/growth rate	Reduction	2146	µg/L		Paredes et al. (2014)
Mytilus galloprovincialis	Mollucs	NOEC	Development larvae/growth rate	Reduction	30	µg/L		Paredes et al. (2014)
Mytilus galloprovincialis	Mollucs	LOEC	Development larvae/growth rate	Reduction	300	µg/L		Paredes et al. (2014)

Table 19 Octocrylene

Species scientific name	Species group	Endpoint	Effect measure	Concentration	Unit	Reference	Column1
Isochryris galbana	Algae	EC10	Development larvae/growth rate	103	µg/L	Giraldo et al 2017	
Isochryris galbana	Algae	NOEC	Development larvae/growth rate	40	µg/L	Giraldo et al 2017	
Isochryris galbana	Algae	LOEC	Development larvae/growth rate	80	µg/L	Giraldo et al 2017	
Mytilus galloprovincialis	Mollucs	EC50	Development larvae/growth rate	>650	µg/L	Giraldo et al 2017	
Mytilus galloprovincialis	Mollucs	EC10	Development larvae/growth rate	511	µg/L	Giraldo et al 2017	
Mytilus galloprovincialis	Mollucs	NOEC	Development larvae/growth rate	20	µg/L	Giraldo et al 2017	
Mytilus galloprovincialis	Mollucs	LOEC	Development larvae/growth rate	40	µg/L	Giraldo et al 2017	
Paracentrotus lividus	Invertebrates	EC50	Mortality	737	µg/L	Giraldo et al 2017	
Paracentrotus lividus	Invertebrates	EC10	Mortality	162	µg/L	Giraldo et al 2017	
Paracentrotus lividus	Invertebrates	NOEC	Mortality	20	µg/L	Giraldo et al 2017	
Paracentrotus lividus	Invertebrates	LOEC	Mortality	40	µg/L	Giraldo et al 2017	

Table 20 nZnO toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference
Algae	*Thalassiosira weissflogii*	NOEC	Growth inhibition	72h	0.01	mg/l	6
Algae	*Thalassiosira weissflogii*	EC20	Growth inhibition	72h	0.07	mg/l	6
Algae	*Thalassiosira weissflogii*	LOEC	Growth inhibition	72h	0.099	mg/l	6
Algae	*Thalassiosira pseudonana*	LOEC	Growth inhibition	96h	0.5	mg/l	3
Algae	*Dunaliella tertiolecta*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Skeletonema marinoi*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Isochrysis galbana*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Dunaliella tertiolecta*	EC50	Growth inhibition	96h	1.94	mg/l	4
Algae	*Skeletonema constatum*	IC50	Growth inhibition	96h	2.36	mg/l	2
Algae	*Thalassiosira pseudonana*	IC50	Growth inhibition	96h	4.56	mg/l	2
Algae	*Thalassiosira pseudonana*	EC100	Growth inhibition	100h	10	mg/l	1
Algae	*Chaetoceros gracilis*	EC100	Growth inhibition	100h	10	mg/l	1
Algae	*Phaeodactylum tricornutum*	EC100	Growth inhibition	100h	10	mg/l	1
Algae (FW)	*Pseudokirchneriella subcapitata*	IC50	Growth inhibition	72h	0.049	mg/l	12
Algae (FW)	*Pseudokirchneriella subcapitata*	IC50	Growth inhibition	72h	0.068	mg/l	12
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	0.75	mg/l	14
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	1.9	mg/l	14
Crustaceans	*Acartia tonsa*	NOEC	Mortality	3days	0.01	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Mortality	3days	0.07	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Mortality	3days	0.099	mg/l	6
Crustaceans	*Acartia tonsa*	NOEC	Mortality	7days	0.099	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Mortality	7days	0.112	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Reproduction	7days	0.143	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Mortality	7days	0.168	mg/l	6
Crustaceans	*Acartia tonsa*	NOEC	Reproduction	7days	0.168	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Reproduction	7days	0.263	mg/l	6
Crustaceans	*Corophium volutator*	LOEC	Mortality	100days	0.5	mg/l	7
Crustaceans	*Tigripus japnicus*	LC50	Mortality	96h	0.85	mg/l	2
Crustaceans	*Elasmopus rapax*	LC50	Mortality	96h	1.19	mg/l	2
Crustaceans	*Artemia salina*	LC50	Mortality	96h	>100	mg/l	5
Crustaceans (FW)	*Thamnocephalus platyurus*	NOEC	Mortality	24h	0.03	mg/l	14
Crustaceans (FW)	*Thamnocephalus platyurus*	EC50	Mortality	24h	0.18	mg/l	14
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	48h	0.5	mg/l	14
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	3.2	mg/l	14
Mollusca	*Mytilus galloprovincialis*	LOEC	Uptake of NP	24days	2	mg/l	10
Mollusca	*Mytilus galloprovincialis*	LOEC	Uptake of NP	4days	2.5	mg/l	9
Mollusca	*Crassostrea gigas*	LC50	Mortality	96h	37.2	mg/l	8
Other invertebrates	*Lytechinus pictus*	EC50	Larval morphology	96h	0.0995	mg/l	11

Table 21 non nZnO toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference
Algae	*Thalassiosira weissflogii*	NOEC	Growth inhibition	72h	0.01	mg/l	6
Algae	*Thalassiosira weissflogii*	EC20	Growth inhibition	72h	0.07	mg/l	6
Algae	*Thalassiosira weissflogii*	LOEC	Growth inhibition	72h	0.099	mg/l	6
Algae	*Thalassiosira pseudonana*	LOEC	Growth inhibition	96h	0.5	mg/l	3
Algae	*Dunaliella tertiolecta*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Skeletonema marinoi*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Isochrysis galbana*	LOEC	Growth inhibition	96h	1	mg/l	3
Algae	*Dunaliella tertiolecta*	EC50	Growth inhibition	96h	1.94	mg/l	4
Algae	*Skeletonema constatum*	IC50	Growth inhibition	96h	2.36	mg/l	2
Algae	*Thalassiosira pseudonana*	IC50	Growth inhibition	96h	4.56	mg/l	2
Algae	*Thalassiosira pseudonana*	EC100	Growth inhibition	100h	10	mg/l	1
Algae	*Chaetoceros gracilis*	EC100	Growth inhibition	100h	10	mg/l	1
Algae	*Phaeodactylum tricornutum*	EC100	Growth inhibition	100h	10	mg/l	1
Algae (FW)	*Pseudokirchneriella subcapitata*	IC50	Growth inhibition	72h	0.049	mg/l	12
Algae (FW)	*Pseudokirchneriella subcapitata*	IC50	Growth inhibition	72h	0.068	mg/l	12
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	0.75	mg/l	14
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	1.9	mg/l	14
Crustaceans	*Acartia tonsa*	NOEC	Mortality	3days	0.01	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Mortality	3days	0.07	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Mortality	3days	0.099	mg/l	6
Crustaceans	*Acartia tonsa*	NOEC	Mortality	7days	0.099	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Mortality	7days	0.112	mg/l	6
Crustaceans	*Acartia tonsa*	EC20	Reproduction	7days	0.143	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Mortality	7days	0.168	mg/l	6
Crustaceans	*Acartia tonsa*	NOEC	Reproduction	7days	0.168	mg/l	6
Crustaceans	*Acartia tonsa*	LOEC	Reproduction	7days	0.263	mg/l	6
Crustaceans	*Corophium volutator*	LOEC	Mortality	100days	0.5	mg/l	7
Crustaceans	*Tigripus japnicus*	LC50	Mortality	96h	0.85	mg/l	2
Crustaceans	*Elasmopus rapax*	LC50	Mortality	96h	1.19	mg/l	2
Crustaceans	*Artemia salina*	LC50	Mortality	96h	>100	mg/l	5
Crustaceans (FW)	*Thamnocephalus platyurus*	NOEC	Mortality	24h	0.03	mg/l	14
Crustaceans (FW)	*Thamnocephalus platyurus*	EC50	Mortality	24h	0.18	mg/l	14
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	48h	0.5	mg/l	14
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	3.2	mg/l	14
Mollusca	*Mytilus galloprovincialis*	LOEC	Uptake of NP	24days	2	mg/l	10
Mollusca	*Mytilus galloprovincialis*	LOEC	Uptake of NP	4days	2.5	mg/l	9
Mollusca	*Crassostrea gigas*	LC50	Mortality	96h	37.2	mg/l	8
Other invertebrates	*Lytechinus pictus*	EC50	Larval morphology	96h	0.0995	mg/l	11

Table 22 nTiO2 toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference
Algae	*Phaeodactylum tricornutum*	EC50	Growth inhibition	72h	10.91	mg/l	17
Algae	*Phaeodactylum tricornutum*	EC50	Growth inhibition	72h	11.3	mg/l	17
Algae	*Phaeodactylum tricornutum*	EC50	Growth inhibition	72h	14.3	mg/l	17
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	>20000	mg/l	14
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	>20000	mg/l	14
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	250	mg/l	15
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	250	mg/l	15
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	500	mg/l	15
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	650.6	mg/l	15
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	940.6	mg/l	15
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	830.8	mg/l	15
Bacteria	*Vibrio fischeri*	LOEC	Growth inhibition	30min	500	mg/l	15
Bacteria	*Vibrio fischeri*	LOEC	Growth inhibition	30min	500	mg/l	15
Bacteria	*Vibrio fischeri*	LOEC	Growth inhibition	30min	1000	mg/l	15
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	15min	>100	mg/l	16
Crustaceans	*Artemia franciscana*	EC50	Feed behaviour	24h	26.52	mg/l	20
Crustaceans	*Artemia franciscana*	EC50	Feed behaviour	24h	17.74	mg/l	20
Crustaceans	*Artemia franciscana*	EC50	Feed behaviour	24h	13.4	mg/l	20
Crustaceans	*Artemia franciscana*	EC50	Feed behaviour	24h	27.13	mg/l	20
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	48h	1	mg/l	13
Crustaceans (FW)	*Daphnia magna*	LOEC	Mortality	48h	2	mg/l	13
Crustaceans (FW)	*Daphnia magna*	LC50	Mortality	48h	5.5	mg/l	13
Crustaceans (FW)	Thamnocephalus platyurus	EC50	Mortality	24h	>20000	mg/l	14
Crustaceans (FW)	Thamnocephalus platyurus	NOEC	Mortality	24h	>20000	mg/l	14
Crustaceans (FW)	*Daphnia magna*	LC50	Mortality	48h	20000	mg/l	14
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	72h	1.3	mg/l	17
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	72h	3.15	mg/l	17
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	72h	3.44	mg/l	17
Molluscs	*Mytilus galloprovincialis*	EC50	Larval malformations	48h	1.23	mg/l	18
Molluscs	*Mytilus galloprovincialis*	EC50	Larval malformations	48h	38.56	mg/l	18
Molluscs	*Mytilus galloprovincialis*	EC50	Larval malformations	48h	1.65	mg/l	18
Molluscs	*Mytilus galloprovincialis*	EC50	Larval malformations	48h	16.39	mg/l	18
Molluscs	*Haliotis diversicolor supertexta*	NOEC	Malformations	10h	2	mg/l	19
Molluscs	*Haliotis diversicolor supertexta*	EC50	Malformations	10h	56.9	mg/l	19
Molluscs	*Haliotis diversicolor supertexta*	EC50	Malformations	10h	345.8	mg/l	19
Rotifers	*Brachionus plicatilis*	EC50	Mortality	48h	5.37	mg/l	17
Rotifers	*Brachionus plicatilis*	EC50	Mortality	48h	10.43	mg/l	17
Rotifers	*Brachionus plicatilis*	EC50	Mortality	48h	267.3	mg/l	17

Table 23 Non nTiO2 toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference
Bacteria	*Vibrio fischeri*	NOEC	Growth inhibition	30min	>20000	mg/l	14
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	>20000	mg/l	14
Crustaceans (FW)	Thamnocephalus platyurus	EC50	Mortality	24h	>20000	mg/l	14
Crustaceans (FW)	Thamnocephalus platyurus	NOEC	Mortality	24h	>20000	mg/l	14

Table 24 Tinosorb M toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference:
Algae (FW)	*Scenedesmus subspicatus*	EC50	Growth inhibition	72h	>2	mg/l	21
Algae (FW)	*Scenedesmus subspicatus*	NOEC	Growth inhibition	72h	>2	mg/l	21
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	21days	>0.025	mg/l	21
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	>65.9	mg/l	21
Fish (FW)	*Danio rerio*	LC50	Malformations	96h	>28.9	mg/l	21

Table 25 Ensulizole toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference
Algae (FW)	*Pseudokirchneriella subcapitata*	EC50	Growth inhibition	72h	>100	mg/l	24
Algae (FW)	*Pseudokirchneriella subcapitata*	LOEC	Growth inhibition	72h	>100	mg/l	24
Algae (FW)	*Pseudokirchneriella subcapitata*	NOEC	Growth inhibition	72h	≥100	mg/l	24
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	72h	>100	mg/l	24
Fish (FW)	*Danio rerio*	NOEC	Malformations	72h	≥1000	mg/l	24

Table 26 Uvinul A plus toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference:
Algae (FW)	*Scenedesmus subspicatus*	EC50	Growth inhibition	72h	>2	mg/l	21
Algae (FW)	*Scenedesmus subspicatus*	NOEC	Growth inhibition	72h	>2	mg/l	21
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	21days	>0.025	mg/l	21
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	>65.9	mg/l	21
Fish (FW)	*Danio rerio*	LC50	Malformations	96h	>28.9	mg/l	21

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference:
Algae (FW)	*Scenedesmus subspicatus*	EC50	Growth inhibition	72h	>100	mg/l	22
Algae (FW)	*Scenedesmus subspicatus*	LOEC	Growth inhibition	72h	>100	mg/l	22
Algae (FW)	*Scenedesmus subspicatus*	NOEC	Growth inhibition	72h	≥100	mg/l	22
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	21days	>0.0143	mg/l	22
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	>100	mg/l	22
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	21days	≥0.0143	mg/l	22
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	48h	≥100	mg/l	22
Fish (FW)	*Danio rerio*	LC50	Malformations	96h	>100	mg/l	22
Fish (FW)	*Pimephales promelas*	NOEC	Malformations	34days	≥0.0088	mg/l	22
Fish (FW)	*Danio rerio*	NOEC	Malformations	96h	≥100	mg/l	22

Table 27 Uvinul T150 toxicity data

Taxonomy	Species	Tox effect	Effect type	Experiment time	Effect	Value	Reference:
Algae (FW)	*Scenedesmus subspicatus*	EC50	Growth inhibtion	72h	>80	mg/l	23
Algae (FW)	*Scenedesmus subspicatus*	NOEC	Growth inhibtion	72h	≥80	mg/l	23
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	>500	mg/l	23
Crustaceans (FW)	*Daphnia magna*	EC50	Mortality	48h	>500	mg/l	23
Crustaceans (FW)	*Daphnia magna*	NOEC	Mortality	21days	≥0.0001	mg/l	23
Fish (FW)	*Danio rerio*	LC50	Malformations	96h	>100	mg/l	23
Fish (FW)	*Danio rerio*	NOEC	Malformations	35days	≥0.00101	mg/l	23

Wageningen Marine Research
T +31 (0)317 48 09 00
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors' address
- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

Wageningen Marine Research is the Netherlands research institute established to provide the scientific support that is essential for developing policies and innovation in respect of the marine environment, fishery activities, aquaculture and the maritime sector.

Wageningen University & Research:
is specialised in the domain of healthy food and living environment.

The Wageningen Marine Research vision
'To explore the potential of marine nature to improve the quality of life'

The Wageningen Marine Research mission
- To conduct research with the aim of acquiring knowledge and offering advice on the sustainable management and use of marine and coastal areas.
- Wageningen Marine Research is an independent, leading scientific research institute

Wageningen Marine Research is part of the international knowledge organisation Wageningen UR (University & Research centre). Within Wageningen UR, nine specialised research institutes of the Stichting Wageningen Research Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment.