Eco-friendly sunscreen products in the marine environment

Potential and relative effects of UV filters

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Preface

In front of you lies the thesis report “eco-friendly sunscreen products in the marine environment”. This thesis is written as part of my graduation of the bachelor program aquatic ecotechnology at the Hogeschool Zeeland, Vlissingen. It is completed at Wageningen Marine Research, Den Helder, from the period of February 2018 till June 2018.

Together with my supervisor, Diana Slijkerman, we set up this research. This research contained a lot of different parts, which made this research difficult, but very challenging. After extensive literature and experimental research I was able to successfully complete this research and gave a lot more insight on the topic of eco-friendly sunscreen products. However this was only the beginning on this topic and this research rose a lot of new questions, which makes continuation of this research very interesting.

With the help of my supervisor Diana Slijkerman, my school supervisor Anne Oele, and colleague Klaas Kaag, I was able to successfully complete this research. Therefore I want to personally thank all of you for helping me with my thesis and by always being there for me when I needed help.

I want to give a special thank you to my supervisor Diana Slijkerman, for all the help, supervision and interesting talks. And not only for supervising me but also for helping me find a job for after my graduation period. I’m really honoured by the potential you see in me.

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Abstract

Coastal tourism and recreation are one of the most rapidly growing fields worldwide and people spend more time outdoors. Skin protection is, in this respect, important to prevent severe skin damage and skin aging. The past decades the awareness and the importance of skin protection against long time exposure to UVR increased. And therefore different types of sunscreen products are available on the market, among which are eco-friendly sunscreen products. The aim of this thesis is to provide a scientific contribution to the knowledge of sunscreen products, by investigating the effects of eco-friendly sunscreen products for the marine environment. The main question is "Can eco-friendly sunscreen products have adverse effects on the marine environment?"

To answer this question 3 approaches were selected. A literature research was performed to investigate which eco-friendly sunscreen products are available, including their claims. And to investigate the individual toxicity of all the UV filters used in these products, toxicity databases were made. Most eco-friendly sunscreen products contain non-nano ZnO as UV filter, but other mineral based UV filters and organic UV filters are also used in some cases. Furthermore the toxicity data showed that both nano and non-nano ZnO showed the strongest toxicity effects similar to the organic UV filters oxybenzone and octocrylene. Whereas TiO_2 and new organic UV filters showed much weaker toxicity effects.

Bioassays were performed to research the potential and relative toxicity of different sunscreen products on marine organisms from various trophic levels. Multiple acute and chronic bioassays were performed on *Vibrio fischeri* bacteria, *Brachionus plicatilis* rotifers and *Skeletonema constatum* algae. This resulted in a clear pattern where eco-friendly show very weak to no relative toxicity effects in the bioassays, whereas the tested products which didn't claim to be eco-friendly showed much stronger relative toxicity effects.

A risk assessment was performed for the case Lac Bay, a touristic hotspot in Bonaire. Estimations on the potential daily emissions of eco-friendly sunscreen products were made and compared to the toxicity data to calculate risk quotients indicating a potential environmental risk for the case. The outcome was that both nano and non-nano ZnO form a potential environmental risk for the case of Lac Bay. However nano-TiO_2 forms no risk. Furthermore it was not possible to perform the risk assessment for the other UV filters, because toxicity data was limited.

Answering the main question results in the following conclusion. From the three approaches the toxicity data and the risk assessment indicates that both nano and non-nano ZnO, which are most used in eco-friendly sunscreen products, show strong toxicity and form a potential environmental risk. Whereas the bioassays show that all eco-friendly sunscreen products show weak to no relative toxicity compared to the not eco-friendly products, which show strong relative toxicity in the bioassays.

List of abbreviations

In this report many abbreviations and technical terms are used, for which this list is included with detailed descriptions for better understanding of the context.

Bioassays	Measurement of the concentration of a substance by its effect on living cells or tissues.
Broad spectrum UV filters	UV filters who make sure of protection 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.
EC50	Effect concentration 50%, the concentration were 50% effect (e.g. mortality, growth inhibition) is reached.
Endocrine system	The chemical messenger system consisting of hormones.
Estrogenic disruption	The interference of chemicals to the endocrine system (hormone system) at certain doses.
Fluorescence	Emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence.
Lipophilicity	The ability of a compound to dissolve in fats, oils and lipids.
LOEC	Lowest observed effect concentration.
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.
NOEC	No observed effect concentration.
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.
UVB	Ultraviolet B.
UVR	Ultraviolet Radiation.
Zooxanthellae	Dinoflagellates that are able to live in symbiosis with marine invertebrates like corals.

1 Introduction

1.1 Background

Coastal tourism and recreation are one of the most rapidly growing fields worldwide and people spend more time outdoors. Skin protection is, in this respect, important to prevent severe skin damage and skin aging. The past decades the awareness and the importance of skin protection against long time exposure to UVR increased (Sánchez-Quiles & Tovar-Sánchez, 2015). As a consequence, the use of sunscreen products increased. And hereby also the emissions into the environment.

Sunscreens typically contain active ingredients that protects the skin from UVR, called UV filters (Danovaro, et al., 2008). UV filters can enter the marine environment in direct (swimming & bathing) and indirect ways (wastewater discharges) (Giokas, Salvador, & Chisvert, 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 K. , Kunz, Zenker, & Rapp, 2010); (Paredes, Perez, Rodil, Quintana, & Beiras, 2014); (Zhang, Ma, Wang, & Ngo, 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. Due to this, alternative/eco-friendly sunscreen products are being introduced on the market. Industries claim that these alternative products are “reef safe” and “eco-friendly” and most of these products do not contain organic UV filters. These products use UV filters based on minerals, such as zinc or titanium, or new organic UV filters.

However, most of these claims are unregulated and the question rises whether eco-friendly sunscreen products are really eco-friendly. Do they not contain polluting substances as well which can have potential effects on the marine environment? In addition, what if everyone changes to using eco-friendly sunscreen products instead? What could be the total emission of these substances into the environment? This are just a few of the questions that arise for eco-friendly products.

1.2 Aim

This study aims to provide a scientific contribution to the knowledge of the potential effects of eco-friendly sunscreen products for marine organisms. To achieve this objective the following main research question is 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 (eco-friendly) 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?

The hypothesis is that claims on eco-friendly products and regulations are still undetermined, but eco-friendly sunscreen products don’t show any adverse effects and contribute to a clean and

healthy marine environment. Furthermore, if everyone changes to using eco-friendly products this process will be enhanced even more.

1.3 Approach

The research questions are answered in 3 steps, according to the following approach.

1. Literature research
    a. Background on sunscreens and behaviour in the marine environment
    b. Toxicity data UV filters
    c. Eco-friendly sunscreen products and their claims
2. Bioassays
    a. Bioassay selection
    b. Range finding experiments
    c. Final tests and end results
    d. Comparison of the bioassays results with the toxicity data
3. Risk assessment
    a. Emission estimations
    b. Risk quotients based on toxicity data

First a literature research is performed where the background of sunscreen products and their behaviour in the marine environment is described. Information from scientific reports and previous studies on this subject, which are obtained via Google Scholar, Science direct and Scopus, are used to research and report this, see chapters 2.1 and 2.2.

Furthermore, toxicity data was gathered for the three different kinds of UV filters described in the background. To obtain this toxicity data, multiple toxicity and chemical databases are used to acquire data on the UV filters such as, databases from the US Environmental Protection Agency (EPA, 2018) and the European Chemical Agency (ECHA, 2008). Furthermore scientific reports that reported toxicity effects on the researched UV filters were used. All this data combined, results in toxicity databases for each individual UV filter, see chapter 2.3 and appendix 5.

Moreover, alternative/eco-friendly sunscreen products are researched in literature. To better understand which eco-friendly products are available, the current market is researched, mainly by Amazon, and an overview list of all the current eco-friendly sunscreen products available is made, including their claims, eco-labels and used UV filters. Continuing on this, the claims and eco-labels are described in more detail to get a better understanding of their meaning, see chapter 3.

Secondly, several lab experiments are performed using bioassays, to compare the potential and relative toxicity of several different sunscreen products. The chosen bioassays are based on testing with different marine organisms, from different trophic levels, to get an overall view of the potential toxicity for the marine environment. In total 7 different products are chosen to compare the different types of UV filters and to compare products that claim to be eco-friendly with products who don't. Because there is very limiting information available in performing bioassays with sunscreen products, range finding tests were performed to not only indicate on what concentrations effects took place, but also to indicate if the bioassays work for sunscreen products.

Furthermore, for each UV filter the results of the bioassays are compared to the toxicity database to indicate differences and/or similarities in the reported toxicity. See chapter 4 for the detailed method and chapter 5 for the results and discussion.

Thirdly, a risk assessment was performed to make estimations on what happens when everyone changes to using eco-friendly sunscreen products. This is done for the case Lac Bay, a touristic hotspot in Bonaire. The risk assessment was based on the daily total UV filter emission estimations combined with toxicity data gathered in this research. This results in a potential risk quotient for each individual UV filter for the case Lac Bay, Bonaire, see chapter 6.

2 Background of sunscreen products and UV filters

2.1 Components of sunscreen products

Sunscreen products are a rich 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).

It is obvious that UV filters can therefore be 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 320-400 nanometre (nm), and ultraviolet B (UVB), ranging from 290-320 nm. These UV filters can be classified in different groups; organic/chemical, inorganic/mineral based and new organic (Sánchez-Quiles & Tovar-Sánchez, 2015).

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

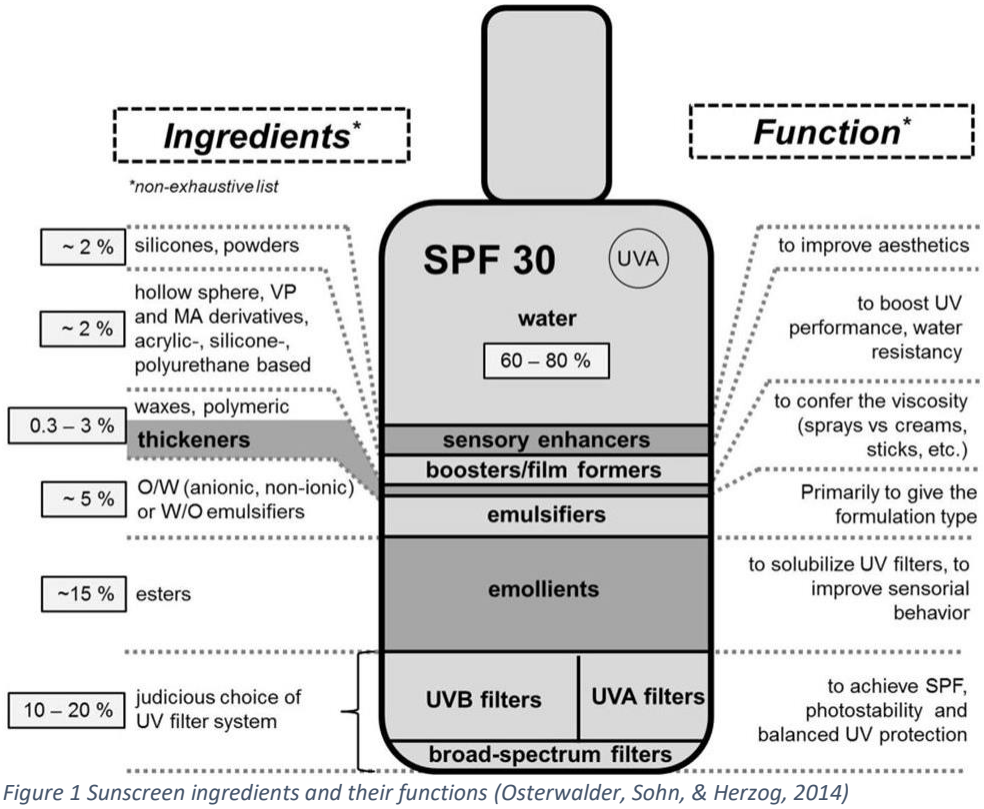

Figure 1 Sunscreen ingredients and their functions (Osterwalder, Sohn, & Herzog, 2014)

An overview of all the described UV filters with their UV protection ranges is presented in Figure 2. From this figure it can be seen that not all UV filters have a broad spectrum protection, which explains why most of the time several UV filters are used together in sunscreen products.

Type	Active ingredients	UVB protection	UVA protection	
Organic UV filter	Oxybenzone			
	Octocrylene			
	Avobenzone			
	Homosalate			
	Octinoxate			
	Octisalate			
Inorganic UV filter	Zinc Oxide (ZnO)			
	Titanium Dioxide (TiO2)			
New organic UV filter	Tinosorb S			
	Tinosorb M			
	Mexoryl SX			
	Mexoryl XL			
	Uvinul A plus			
	Uvinul T 150			
	Ensulizole			
	Protection (nm):	290-320	320-340	340-400

m

Figure 2 UV filters and their UV protection range based on (Skinacea, 2012)

Organic (chemical) UV filters

Organic UV filters work via an action mode whereby UVR is scattered, reflected and/or absorbed by skin contact. Organic UV filters show similar characteristic properties of persistent organic pollutants (POP), such as Polychlorinated Biphenyls (PCBs), dioxins and dichlorodifenyltrichloroethane (DDT) (Díaz-Cruz & Barceló, 2009). Common examples of organic UV filters are salicylates, benzophenones, avobenzone, homosalate, octocrylene and cinnamates. Benzophenones are frequently used in organic sunscreen products and the most widely used one is oxybenzone (Antoniou, Kosmadaki, Stratigos, & Katsambas, 2008). Mostly for oxybenzone and octocrylene, effects on marine organisms have been reported (2.3.1). All products are approved by the Federal Drug Agency (FDA) however, the counsel of Bonaire voted to ban oxybenzone and octinoxate as of 2021 (Pappas, 2018).

Inorganic (mineral based) UV filters

Mineral based UV-filters work via an action mode whereby UVR is scattered, reflected and/or absorbed by skin contact. The extent of absorbance and application depends on its structural properties and metal origin. Zinc oxide (ZnO) and titanium dioxide (TiO_2) are mineral based UV filters (Manaia, Kaminski, Corrêa, & Chiavacci, 2013), for which the particle size ranges from nano to non-nano sizes. Mainly for nano sized mineral UV filters effects on the marine environment are reported (2.3.2 and 2.3.3). ZnO and TiO_2 sufficiently reflect UVR and show no absorption of visible light, which qualifies them to be efficiently, approved by the FDA and therefore the most used in these type of sunscreen products (Osterwalder, Sohn, & Herzog, 2014).

New organic UV filters

Other products are being introduced on the market containing so called ‘new organic’ UV filters. The main reason for this is that it is stated that these UV filters have a lower chance of resulting in skin irritation and skin allergies (Dr. Jetske Ultee, 2018). Common examples of new organic UV filters are Tinosorb S/M, Uvinul A plus/T150, ensulizole and mexoryl SX/XL. Most of these are not yet approved by the FDA, because they are relatively new UV filters and are therefore mainly used in EU products (Skinacea, 2012).

2.2 UV filters in the marine environment

The presence of UV filters in the environment has been reported since the early 1980s, however, no connection between UV filters occurrence and personal care products, such as sunscreen was made. Currently, several studies recognize the importance of the accumulation of personal care products, as well as sunscreen, in the marine environment. The concentrations of this relatively new class of pollutants vary depending on the location of sampling and the extent of recreational usages, and thus their potential effect too (Giokas, Salvador, & Chisvert, 2007).

It is known that UV filters may enter the aquatic environment via direct or indirect paths (Giokas, Salvador, & Chisvert, 2007); (Richardson & Kimura, 2015). Direct discharge of sunscreens and their ingredients to natural water bodies originate from swimming and bathing activities and indirectly from industrial wastewater effluent (Giokas, Salvador, & Chisvert, 2007). Several studies show a correlation between beachgoers, swimming activities and the amount of sunscreen ingredients released into seawater (Balmer, Buser, Müller, & Poiger, 2005); (Tovar-Sánchez, et al., 2013); (Sánchez-Quiles & Tovar-Sánchez, 2015).

UV filters can be distributed, influenced by oceanic currents, seasons and flow conditions (Emnet, Gaw, Northcott, Storey, & Graham, 2015). The presence of organic UV filters is even found at Antarctic coastal areas and in the middle of the Pacific Ocean at concentrations between 6 and 55 ng/l. The presence of sunscreen ingredients at remote locations may be due to the combination of long ranging oceanic currents and/or atmospheric transport (Tsui, et al., 2014); (Ramos, Homem, Alves, & Santos, 2015). Especially UV filters with high photo stability, such as oxybenzone and octocrylene may be able to undergo long range or local transport via oceanic currents, see Figure 3 (Tsui, et al., 2014). Flow conditions can influence distribution behaviour steered by seasonality (Giokas, Salvador, & Chisvert, 2007); (Amine, Gomez, Halwani, Casellas, & Fenet, 2012).

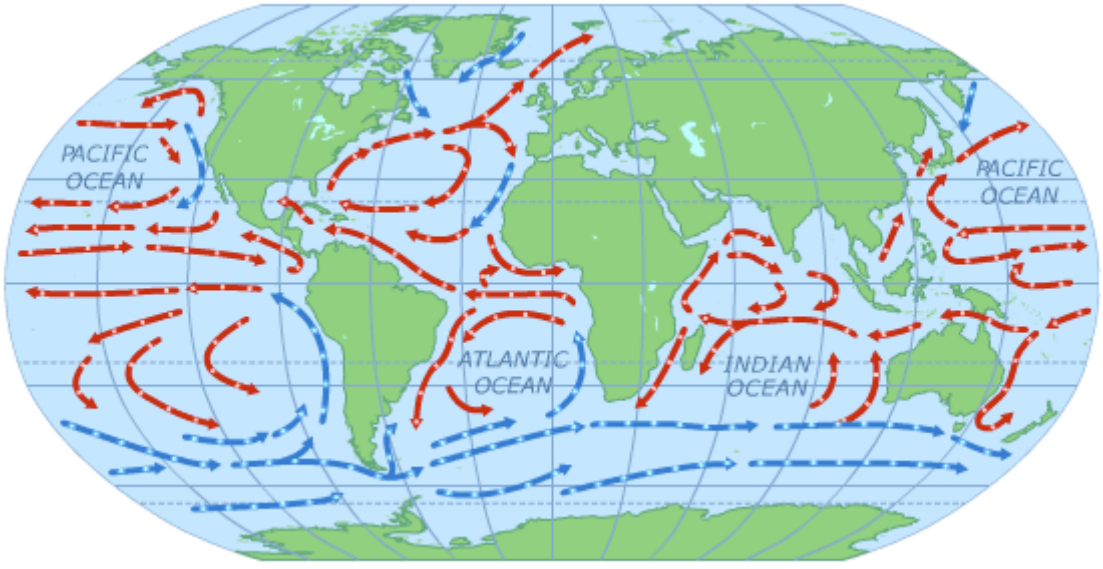

Figure 3 Overview of ocean currents (Earth labs, 2010)

Distributional behaviour of UV filters in coastal waters can also be explained as vertical (water column) distribution, whereby the highest concentrations of oxybenzone, ZnO and TiO_2 were measured in the surface microlayer (SML) (Tovar-Sánchez, et al., 2013); (Sánchez-Quiles & Tovar-Sánchez, 2015). Due to UV filters' lipophilicity and relative insolubility, UV filters are more likely to concentrate in the SML than to accumulate in soils and natural organic matter (Tovar-Sánchez, et al.,

2013). Concentrations of some of these UV filters in the SML are found up to 42% higher, for oxybenzone, than in the subsurface water column (Sánchez-Quiles & Tovar-Sánchez, 2015).

Sorption behaviour

Sorption of chemicals to organic substances, such as sediment or dissolved organic matter (DOM), affects the amount of available compounds which can be taken up by organisms. The $\log K_{ow}$ is the main indicator for water solubility. A $\log K_{ow}$ of <1 suggests a compound that is highly soluble in water (hydrophilic). A $\log K_{ow}$ of >4 suggest a compound that is low soluble in water (hydrophobic). However, compounds with a $\log K_{ow}$ of >8 can be considered to not be bioavailable in aquatic systems (Ramos, Homem, Alves, & Santos, 2015). Sunscreen products are water resistant and the majority of organic UV filters have a relatively high $\log K_{ow}$. This indicates that these compounds may be removed from the water column via sorption processes by means of binding to sediment or DOM particles. However it is still obscure if these removal mechanisms massively influence the bioavailability of UV filters in the marine environment (Giokas, Salvador, & Chisvert, 2007).

Persistence of organic UV filters

Organic UV filters contain similar properties as POP (persistent organic pollutants). Therefore organic UV filters can be classified as environmental stable and not readily biodegradable (Díaz-Cruz & Barceló, 2009). Nevertheless, organic UV filters are still subjected to degradation in multiple ways including, photo degradation, photo isomerization and degradation by disinfection (Santos, Miranda, & Esteves da Silva, 2012). As mentioned before the intact UV filter can enter the aquatic environment. But also its metabolites may enter the aquatic environment due to these degradation ways (Díaz-Cruz & Barceló, 2009).

Persistence of (nano) ZnO and TiO_2

Once entered into the marine environment, minerals like ZnO and TiO_2 can undergo several processes. In general these minerals can stay in suspension as individual particles, dissolve in seawater, aggregate and form larger particles which are subsequently deposited in sediment, adsorption by DOM, and transform both chemically and biologically in the marine environment, see Figure 4 (Yung, Mouneyrac, & Leung, 2015).

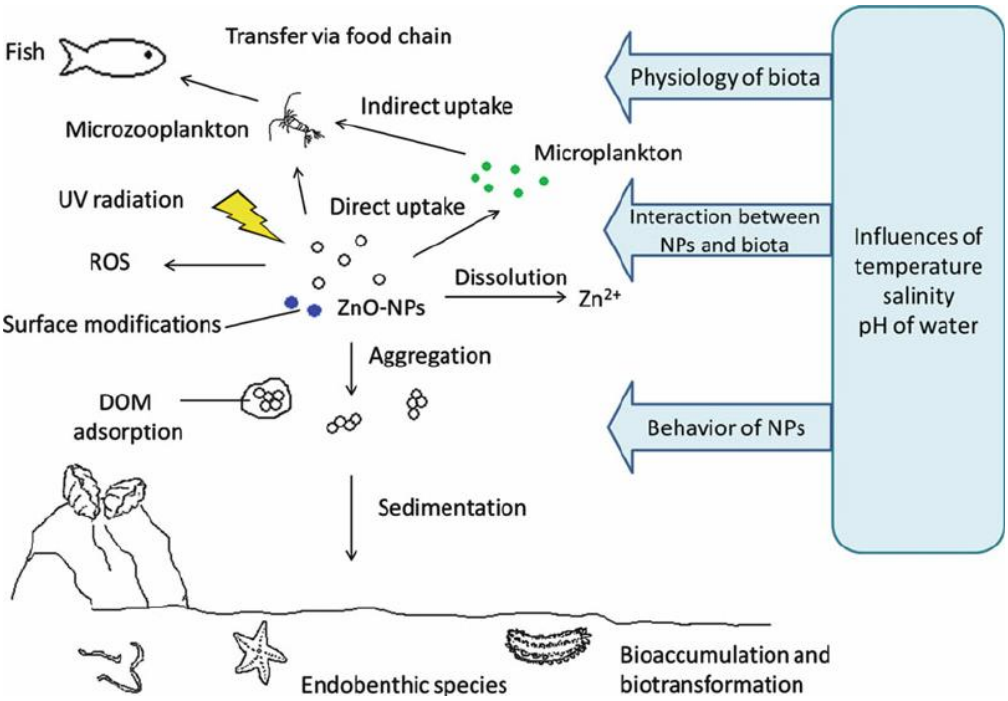

Figure 4 Fate and behaviour of ZnO and TiO2 (Yung, Mouneyrac, & Leung, 2015)

These processes can result in changes in the bioavailability of ZnO and TiO_2. Elimination of aggregates from the water column through deposition or sedimentation decreases the concentration of bioavailable ZnO and TiO_2 for pelagic species. Even though these aggregates become less mobile, uptake by sediment-dwelling organisms or filter feeders is still possible (Yung, Mouneyrac, & Leung, 2015).

Chemical transformation of ZnO and TiO_2 will significantly influence their size, shape, and surface chemistry. Because of their high $logK_{ow}$ and their high surface reactivity, ZnO and TiO_2 can adsorb onto pollutants or DOMs in the marine environment. DOM can alter their surface charge and inhibit aggregation, which may increase their mobility in the environment. Redox reactions occurring on the surface of ZnO and TiO_2 can also lead to the production of Reactive Oxygen Species (ROS). ROS are chemically reactive chemical species that are able to oxidize organic compounds in the environment and can lead to a variety of effects including lipid peroxidation, DNA damage and oxidative stress (Yung, Mouneyrac, & Leung, 2015). The adsorption of DOMs onto ZnO and TiO_2 can increase their toxicity due to ROS production. ZnO and TiO_2 may also dissolve, releasing free Zn^{2+} or Ti ions. These ions can be part of various chemical reactions, including interacting with trace metals which can induce toxic effects (Franklin, et al., 2007).

These processes can take place for both ZnO and TiO_2. However dissolved ions and its processes have been implicated as the major mechanism driving toxicity of ZnO. TiO_2 toxicity effects are mostly related to oxidative stress by ROS (Minetto, Libralato, & Ghirardini, 2014); (Yung, Mouneyrac, & Leung, 2015). Most of these effects have been described to ZnO and TiO_2 in nano particle form. However there are few studies which implicate that non-nano particle ZnO and TiO_2 are resulting in similar toxicity effects (2.3.3).

2.3 Pollution to the marine environment

Information on toxicity data for various UV filters is gathered. To obtain this toxicity data, multiple toxicity and chemical databases are used to acquire data on the UV filters such as, databases from the US Environmental Protection Agency (EPA, 2018) and the European Chemical Agency (ECHA,

2008). Furthermore scientific reports that reported toxicity effects on the researched UV filters are used. For the complete toxicity databases, see appendix 5.

2.3.1 Toxicity data organic UV filters

Organic UV filters can be hazardous to the aquatic environment and can result in several types of impacts in the marine environment 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, Perez, Rodil, Quintana, & Beiras, 2014); (Downs, et al., 2015); (Bachelot, et al., 2012); (Fent, Kunz, & Gomez, 2008). Oxybenzone and octocrylene are studied the most. In addition, a toxicity database for oxybenzone and octocrylene is made, see appendix 5.1. The coral species *Pocillopora damicornis* is the most sensitive specie for oxybenzone at LC20 0.062 µg/l. The mollusc *Mytilus galloprovincialis* is the most sensitive specie for octocrylene at NOEC of 20 µg/l.

The following effects have been described:

- Genotoxicity; DNA damage to aquatic organisms by oxybenzone (Downs, et al., 2015).
- Endocrine toxicity; alteration of endocrine activities in the endocrine system, causing estrogenic disruption by oxybenzone, octocrylene, homosalate and other salicates (Fent, Kunz, & Gomez, 2008).
- Decreasing reproduction success by oxybenzone and octocrylene (Kim, Jung, Kho, & Choi, 2014).
- Developmental toxicity effects in zebra fish embryos by oxybenzone and octocrylene (Balázs, et al., 2016); (Blüthgen, Meili, Chew, Odermatt, & Fent, 2014).
- Phototoxicity by photo degradation of UV filters resulting in lipid, proteins and DNA damage by oxybenzone and octocrylene (Sánchez-Quiles & Tovar-Sánchez, 2015).
- Coral toxicity; organic UV filters induce coral bleaching (Figure 5) 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).

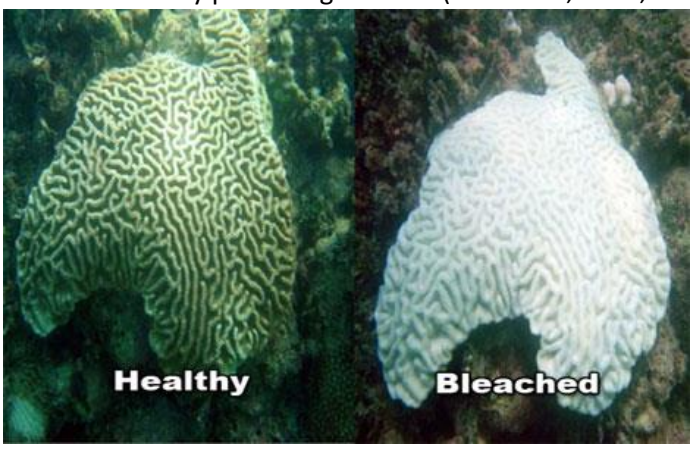

Figure 5 The effect of coral bleaching (Thompson, 2013)

- Coral toxicity; oxybenzone acts as a genotoxicant to corals and can also act as an endocrine disruptor (Downs, et al., 2015).
- Reef toxicity; declining populations in reef ecosystems by oxybenzone, octocrylene and salicates (McCoshum, Schlarb, & Baum, 2016).

- Disbalance of cycles; discharges of sunscreen ingredients can affect important cycles e.g. alterations in the nitrogen and phosphorus cycles. This can lead to eutrophication and algae blooms which can affect several coral species (Danovaro & Corinaldesi, 2003).

2.3.2 Toxicity data nano mineral based UV filters

Mineral based UV filters containing nano-ZnO and/or nano-TiO_2 are also known to be toxic for the marine environment. An overview of all the reported effects, including their effect concentrations, can be seen in appendix 5.2. The algae species *Thalassiosira weissflogii* is the most sensitive specie for nano-ZnO at NOEC of 0.01 mg/l. The freshwater crustacean *Daphnia magna* is the most sensitive specie for nano-TiO_2 at NOEC of 1 mg/l. The following effects are described:

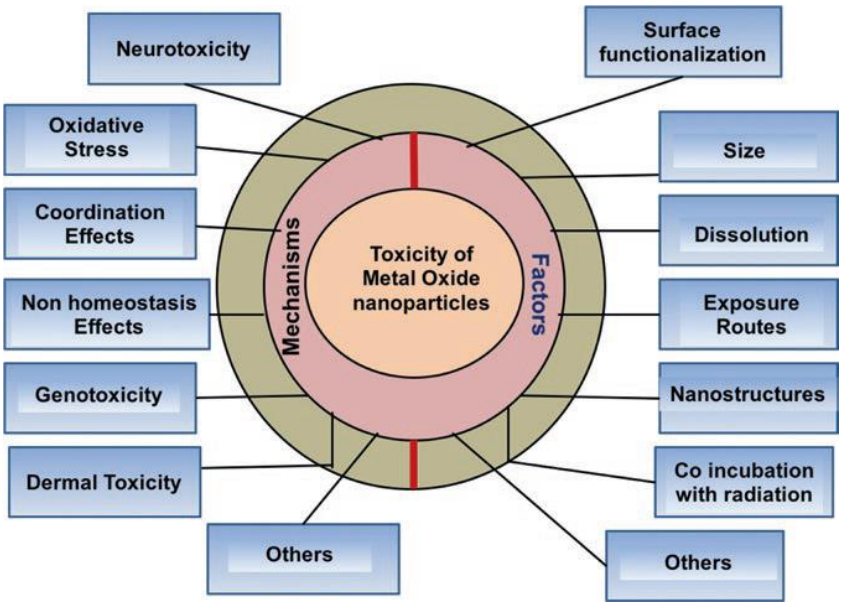

Figure 6 The toxicity mechanisms of metal oxide nano-particles (Thompson, 2013)

Several studies suggested that the generation of ROS as a likely toxic pathway of nano-ZnO to marine algae. None of those studies however measured ROS. The photocatalytic activity of nano-ZnO in seawater is still unknown as is the associated phototoxicity to the marine algae (Wong, Leung, Djurisic, & Leung, 2010); (Manzo, Miglietta, Rametta, Buono, & Di Francia, 2013b); (Miller, et al., 2010).

Nevertheless, growth inhibition is measured and reported in some studies. The median inhibition concentrations (IC50) of nano-ZnO to the algae species *Thalassiosira pseudonana* and *Skeletonema costatum* are 4.56 and 2.36 mg/L respectively (Wong, Leung, Djurisic, & Leung, 2010).

Due to the aggregates formed by nano-ZnO, benthic invertebrates, especially filter feeders and suspension feeders, face a higher exposure to nano-ZnO (Keller, et al., 2010). Several studies are conducted to study the bioaccumulation of nano-ZnO in bivalve molluscs including clams, oysters, and mussels. Exposure to oyster *Crassostrea gigas* caused an accumulation of zinc in gills and later in their digestive system. Mitochondrial damage and oxidative stress is observed, eventually resulting in mortality at LC50 of 37.2 mg/l (Trevisan, et al., 2014). Oxidative stress is also observed for *Artemia salina* crustaceans resulting in mortality at LC50 of >100 (Ates, Daniels, Arslan, Farah, & Rivera, 2013).

Exposure of zooplankton to nano-ZnO is associated with a number of harmful effects which are often linked to the physicochemical nature of the nanoparticles, such as the primary particle size, the

concentration of free zinc ions, and the aggregates of the nano-ZnO. Effect assessments for nano-ZnO on marine crustaceans seems to be species dependent. Copepods and amphipods seem to be more sensitive to nano-ZnO than brine shrimps. Some studies reported that toxic effects of nano-ZnO may also be occurring at environmentally realistic concentrations. For example, for the algae specie *Thalassiosira weissflogii* an EC20 of 0.07 mg/l is reported. (Jarvis T. , Miller, Lenihan, & Bielmyer, 2013); (Fabrega, et al., 2012); (Larner, et al., 2012).

TiO_2

Nano-TiO_2 has no obvious impact on the size and reproducibility of algal cells, but they cause a negative effect on algal photosynthesis at concentrations of 200 mg/l, and decrease at levels of 50 mg/l (Hu, et al., 2018). However, concentration dependent inhibitory effects with no significant differences amongst the sizes of nano-TiO_2 are found for several algal species (Clement, Hurel, & Marmier, 2013).

On the other hand, an EC50 for the loss in bioluminescence of *Vibrio fischeri* bacteria exposed to nano-TiO_2 is observed at concentrations of 650,6 mg/l (Lee, et al., 2008).

But much stronger toxicity effects are shown for rotifers, molluscs and fish. Size dependant toxicity effects are found for *Brachionus plicatilis* rotifers with an EC50 of 5.37, 10.43 and 267.3 mg/l at nanoparticle sizes of 15, 25 and 32 nm (Clement, Hurel, & Marmier, 2013). Larval malformations are found for the mollusc *Mytilus galloprovincialis* with EC50 concentrations of 1.23, 1.65, 16.39 and 38.56 mg/l (Libralato, et al., 2013).Toxicity effects are found for fish embryos (*Oryzias latipes*), including reduction of hatching time, altered swimming activities and malformations at concentrations of 0.7 mg/l (Paterson, Ataria, Hoque, Burns, & Metcalfe, 2010).

Overall it is observed that nano and non-nano TiO_2 shows weaker toxicity effects than nano and non-nano ZnO. From the toxicity databases there is one research that tested both ZnO and TiO_2, (Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008). The species *Vibrio fischeri* and *Daphnia magna* can be directly compared for both ZnO and TiO_2, see Table 1. This confirms that, following the same method and testing under the same conditions, nano-ZnO shows stronger toxicity effects than nano-TiO_2.

Table 1 Comparing toxicity effects of 2 species for both nano-ZnO and nano-TiO2

					Effects per UV filter in mg/l			
Taxonomy	Species	Tox effect	Effect type	Experiment time	Nano-ZnO	Nano-TiO2	Non-nano ZnO	Non-nano TiO2
Crustaceans (FW)	*Daphnia magna*	LC50	Mortality	48h	3.2	20000	8.8	
Bacteria	*Vibrio fischeri*	EC50	Growth inhibition	30min	1.9	>20000	1.8	>20000

2.3.3 Toxicity data non-nano mineral based UV filters

There are both nano and non-nano mineral based UV filters. The difference is the particle size, where a UV filter is considered as nano when the dimensions of the particles are smaller than 100nm and non-nano UV filters have particles bigger than 100nm (National Nanotechnology Initiative, 2018a).

Overall there is a relatively low number of studies that reported toxicity effects for non-nano-ZnO and TiO_2. An overview of all the reported toxicity data is presented in appendix 5.3.

For non-nano-ZnO the strongest toxicity effect reported is an 72 hour IC50 of 0.063 mg/l for the freshwater algae *Pseudokirchneriella subcapitata* (Franklin, et al., 2007). Keeping in mind that marine algae species show weaker toxicity with E/IC50 values ranging between 3 and 6.5 mg/l. Furthermore toxicity effects are reported for bacteria species at 1.8 mg/l and crustaceans species ranging between 0.24 and 8.8 mg/l. In general the sensitivity for non-nano-ZnO is crustaceans>bacteria>algae.

For non-nano TiO_2 the strongest toxicity effect reported is an 30 minutes EC50 of >20.000 mg/l for the bacteria *Vibrio fischeri* (Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008). The same sensitivity is reported for freshwater crustaceans (Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008). Apart from this one source no other toxicity effects are reported for non-nano TiO_2.

2.3.4 Toxicity data new organic UV filters

For new organic UV filters there is a relatively low number of studies that reported toxicity effects. An overview of all the reported toxicity data can be seen in appendix 5.4, which originates from classification and regulation reports and only represent freshwater species. No other scientific reports reporting (toxicity) effects are found for these UV filters. Furthermore all the available data resulted in effects that exceed the highest test concentration.

Due to this and the very limited data it isn't possible to accurately name a most and least toxic substance, even though both Uvinul A plus and T150 were tested at lower concentrations compared to the other new organic UV filters. Where the strongest toxicity reported for Uvinul A plus is an 34 days NOEC of >0.0088 mg/l for the freshwater fish *Pimephales promelas* (European chemicals agency, 2018). And the strongest toxicity reported for Uvinul T150 is an 21 days NOEC of ≥0.0001 mg/l for the freshwater crustacean *Daphnia magna* (Federal Institute for Occupational Safety and Health, 2016).

Furthermore for Tinosorb S and Mexoryl XL no environmental effects are reported. However there are some toxicity effects reported for Tinosorb M and Mexoryl SX. Where the strongest toxicity reported for Tinosorb M is an 21 days NOEC of >0.025 mg/l for the freshwater crustacean *Daphnia magna* (Federal Institute for Occupational Safety and Health, 2016). And the strongest toxicity reported for Mexoryl SX is an 96 hour NOEC of 100 mg/l for the freshwater fish *Oncorhynchus mykiss* (European chemical agency, 2018).

Moreover, the strongest toxicity reported for Ensulizole is an 72 hour NOEC of ≥100 mg/l for the freshwater algae *Pseudokirchneriella subcapitata* (European chemicals agency, 2018).

2.3.5 Conclusion toxicity data

Table 2 and Table 3 show an overview of the strongest reported toxicity data for the 11 UV filters of which a toxicity database is made. Table 2 is based on NOEC's and Table 3 is based on EC50's. The taxonomy and species is also given, including the type of effect and the reference. Furthermore the total amount of recorded NOEC or EC50 values is given per UV filter as "hits". It has to be kept in mind that the effects of the new organic UV filters, are reported as > or ≥, which indicates that the effect is shown at concentrations exceeding the highest tested concentration. This doesn't accurately represent the toxicity of these UV filters, nevertheless this is used because no other data is available.

Concluding the toxicity data it is observed that the organic UV filters oxybenzone and octocrylene relatively show the strongest toxicity. However both nano and non-nano ZnO also relatively shows strong toxicity, especially when comparing this to the toxicity of both nano and non-nano TiO_2, which shows much weaker toxicity. Furthermore it is observed that nano mineral UV filters show stronger toxicity than the non-nano counterparts for both ZnO and TiO_2, keeping in mind that there is less reported data available for non-nano UV filters than for nano UV filters.

Giving that there is limiting data and effects exceed the highest test concentrations, new organic UV filters show, especially at EC50 level, weak toxicity similar to that of non-nano TiO_2.

Table 2 Overview strongest toxicity of 11 UV filters based on NOEC's in µg/l

Type of UV filter	UV filter	Taxonomy	Species	Effect type	Strongest effect (ug/l)	Reference	Hits
Organic	Oxybenzone	Molluscs	*Mytilus galloprovincialis*	Larval development	30	(Paredes, Perez, Rodil, Quintana, & Beiras, 2014)	23
Organic	Octocrylene	Molluscs	*Mytilus galloprovincialis*	Larval development	20	(Girlado, et al., 2017)	6
Mineral	Nano-ZnO	Algae	*Thalassiosira weissflogii*	Growth inhibition	10	(Jarvis T. A., Miller, Lenihan, & Bielmyer, 2013)	18
Mineral	Non-nano-ZnO	Crustaceans (FW)	*Thamnocephalus platyurus*	Mortality	50	(Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008)	3
Mineral	Nano-TiO2	Crustaceans (FW)	*Daphnia magna*	Mortality	1000	(Lovern & Klapper, 2006)	11
Mineral	Non-nano-TiO2	Bacteria	*Vibrio fischeri*	Growth inhibition	>20000000	(Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008)	2
New organic	Uvinul A plus	Fish (FW)	*Pimephales promelas*	Malformations	≥8.8	(European chemicals agency, 2018)	6
New organic	Uvinul T150	Crustaceans (FW)	*Daphnia magna*	Mortality	≥0.1	(Federal Institute for Occupational Safety and Health, 2016)	3
New organic	Tinosorb M	Crustaceans (FW)	*Daphnia magna*	Mortality	>25	(Federal Institute for Occupational Safety and Health, 2016)	2
New organic	Mexoryl SX	Fish (FW)	*Oncorhynchus mykiss*	Mortality	100000	(European chemical agency, 2018)	1
New organic	Ensulizole	Algae (FW)	*Pseudokirchneriella subcapitata*	Growth inhibition	≥100000	(European chemicals agency, 2018)	4

Table 3 Overview strongest toxicity of 11 UV filters based on EC50's in µg/l

Type of UV filter	UV filter	Taxonomy	Species	Effect type	Strongest effect (ug/l)	Reference	Hits
Organic	Oxybenzone	Corals	*Pocillopora meandrina*	Calicoblast cells mortality	8	Downs et al 2016	22
Organic	Octocrylene	Invertebrates	*Paracentrotus lividus*	Mortality	737	Giraldo et al 2017	2
Mineral	Nano-ZnO	Algae (FW)	*Pseudokirchneriella subcapitata*	Growth inhibition	49	(Franklin, et al., 2007)	13
Mineral	Non-nano-ZnO	Algae (FW)	*Pseudokirchneriella subcapitata*	Growth inhibition	63	(Franklin, et al., 2007)	9
Mineral	Nano-TiO2	Molluscs	*Mytilus galloprovincialis*	Larval development	1230	(Libralato, et al., 2013)	27
Mineral	Non-nano-TiO2	Bacteria	*Vibrio fischeri*	Growth inhibition	>20000000	(Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008)	2
New organic	Uvinul A plus	Crustaceans (FW)	*Daphnia magna*	Mortality	>14.3	(European chemicals agency, 2018)	4
New organic	Uvinul T150	Algae (FW)	*Scenedesmus subspicatus*	Growth inhibition	>80000	(Federal Institute for Occupational Safety and Health, 2016)	4
New organic	Tinosorb M	Algae (FW)	*Scenedesmus subspicatus*	Growth inhibition	>2000	(Federal Institute for Occupational Safety and Health, 2016)	3
New organic	Mexoryl SX	Fish (FW)	*Oncorhynchus mykiss*	Mortality	>100000	(European chemical agency, 2018)	4
New organic	Ensulizole	Algae (FW)	*Pseudokirchneriella subcapitata*	Growth inhibition	>100000	(European chemicals agency, 2018)	1

3 Alternative sunscreen products

Due to the increase in awareness, research and knowledge about the effects of sunscreen ingredients on the marine environment and human skin health, alternative/eco-friendly products are introduced. These products claim to be reef safe, eco-friendly, free of oxybenzone, etc. Overall the knowledge of the claims and labels are still undetermined. Therefore this chapter describes which alternative products there are and what claims are being stated.

3.1 Products and their claims

Currently on the market there are many alternative sunscreen products available. To get a better understanding of all the types of "eco"-products available, including their active ingredients, a detailed overview list is made (appendix 1). This list is made by researching the current market (March 2018).

By reviewing the current market 91 different sunscreen products are reported which claim to be safe for the environment, see Table 4 for the overview.

In total 53% of the total products contain the UV filter non-nano-ZnO, and 16% of the total products contain non-nano-TiO_2, of which 21% of these products contain both UV filters.

Furthermore, 19% of the total products contain the UV filter (nano) ZnO and 14% of the total products contain (nano) TiO_2, of which 40% of these products contain both UV filters. For these products no specific particle size was reported so it could not be determined if nano or non-nano particles are used.

Moreover, 30% of the total products contain one or a combination of organic UV filters, of which 15% of these products also contain mineral based UV filters. Furthermore 9% of the total products still contain oxybenzone.

Table 4 Overview of the active ingredients used in eco-products

UV filter	Number
Non-nano ZnO	48
Non-nano TiO2	15
Combination	13
ZnO	17
TiO2	13
Combination	12
Organic UV filters	27
Organic + mineral	4
Oxybenzone	8

Continuing on this, many different claims are present in eco-friendly sunscreen products for which the exact meaning is undetermined. To make this more clear, the eco-labels and some of the claims of the products are described in more detail, according to what companies and industries state about this.

3.1.1 Eco labels

Ecocert:

The Ecocert label was the first certification label for cosmetica products that are completely eco- and bio-logical. Before it can be guaranteed that a product is eco-friendly and can get the Ecocert label, products have to follow several rules. Firstly 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).

Figure 7 Ecocert label (Naturalshop, 2018)

EWG verified:

EWG (Environmental Working Group) is an organization that performs ground-breaking research in personal care products if they are safe for human health and the environment. There is a whole database which lists and scores many 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).

Figure 8 EWG verified label (Environmental Working Group, 2018)

Beyond that EWG has "Skin Deep ratings". This is a list on the EWG website which provides information on ingredients in personal care product from the published scientific literature, to supplementing incomplete 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):

Sunscreen manufacturers can obtain the label of being a certified B cooperation. The manufacturers are certified by the non-profit B lab to meet rigorous standards of social and environmental standards. On their website certified manufacturers are given a score in multiple factors among which is the environment (B corperation, 2018). Although this label isn't directly referring to products being safe for the marine environment, it does indicate some aspects of the manufacturers taking the environment into account.

Figure 9 B certified corporation label (B corperation, 2018)

3.1.2 Claims

In general, most products that state a reference for their claims refer to the coral toxicity and bleaching researches of the Haereticus Environmental Lab of C. Downs (Haereticus Environmental Laboratory, 2018), or the scientific reports on oxybenzone and coral bleaching of R. Danovaro (Danovaro & Corinaldesi, 2003); (Danovaro, et al., 2008). 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. Some claims will be described in more detail.

Non-nano vs nano

The claim "non-nano" is stated many times in products that use mineral based UV filters. Although an explanation on why non-nano particles are used is most of the time not present. Nevertheless (Badger healthy body care, 2018) is one of the few products that claims to be non-nano and explains the reason behind the products being non-nano.

"*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).

Additionally, many mineral sunscreens use nano sized ZnO because it is less whitening and therefore more aesthetically appealing than larger particle zinc oxide. Although customers have insisted that they don't want nanoparticles in sunscreens. Badger figured out a method of working with larger particle ZnO that allows them to use a minimal amount of ZnO with minimal whitening effect. Badger would rather not use nanoparticles if they don't need to because of the health issues and their potential environmental concerns (Badger healthy body care, 2018).

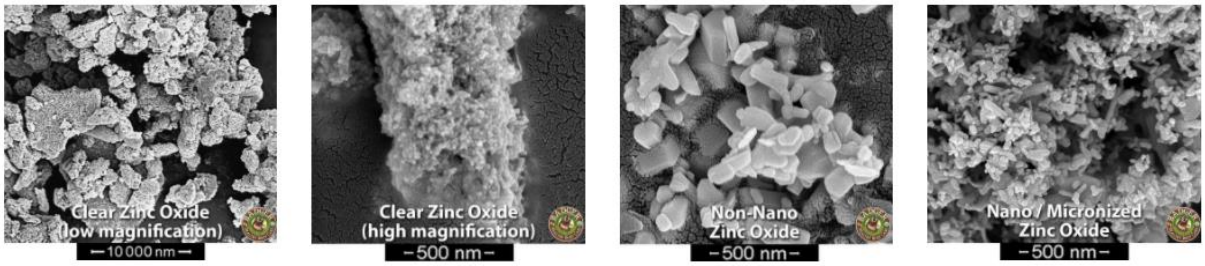

Figure 10 Different zinc oxide particles (raw zinc oxide) at microscopic level (Badger healthy body care, 2018).

To sum up the differences in nano, non-nano and clear particles, see Figure 10. Clear zinc oxide at low magnification is composed of large aggregates ranging between 500 and 9000nm. At high magnification the aggregates show smaller nanoparticles fused together into a larger particle, without any loose nanoparticles. Furthermore, non-nano zinc oxide shows to contain only few particles smaller than 100nm. Most particles appear to be in the range of 100-500nm. Finally, nano zinc oxide is made of a variety of smaller particle sizes, where there certainly are particles smaller than 100nm, classifying it as nano (Badger healthy body care, 2018).

Another explanation: "*When ingredients are uncoated and nano-size (less than 35nm in diameter), they can enter the cells of invertebrates and cause oxidative stress in sunlight. This blows up the cells so they die. Your best bet is to go for non-nano zinc oxide larger than 150nm. At that point, the toxicity drops off and there is no threat"* (Raw elements, 2018).

Mineral based

Almost all products who claim to be 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. Most of the time the claim mineral based is referred to these UV filters being more safe and more effective against UV rays. And only a few products also refer mineral based to being better for the environment compared to chemical UV filters. Furthermore, some products describe that to ensure a broad spectrum coverage it is better to use sunscreen products that use mineral based active ingredients, instead of chemical UV filters, see Figure 11 (Badger healthy body care, 2018); (Beyond coastal, 2018).

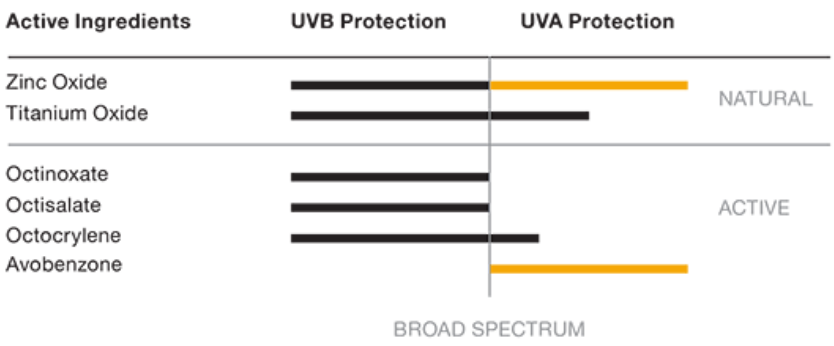

Figure 11 Active ingredient required to ensure broad spectrum coverage (Beyond coastal, 2018).

(Raw elements, 2018) adds to this by means of their claim that mineral based active ingredients (ZnO and TiO_2) are more effective against UV rays, because they have a broader protection spectrum for both UVA and UVB. They say that zinc oxide is the only FDA approved broad spectrum UVA/UVB protection ingredient. Continuing on zinc oxide, (Raw elements, 2018) says that zinc oxide won't degrade or lose potency over time, won't produce the skin sensitivities and rashes, or initiate the creation of free radicals or hormone disruptors commonly caused by chemical sunscreens.

Reef safe

The claim reef safe or coral reef friendly is the most stated claim on alternative products. Many products say that 14,000 tons of sunscreen washes off swimmers, scuba divers and snorkelers into coral reef environments each year, see Figure 12. That's a big problem because common chemical sunscreen ingredients such as oxybenzone, butylparaben and octinoxate can bleach and seriously damage coral reefs, referring to the research of (Downs, et al., 2015) which is about coral bleaching due to chemical UV filters (Alba Botanica, 2018); (Badger healthy body care, 2018); (Beyond coastal, 2018); (Raw elements, 2018).

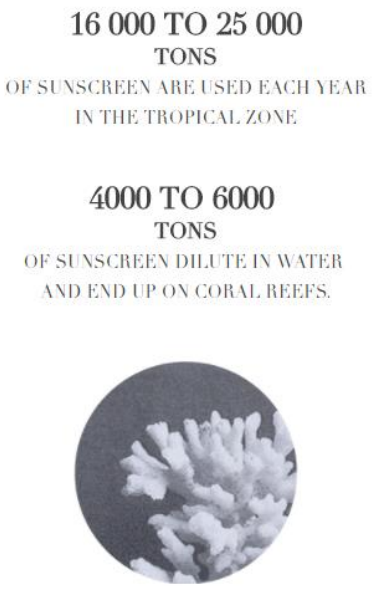

Figure 12 sunscreens end up in the marine environment (EQ love, 2018)

(Raw elements, 2018) says that the problem originates from the fact that many people are unknowingly using sunscreens that damage corals. They say that there is so much misinformation and little regulations on the terminology. They say that words like natural, eco-safe or reef safe are in the description of very toxic products. Furthermore they claim that some brands add minerals or organic ingredients into the products, distracting from dangerous active ingredients. To help the customers choosing the right products they made a list of cautionary ingredients that are many times not allowed in eco-marine reserves. They are not allowed because they have a negative effect on corals from damaging DNA to bleaching. Furthermore they say that one or more of the ingredients listed are in over 90% of all sunscreens on the market. The list includes; avobenzone, octocrylene, titanium coated in aluminium, oxybenzone, octyl salicate, homosalate, nano particles, octinoxate, padimate O/PABA and many parabens.

To indicate if a sunscreen is really reef/coral safe (Badger healthy body care, 2018) considers the following:

- Look at the active and inactive ingredients on the sunscreen product. Avoid using products containing oxybenzone or other ingredients listed by the Haereticus Laboratory to be harmful to coral reefs.
- Don't instantly believe reef safe or reef friendly claims on products. Claims are unregulated and therefore could be incorrect. Look at the ingredients and judge for yourself.
- Use a water resistant sunscreen, which will be more likely to stay on your skin and out of the water.

Similar as Raw elements, (AllGoodproducts, 2018) considered the following for their sunscreen products to be reef safe;

Figure 13 Reef friendly criteria (AllGoodproducts, 2018)

These criteria are obtained by referring to several sources which are articles that state the research of (Downs, et al., 2015).

Eco-friendly:

Many products claim to be eco-friendly without proper explanation. The claim eco-friendly can be referred best to products and industries being green in production, manufacturing, carbon footprint reduction etc. Instead of 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, without any further explanation on their website. Although some products give some explanation.

A good example is (Alba Botanica, 2018) who claims that when you see a biodegradable claim on their label, it means the formula has been tested by an accredited third party laboratory in accordance with industry standard methodology for biodegradability claims. These formulas meet the required standards necessary for “Biodegradable Certification”. This means the formula is designed to break down in nature with minimal impact on the earth.

No oxybenzone and/or octinoxate:

Multiple products state that their products doesn't contain the UV filters oxybenzone and octinoxate. Where especially oxybenzone is known to have effects on marine organisms (2.3.1). 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.

4 Bioassays; method

To study the relative toxicity of the selected sunscreen products, lab experiments were performed. This was done with 4 bioassays. The bioassays include a micro toxicity (acute) test, algae growth inhibition test, rotifer acute (mortality) toxicity test and rotifer chronic (reproduction) toxicity test. These bioassays, including their method, are briefly explained in this chapter. The sunscreen products tested with these bioassays include 7 products using a variety of UV filters. In paragraph 4.1 these products are further explained, including why they are chosen. Furthermore, this chapter includes a description of the boundary conditions and the reference tests.

4.1 Tested products

For the lab experiments a total of 7 products is selected to test their potential and relative toxicity. The products are selected based on their different UV filters, (Table 5), and products from all 3 different UV filter groups are chosen to compare potential and relative toxicity for all available sunscreen groups.

The products which claim to be eco-friendly are coloured green and the other products aren't. According to (Environmental Working Group, 2018) the UV filters are given a score based on their overall hazard ranging from green (low hazard), yellow (moderate hazard) and red (high hazard). Regarding the respect towards the sunscreen producers, the names of the tested products are classified and therefore numbered A till G.

Table 5 Overview of UV filters of the selected products

		Sunscreen products						
Group	**UV filter**	**Product A**	**Product B**	**Product C**	**Product D**	**Product E**	**Product F**	**Product G**
Organic (chemical)	Avobenzone	x	x					
	Homosalate	x	x					
	Octisalate	x						
	Octocrylene	x	x					
	Oxybenzone	x						
Inorganic (mineral based)	ZnO				x			
	ZnO (nano)			x				
	TiO2			x	x	x		
New organic	Ensulizole						x	
	Uvinul A plus						x	x
	Uvinul T150						x	x
	Tinosorb M							x
	Tinosorb S						x	

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, which is known to be toxic (2.3.1). 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 nano-ZnO. The product claims to be paraben free and biological and has the ecolabel of ecocert (3.1.1). The principles of this label indicate that a product needs to be free of nano particles. However, product C uses nano zinc oxide as one of the active ingredients. Furthermore, nano particles are known to cause effects for marine organisms (2.3.2). 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 show 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

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 to test if their claims on being eco compatible are true. 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.

4.2 Boundary conditions

Before performing the bioassays, some boundary conditions are set up which will be explained in this chapter.

Concentration series

To test the different sunscreen products in the bioassays a concentration series is used, which is determined from the range finding experiments performed beforehand. See appendix 3 for the results. Knowing the relevant concentration ranges, the concentrations used in the bioassays are made based on log scales, because calculations on concentrations series based on a log scale are more accurate. The concentration series is obtained via dilution of a stock solution. This stock solution is made according to the protocol described in appendix 2. Weighing each individual sunscreen product to calculate the concentration of which a stock solution is made.

Water quality parameters

To make sure the circumstances in the stock solutions of all sunscreen products are similar, some water quality parameters are measured before the samples are taken for the bioassays. More specifically, pH, oxygen, salinity and temperature are measured. This is done using the "Mettler Toledo" pH meter (pH and temperature), and the "HQ40d multimeter" (oxygen, salinity). If any major differences occur between the different stock solutions this is taken into account during the result analysis. The conditions for the parameters are, pH of ± 8, oxygen of ± 8 mg/l, salinity of ± 32,5 ‰ and temperature between 20-25 ^{o}C, which are based on the conditions stated in the methods of the bioassays.

Reference toxicant

To make sure that the experiments are performed correctly by the operator and to make sure the used organisms are in representable shape a reference toxicity test is performed for all the bioassays. This is done with the toxicant potassium dichromate ($K_2Cr_2O_7$) and with fenol for the micro toxicity test. These toxicants are as described in the standard test protocol and are performed following the method description. If the reference toxicity test is not according to the standards, the test needs to be redone.

4.3 Micro toxicity test (*Vibrio fischeri*)

Bacteria are important for the nutrient cycle in ecosystems (Waggoner & Speer, 2006). A lot of research has been performed with the micro toxicity test and the outcome was that *V. fischeri* is sensitive to a broad spectrum of toxicants. Therefore this 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 (Figure 14), 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 (appendix 3) 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.

Figure 14 Micro toxicity analyser (Lab&process, 2018)

4.4 Algae growth inhibition test (*Skeletonema constatum*)

Algae are very important organisms in ecosystems. They are primary producers, so they form the basis of the aquatic food web (Kolak, 2017). So it is relevant to determine if eco-friendly UV filters have any toxicity effects on marine algae. Therefore 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 (Table 6), *S. constatum* is exposed to the different sunscreen products, over a period of 96 hours. Based on the range finding experiment (appendix 3.2) 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 (Figure 16). The way the plate is filled is as following; each test concentration has 8 replicates, apart from the blanc which has 16 replicates. Furthermore 4 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 (Figure 15) 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 6 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

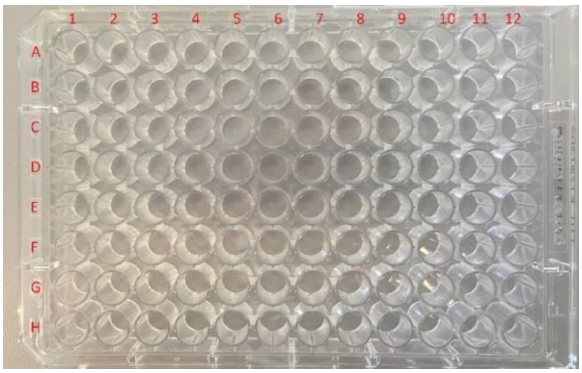

Figure 16 Algae growth inhibition test plate setup

Figure 15 Biotek microtiter plate reader Fx800

4.5 Rotoxkit M, acute and chronic test (*Brachionus plicatilis*)

Rotifer tests are widely used as indicators for toxicity. This because rotifers are ecologically important primary consumers of many aquatic communities. The *B. plicatilis* have a wide distribution and are found in diverse aquatic habitats on all continents. Furthermore the life cycle and size of rotifers makes them well suited for toxicity experiments (Wells, Lee, & Blaise, 1997). Therefore both an acute/mortality and chronic/reproduction 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 for this bioassay in the range finding tests and are therefore excluded in the final tests. For the Rotoxkit M chronic test some products are only tested for 1 or 2 concentrations (limit test). This is done because no strong effect were observed for these products in the range finding tests, see appendix 3.4.

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 of the *B. plicatilis* is measured at 24 and 48 hours, by counting the alive and death organisms (mortality) 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.

The general protocol for the chronic/reproduction test is similar as for the acute/mortality test. But for this test the reproduction of *B. plicatilis* is tested instead of mortality. Each concentration has 8 replicas instead of 6, see Figure 18. Each test well only has 1 *B. plicatilis* instead of 5 and all test wells are provided with *Phaeodactylum tricornutum* algae, serving as food for the *B. plicatilis*. And the chronic test lasts 6 days, counting the *B. plicatilis* after the 1st day, to check if there is one *B. plicatilis* in each test well, and after the 6th day, to count the reproduction of *B. plicatilis*. It has to be taken into account that the chronic/reproduction test is made based on the acute/mortality rotifer test of (MicroBioTests Inc., 2018), but is not yet an official toxicity test.

Table 7 Concentration series Rotoxkit M 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

Table 8 Concentration series Rotoxkit M chronic

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)	5.5	3.2	75.0	150.0	150.0	3.8	75.0
C2 (µl/l)	8.8	5.0	150.0	150.0	150.0	5.3	150.0
C3 (µl/l)	13.9	8.0	0.0	150.0	150.0	7.5	0.0
C4 (µl/l)	22.1	12.6	75.0	150.0	150.0	10.6	75.0
C5 (µl/l)	35.0	20.0	150.0	150.0	150.0	15.0	150.0

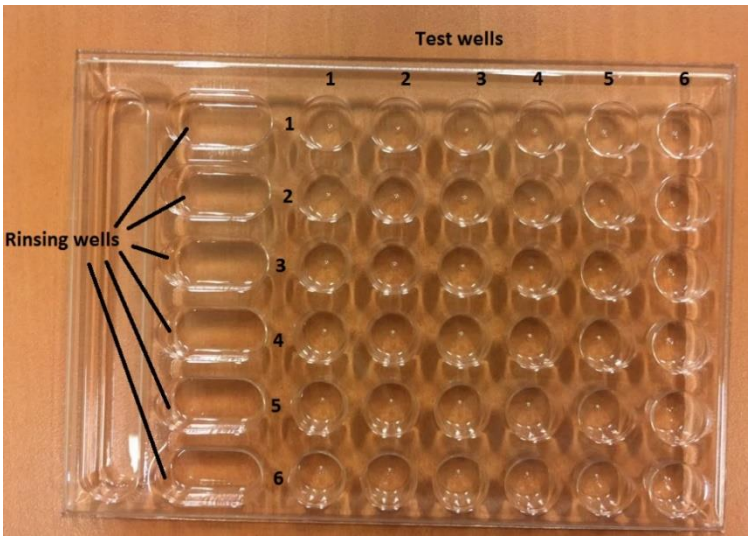

Figure 17 Overview acute rotifer test plate

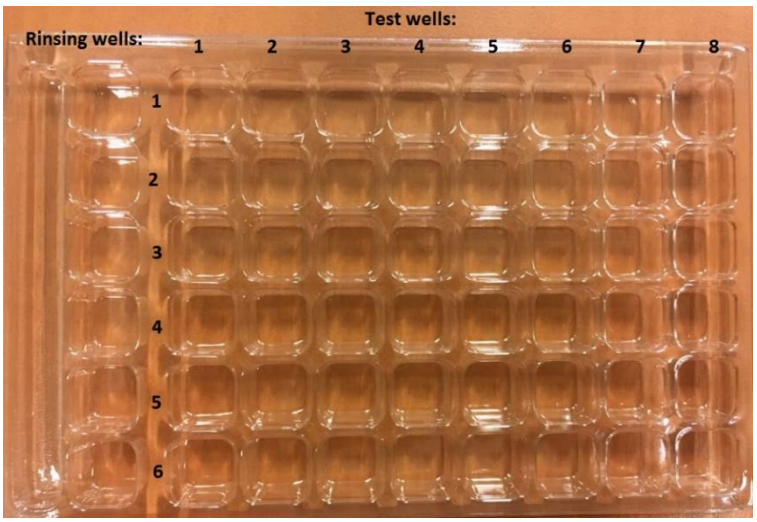

Figure 18 Overview chronic rotifer test plate

4.6 Reporting endpoints

When the reported luminescence inhibition, mortality, reproduction inhibition and growth inhibition of all bioassays was strong enough, the data is translated to EC50's. This is done by using excel sheets, as described in the methods, where the mortality/inhibition values are inserted and used to calculate the concentration where 50% effect is reached, resulting in the EC50. Sometimes the effect doesn't reach 50%, but it is still possible to calculate the EC50 based on extrapolated (estimated) data. However this is less accurate, so therefore 95% confidence intervals are included as well, to indicate how accurate the calculated EC50 is. The bigger the interval range, the less accurate the calculated EC50.

For some tests the effect wasn't strong enough to calculate an EC50, so therefore the effect is noted as 'exceeding the highest test concentration' and the highest mortality or inhibition percentage is noted. Furthermore, resulting from making the concentrations series, it was concluded that concentrations higher than 200 µl/l can't be tested because of maximum solubility of the sunscreen products.

The end result is comparing all the products based on their relative toxicity for all the bioassays to determine which products show the strongest and weakest relative toxicity.

5 Bioassays; results and discussion

This chapter describes the results and discussion of the performed bioassays. First the general performance of the bioassays is described and discussed, after that a summary of the combined end result is given, and the results of each sunscreen product will be described and discussed individually. After that the results are compared to the toxicity data and a conclusion on the bioassays is derived. For the detailed results, see appendix 4.

5.1 Performance of the bioassays

For all bioassays reference tests are performed. All EC50's are within the range, apart from the Rotoxkit M chronic test, because no known effect range for the reference is known yet. Nevertheless it can be concluded that all bioassays are performed correctly, because the procedure of making the concentration series and transferring the rotifers is the same as for the Rotoxkit M acute test, which is performed correctly. Therefore it can be concluded that the chronic test is also performed correctly, according to the method.

Before every bioassay water quality parameters of the stock solutions are measured to make sure that the circumstances are the same. Quality parameters met the criteria in all performed bioassays (Table 9).

Table 9 Summary of the water quality parameters

Parameter	**pH**	**Oxygen (mg/l)**	**Salinity (‰)**	**Temperature (°C)**
Average	7.95	8.19	33.1	23.4
Criteria	± 8	± 8	± 32.5	20-25

When preparing the stock solutions it is observed that the solutions are not completely homogenous. Especially at higher concentrations undissolved particles remain in the stock solution (Figure 19). Possible causes are that some substances in the sunscreen products don't completely dissolve because of their high logK_{ow} or low water solubility, or some substances form agglomerates (2.2). To prevent that the test concentrations are influenced by this phenomenon and because it is expected that the big undissolved particles can't be taken up by the tested organisms, it is made sure that these undissolved particles are taken up as little as possible. Although it has to be kept in mind that initial concentrations in the test series can variate because of this.

Figure 19 Example of a stock solution that is not homogenous

For the general performance of the bioassays, some discussion points are noted;

Micro toxicity; 95% confidence interval

It is observed that within the micro toxicity test the EC50 values have a big 95% confidence interval. This is because for all products, apart from product F, the effect doesn't reach 50%. Therefore the EC50 is calculated from extrapolated data and is estimated, which explains this big interval. So the accuracy of the EC50 is questionable.

Micro toxicity; normal vs centrifuged

As explained in 4.3, within the micro toxicity test both normal and centrifuged samples are tested. No big differences in effects are observed comparing the two samples. So the hypothesis that by centrifuging, more particles will dissolve and thereby influences the toxicity, can be rejected.

Rotoxkit M acute and chronic; physical effects

In the Rotoxkit M acute test physical effects are observed, especially for product A and B. Where with most tested substances, including the reference test, death rotifers lay on the bottom of the test well, now dead rotifers are found in the fat/oil top layer of the water column. The rotifers could have gotten stuck in this layer, where they are not able to breathe and move, eventually leading to death. When this effect is a big portion of the total effect, it overrules the potential toxicity effect.

Even though this is another type of effect, this can still be translated as a field relevant effect. Fat and oil layers tend to coagulate in the top layer of the test wells, forming a film. Translating this to the field, these substances could end up in the SML of the water column, which is reported and could cause effects in the environment (2.2).

Rotoxkit M chronic; variations in the test results

Overall it is observed that the results for the Rotoxkit M chronic test variate a lot, which is the case for all products. This can be because this test is not yet finalised. Observations suggest that possible causes of these variations can be difference in age of the rotifers before exposure and differences in irregular reproduction success between different controls and toxicants.

It was also observed that in the control wells, quite a lot of mortality was present. This was the case for most sunscreen products. To not influence the mean reproduction value of the control, these zero values were taken out of the calculation. A possible explanation for this is that errors are made while filling the test plates.

Overall there are multiple factors that have influenced the results of the chronic rotifer bioassay. Even though some results were reported for this bioassay, it is possible that the results don't accurately represent the toxicity of the sunscreen products, hence more testing is required when the test is finalised.

5.2 Test results of the bioassays

Table 10 (next page) shows the combined end result of all the sunscreen products (on the left) for all the conducted bioassays (at the top). For the detailed results see appendix 4. If a significant toxicity effect is reported, an exact EC50 in (µl/l) is calculated. When this isn't possible the EC50 is noted as exceeding the highest tested concentration. In between brackets the 95% confidence interval of the EC50 is given. For the micro toxicity the actual intervals aren't reported, because the EC50's are calculated as a mean from 2 replicates, resulting in multiple intervals. Therefore the intervals are only shown in appendix 4.1.

Table 10 EC50 in (µl/l) for the bioassays

	Bioassays EC50 in µl/l (95% CI)					
Product	**Microtox**	**Microtox centrifuged**	**Rotifer acute**	**Rotifer chronic**	**Algae 1day**	**Algae 4days**
Product A	138	107	38.06 (31.4-46.1)	>35	16.1 (10.9-23.6)	27.5 (16.6-45.5)
Product B	>90	180	3.93 (>>>)	6.7 (0.9-50.4)	69.9 (54.9-88.9)	>100
Product C	>90	>90	>150	>150	21.5 (18.0-25.6)	18.9 (16.7-21.3)
Product D	>90	>90	>150	>150	127 (111.9-144.1)	94.6 (74.1-120.7)
Product E	>90	>90	>150	>150	>200	>200
Product F	23	44	>150	1.97 (>>>)	>125	>125
Product G	235	242	>150	>150	>200	>200

Product A

Product A shows relatively strong effects at all bioassays compared to other products, apart from the Rotoxkit M chronic test. The effects are dose-response related and EC50's could be calculated. The algae growth inhibition test with 1 day stock shows the strongest effect at an EC50 of 16.1 µl/l and the Rotoxkit M chronic test shows the weakest effect at an EC50 of >35 µl/l, where the maximum reported reproduction inhibition is 15%. These effects can be explained by the fact that product A contains substances that are known to cause effects on marine organisms (2.3.1).

Micro toxicity

The micro toxicity test shows luminescence inhibition effects at anEC50 of 138 and 107 µl/l, which are dose-response related, but decrease over time. So the toxicity can be explained as an acute effect on *V. fischeri* bacteria, which, to some extent, re-establish over time, see appendix 4.1.

Rotoxkit M acute

For the Rotoxkit M acute test product A is one of only two products that show mortality effects on *B. plicatilis* rotifers, with an EC50 of 38.06 µl/l. Although it has to be kept in mind that some of the mortality measured is because of physical effects instead of toxicity effects, explained in 5.1. This slightly influences the results of product A.

Rotoxkit M chronic

In the Rotoxkit M chronic test product A shows no reproduction effects with an EC50 of >35 µl/l. 15% is the strongest measured reproduction inhibition. But as described in 5.1 the results variate a lot and differ from previous range finding tests, as can be seen in Figure 20. Comparing the two overlapping concentrations (25 and 35 µl/l), huge differences between the two test results are observed.

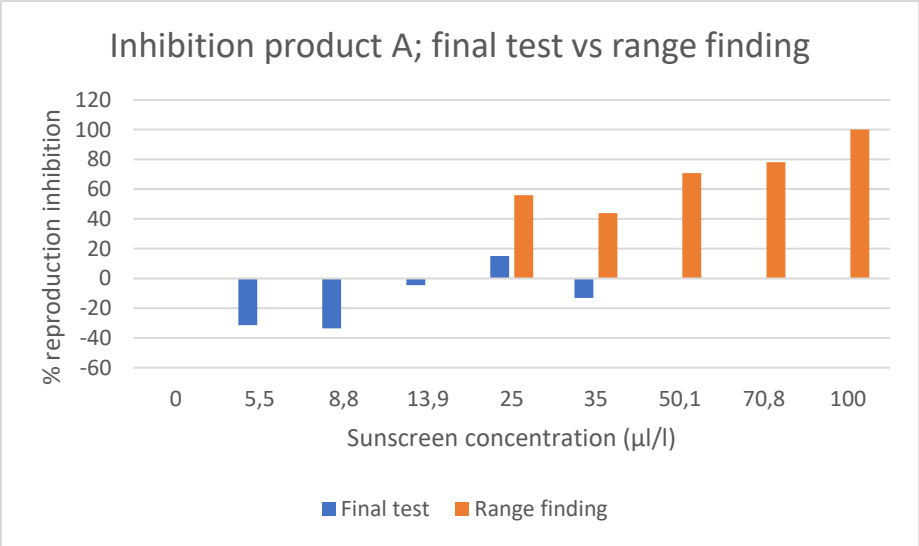

Figure 20 Rotoxkit M chronic reproduction inhibition results of 2 tests

The range finding test shows way higher mortality rates than the final test result. This can be caused by the fact that overall the range finding test shows very low reproduction success, already starting in the control. And because, for the end result the reproduction success of the test concentrations is compared to the control, the test is more vulnerable to small changes in reproduction. On the other hand the final test shows very high reproduction success. This causes lots of variation within the test, where the extremes will strongly influence the inhibition values. Giving a possible explanation on the difference between the two tests. So even though no effects are shown in the final test, it is possible that this isn't an accurate result. When this bioassay is finalised, more testing should give a more accurate insight in the effects of product A.

Algae growth inhibition test

The algae growth inhibition test shows growth inhibition effects with an EC50 (1day) of 16.1 and an EC50 (4days) of 27.5 µl/l, which decrease over time and also decrease with "older" stock solution. The decrease in effect with older stock solution can be explained by the fact that substances agglomerate and attach to the Erlenmeyer's over time, which could have decreased the initial concentration of the stock solution. Another possible cause is that the mixture of sunscreen chemically reacts and changes over time. Resulting in substances becoming, in this case, less bioavailable.

Product B

Product B shows relatively strong effects at the Rotoxkit M acute and chronic test and the algae growth inhibition test 1 day stock, compared to other products. The effects are dose-response related and EC50's could be calculated. The Rotoxkit M acute test shows the strongest effect at an EC50 of 3.93 µl/l and the weakest effects are observed in the micro toxicity at an EC50 of >90 µl/l with a maximum luminescence inhibition of 11.23% and 4 days algae test with an EC50 of >100 µl/l with a maximum growth inhibition of 37%. The effects can be explained by product B containing substances that are known to cause effects for marine organisms, such as octocrylene, see 2.3.1 and appendix 5.1.

Micro toxicity

For the micro toxicity test the luminescence inhibition effect is weak, but dose-response related at the beginning of the test. This decreases over time results in a weak effect which is the same for all concentrations after 30 minutes of exposure. So the effect can be explained as an acute effect, which decreases over time and eventually ceases away. In addition, the 95% confidence interval is very high, explained in (5.1).

Even though for the centrifuged sample an EC50 could be calculated, the effect is almost the same as for the normal solution, see appendix 4.1. The only difference being that the effect doesn't completely cease away over time. A possible explanation can be that due to centrifuging more substances become available for *V. fischeri*, which could have affected the toxicity. Although this only results in a minor difference.

Rotoxkit M acute

The Rotoxkit M acute test shows relatively strong mortality effect, compared to other products. The effect is, however, uniform for all concentrations (Figure 21). It is observed that physical effects are the cause of the mortality instead of an aquatic toxicity effect. Different from product A this effect is even stronger for product B, overruling any possible toxicity effect. Therefore the mortality caused by physical effects is reported during the bioassay and, if withdrawn from the initial mortality values, almost no mortality remains (Figure 22). Therefore the effect of product B on *B. plicatilis* can be described as a physical effect.

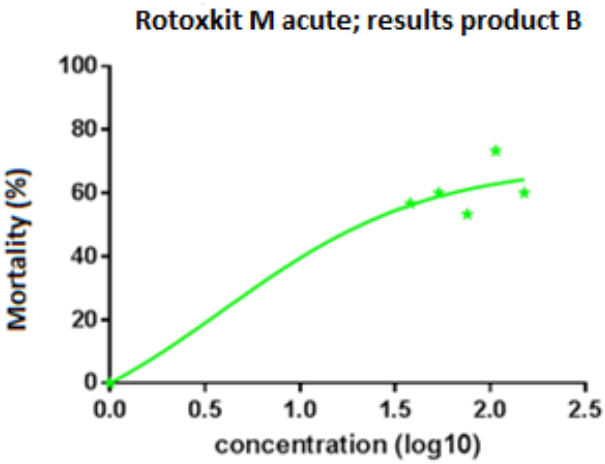

Figure 21 Rotoxkit M acute test results product B with log10 concentration

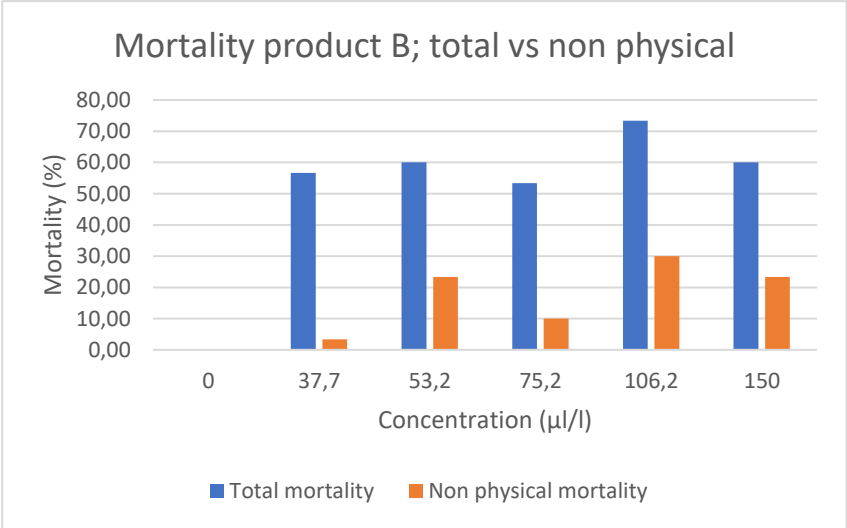

Figure 22 Mortality product B; comparing the total and the non-physical effect

Algae growth inhibition

The algae growth inhibition test shows a relatively strong growth inhibition effect with the 1 day stock, whereas the 4 days stock shows very weak inhibition effect. This stronger growth inhibition in the 1 day stock can be seen in Figure 23, which compares the two highest concentrations, C4= 56.2 µl/l and C5= 100 µl/l, for the 1 day and 4 days stock.

The weaker observed effect with older stock solution can be explained by the fact that substances agglomerate and attach to the Erlenmeyer's over time. This could have decreased the initial concentration of the stock solution. Another possible cause is that the mixture of sunscreen

chemically reacts and changes over time. Resulting in substances becoming, in this case, less bioavailable.

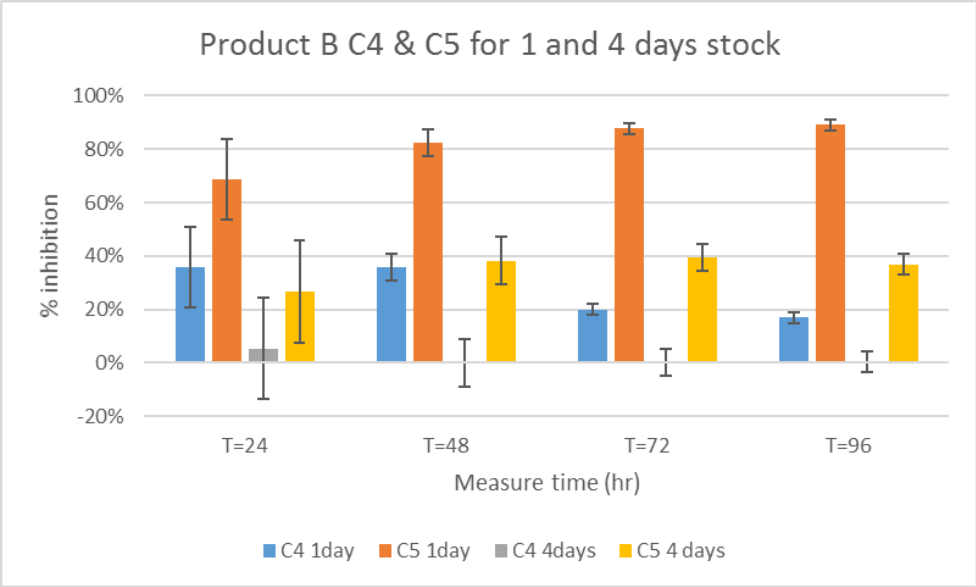

Figure 23 Decrease in growth inhibition with older stock for the algae growth inhibition test

Product C

Product C only shows relatively strong effects in the algae growth inhibition test, compared to other products, with an EC50 (1day) of 21.5 µl/l and an EC50 (4days) of 18.9 µl/l. A relatively weak effect at the Rotoxkit M chronic test is observed, for which no EC50 could be calculated. Even though the maximum effect is 72% reproduction inhibition, the test is only performed for 2 concentrations. The other bioassays show no effects with EC50's exceeding the highest test concentration. The micro toxicity test shows a maximum luminescence inhibition of 7.30% and the Rotoxkit M acute test shows a maximum mortality of 17%. The effects can be explained by product C using nano-particle ZnO, which is known to cause effects for marine organisms (2.3).

Rotoxkit M acute

For the Rotoxkit M acute test product C shows no mortality effect towards *B. plicatilis* rotifers. Apart from two random test wells which show 100% mortality, see appendix 4.2. But because they are random they can be neglected.

Rotoxkit M chronic

For the Rotoxkit M chronic test product C shows a weak reproduction effect compared to some other products which show no effect. Although this effect has only been observed for concentrations of 75 and 150 µl/l, compared to other products who show the same toxicity at concentrations <35 µl/l, see appendix 4.3.

Algae growth inhibition

For the algae growth inhibition test product C shows a relatively strong growth inhibition effect, compared to other products. The effect is already present after the 24h measurement, is dose-responsive (Figure 24) and doesn't change a lot over time, so it can be described as an acute effect.

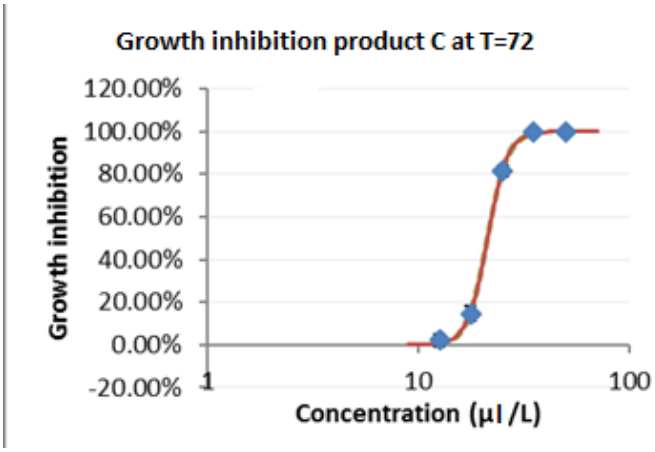

Figure 24 Dose-response curve product C for the algae growth inhibition test at T=72

Different from product A and B, the growth inhibition effect becomes stronger comparing the 1 day with 4 days stock solutions. A possible explanation for this is that ZnO and TiO_2 particles can undergo modifications which can influence the interaction with organisms in the marine environment, potentially increasing their bioavailability and/or toxicity (2.2).

Product D

Product D only shows a relatively weak growth inhibition effect, compared to other products, at the algae growth inhibition test with an EC50 (1day) of 127 µl/l and an EC50 (4days) of 94.6 µl/l. For the other bioassays no relative effects are observed, with EC50's exceeding the highest test concentration. The micro toxicity shows a maximum luminescence inhibition of -12.02%, the Rotoxkit M acute test shows a maximum mortality of 3% and the Rotoxkit M chronic test shows a maximum reproduction inhibition of 35%.

Algae growth inhibition

For the algae growth inhibition test a weak growth inhibition effect is observed, even though the effect only increases at the highest concentrations. Although, for the 1 day stock, this is only observed at 200µl/l compared to other products which show the same effect at much lower concentrations.

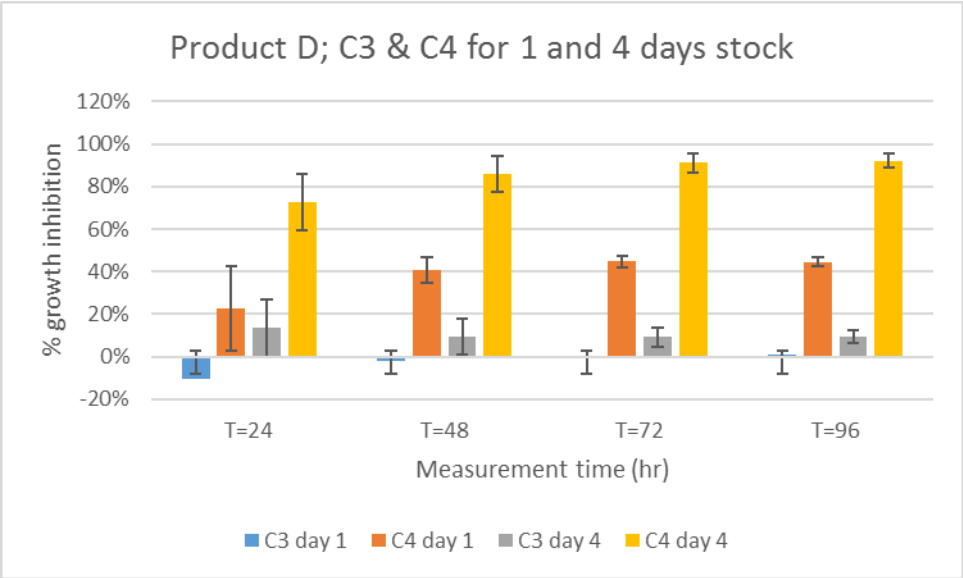

Figure 25 Increase in growth inhibition effect comparing 1 and 4 days stock solutions

However, the effect of the 4 days stock has significantly become stronger compared to the 1 day stock. This is clearly shown in Figure 25, where concentration C3 (79.6µl/l) and C4 (126.2µl/l) are compared to each other for both 1 day and 4 days stock solutions. A possible explanation for this increase is that ZnO and TiO_2 particles can undergo modifications which can influence the interaction with organisms in the marine environment, potentially increasing their bioavailability and/or toxicity (2.2).

Product E

Product E doesn't show any relative effects, compared to other products, for all the bioassays. EC50's are exceeding the highest test concentrations with maximum effects of -11.50% luminescence inhibition for the micro toxicity test, 3% mortality in the Rotoxkit M acute test, 42% reproduction inhibition in the Rotoxkit M chronic test and 47% and 38% growth inhibition for the 1 day and 4 days algae growth inhibition test.

Algae growth inhibition

Only some effects are observed for the algae growth inhibition test, which shows 47% growth inhibition effect at the highest concentration of 200µl/l, for the 1 day stock solution. However, no EC50 could be calculated, see appendix 4.4.

It is, however, interesting to note that product E, showing a relatively weaker effect at the algae growth inhibition test compared to other products, the effect does not increase comparing the 1 day and 4 days stock solutions. While it contains mineral based active ingredients who typically show this effect, like product C and D. This is probably because this increase in effect is only shown when a stronger toxicity effect is shown.

Product F

Product F does show relatively strong effects, compared to the other products, for the micro toxicity test with an EC50 of 23 and 44 µl/l and the Rotoxkit M chronic test with an EC50 of 1.97 µl/l. The Rotoxkit M acute test shows no relative effects with a maximum mortality of 13%. Furthermore a relatively weak effect is observed for the algae growth inhibition test, of which no EC50 could be

calculated, with a maximum growth inhibition of 58% and 66%. The active ingredients of product F are not known to show toxicity effects in aquatic organisms (2.3.4). The toxicity effect of product F is probably caused by other inactive ingredients.

Micro toxicity

For the micro toxicity test product F shows an acute effect which is dose related, but as for all the products, decreases significantly over time (Figure 26). The effect is plotted against the concentration in %, at measurement times of 5, 15 and 30 minutes.

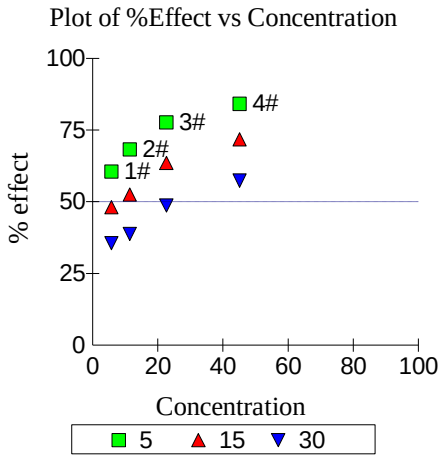

Figure 26 Product F micro toxicity luminescence inhibition effect

Rotoxkit M chronic

For the Rotoxkit M chronic test product F shows an reproduction effect that doesn't seem to be dose responsive (appendix 4.3). For both the range finding and final test, the observed effects were similar for all concentrations. This could be caused by physical effects, as is the case in the Rotoxkit M acute test in product A and B. But the symptoms of physical effects, like floating, are however not observed. So there is no clear explanation for these effects.

Algae growth inhibition

For the algae growth inhibition test product F shows a relatively weak effect, which increases over time and also increases comparing the 1 and 4 days stock solutions. This suggests that new organic UV filters show the same effects as mineral based UV filters, increasing in toxicity over time. Although this relatively weak effect is only observed at the highest test concentration of 125µl/l, where other products show the same effects at lower concentrations.

Product G

Product G does only show relatively weak effects, compared to the other products, for the micro toxicity test, with EC50's of 235 and 242 µl/l, and for the Rotoxkit M chronic test, of which no EC50 could be calculated with a maximum reproduction inhibition effect of 52%. For the other bioassays no effects are observed, with EC50's exceeding the highest test concentration. With maximum effects of 13% mortality in the Rotoxkit M acute test and 12% and 58% growth inhibition in the 1 day and 4 days algae growth inhibition test.

Micro toxicity

For the micro toxicity test a relatively weak luminescence inhibition effect is observed, for which EC50's of 235 and 242 µl/l are calculated. The effect is dose related, and is already present at the first measurement, which suggests an acute toxicity effect that decreases over time. Even though a toxicity effect is shown, this effect is still weak compared to some other products.

Rotoxkit M chronic

For the Rotoxkit M chronic test product G shows a weak reproduction effect, which is similar to the effect for product C. The effect is however only at high concentrations of 75 and 150µl/l.

5.3 Comparing results bioassays with toxicity data

All the calculated EC50's are translated to the concentration of each individual UV filter in order to derive an EC50 in (mg UV filter/l). These concentrations are estimations and are only valid if the total effect of the sunscreen is caused by the specific UV filter. Which is most likely not the case as most sunscreen products contain multiple UV filter that can have effects. Table 11 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. In the case of product C the percentage is derived from the average of all found products in appendix 1, which contains nano-ZnO and non-nano TiO_2. And the percentages of product F and G are derived from maximum approved concentrations by European and USA regulations (Skinacea, 2012). After that for all the bioassays the concentrations in mg/l UV filter are calculated.

Table 11 Concentration of active ingredients/UV filters at EC50's in mg/l

			mg/l of active ingredient at EC50					
Product	**Active ingredients**	**% substance**	**Microtox**	**Microtox (centrifuged)**	**Rotoxkit M acute**	**Rotoxkit M chronic**	**Algae 1day**	**Algae 4days**
A	avobenzone	3	3.99	3.10	1.10		0.47	0.80
A	homosalate	7.5	9.98	7.74	2.75		1.16	1.99
A	octyl salicate	5	6.66	5.16	1.84		0.78	1.33
A	octocrylene	2.75	3.66	2.84	1.01		0.43	0.73
A	oxybenzone	2	2.66	2.06	0.73		0.31	0.53
B	homosalate	7		12.44	0.27	0.46	4.83	
B	octocrylene	3		5.33	0.12	0.20	2.07	
B	avobenzone	3		5.33	0.12	0.20	2.07	
B	octyl salicate	3		5.33	0.12	0.20	2.07	
B	TiO2 (nano)	5		8.88	0.19	0.33	3.45	
C	ZnO (nano)	10					2.23	1.96
C	TiO2	5.8					1.29	1.14
D	TiO2	6.4					8.42	6.27
D	ZnO	6					7.90	5.88
E	TiO2	8.8						
F	Uvinul A plus	10	2.19	4.18		0.19		
F	Uvinul T 150	15	3.28	6.27		0.28		
F	Tinosorb S	10	2.19	4.18		0.19		
F	Ensulizole	4	0.87	1.67		0.07		
G	Tinosorb M	10	23.45	24.15				
G	Uvinul T 150	5	11.73	12.08				
G	Uvinul A plus	10	23.45	24.15				

Comparing this with the toxicity database of 2.3, it is observed that, in most cases, the toxicity of the bioassays differs from the toxicity data. For example, for oxybenzone, in the toxicity data the EC50 of *Haptophyte* algae species is 0.01 mg/l while the toxicity for *S. constatum* algae in the bioassays is 0.31 and 0.53 mg/l. Unfortunately no other species can be compared with the toxicity data, but this confirms that, for oxybenzone, the toxicity data shows stronger toxicity than observed in the bioassays. Another example is when comparing non-nano-ZnO. The toxicity data shows an EC50 of 2.97 mg/l for *S. constatum* algae, while the bioassays show EC50's of 7.90 and 5.88 mg/l, indicating

that non-nano ZnO shows stronger toxicity effects in the toxicity data than it does in the bioassays. A possible explanation for these differences is that the UV filters in sunscreen products are less bioavailable because of sunscreens being a complex mixture of many substances, while the chemical on itself shows much stronger toxicity effects.

However, when comparing the toxicity observed for nano-ZnO with the toxicity data, the outcome is quite similar. The EC50 for *S. constatum* in the toxicity data is 2.36 mg/l where the bioassays show EC50's of 2.23 and 1.96 mg/l. However the toxicity calculated from the bioassays is still estimated, and it is unsure if the toxicity effects originates from nano-ZnO or from another ingredient in the sunscreen product.

Even though, some studies used in the toxicity database state that there observed toxicity effects are field relevant. However, all bioassays, including these bioassays, are performed in a laboratory environment. Therefore it can't directly be translated to the field. However the toxicity of oxybenzone and octocrylene is compared with field measured concentrations by (Schaap & Slijkerman, 2018), which resulted in a similarity between toxicity data and field concentrations. Nevertheless it is still very hard, or impossible to measure ZnO, TiO_2 or new organic UV filter concentrations in the field, so as of yet it is unsure if these toxicity effects are field relevant.

5.4 Conclusion bioassays

Concluding the bioassays the effects of all products are compared for each bioassay (Table 12). This is based on EC50 values. For each individual bioassay the relative effect of each product is compared. This is categorized with 4 scores ranging from the relatively strongest measured effect to no effect. These categories are coupled to a score, resulting in the average score per product.

Table 12 Relative effect of all products for each bioassay

	= strongest effect (3)		= moderate effect (2)		= weakest effect (1)		= no effect (0)
	Bioassays EC50 (µl/l)						
Product:	**Microtox**	**Microtox centrifuged**	**Rotoxkit M acute**	**Rotoxkit M chronic**	**Algae 1day**	**Algae 4days**	**Score:**
Product A							2.17
Product B							1.33
Product C							1.17
Product D							0.33
Product E							0.00
Product F							1.83
Product G							0.50

The outcome of this is that the eco-friendly products D, E and G have much lower scores, so show relatively weaker effects, than the products A, B, C and F, which don't claim to be eco-friendly.

Product A, which contains oxybenzone, shows the highest score, followed by product F. Which is remarkable, as product F contains similar active ingredients as the eco-friendly product G and the ingredients are not reported to show strong effects on aquatic organisms in the toxicity data.

Furthermore, product B, containing organic UV filters excluding oxybenzone, and product C containing nano-ZnO, both have a relatively higher score than the eco-friendly products.

In addition, product E shows no relative toxicity effects for any of the bioassays, compared to the other products. This can be explained by the fact that product E only contains non-nano TiO_2 as UV filters, for which very weak toxicity effects are reported in the toxicity data. Compared to product D, which also contains non-nano ZnO, for which stronger toxicity effect are reported in the toxicity data, resulting in a higher score than product E.

6 Risk assessment; case Lac Bay, Bonaire

Another part of this research is performing a risk assessment for mineral based and new organic UV filters. With the intention to make estimations on the potential risk if everyone changes to using sunscreen products containing these types of UV filters. This is investigated for Lac Bay, Bonaire (Figure 27). Lac Bay is a tropical marine lagoon on the eastern coast of Bonaire and covers an area of approximately 700 ha and is popular for various recreational and touristic activities (Debrot, Meesters, & Slijkerman, 2010); (Debrot A. , 2012); (Hylkema, Vogelaar, Meesters, Nagelkerken, & Debrot, 2015). The number of tourists at Lac Bay has been increasing, especially the last decades, due to growing cruise ship visits (Debrot A. , 2012). So this makes it interesting to see what the emissions will be in this area. The bay's depth ranges between 0 and 3 meters deep and a shallow coral barrier protects the bay from waves of eastern onshore winds. A main channel of approximately 5 meters deep connects the bay with the coral reef (Debrot A. , 2012); (Hylkema, Vogelaar, Meesters, Nagelkerken, & Debrot, 2015). Lac Bay consists of unique and environmental important habitats, including mangroves and seagrass beds, and houses rare and threatened species like, sea turtles and the Caribbean queen conch (Debrot, Meesters, & Slijkerman, 2010); (Debrot A. , 2012); (Hylkema, Vogelaar, Meesters, Nagelkerken, & Debrot, 2015). Which makes it very interesting and relevant to estimate the emissions in an vulnerable area like Lac Bay. Furthermore, uncontrolled development of marine recreation sites at Lac Bay will result to marine water contamination whereby the carrying capacity of this area could be (irreversibly) affected (Debrot A. , 2012).

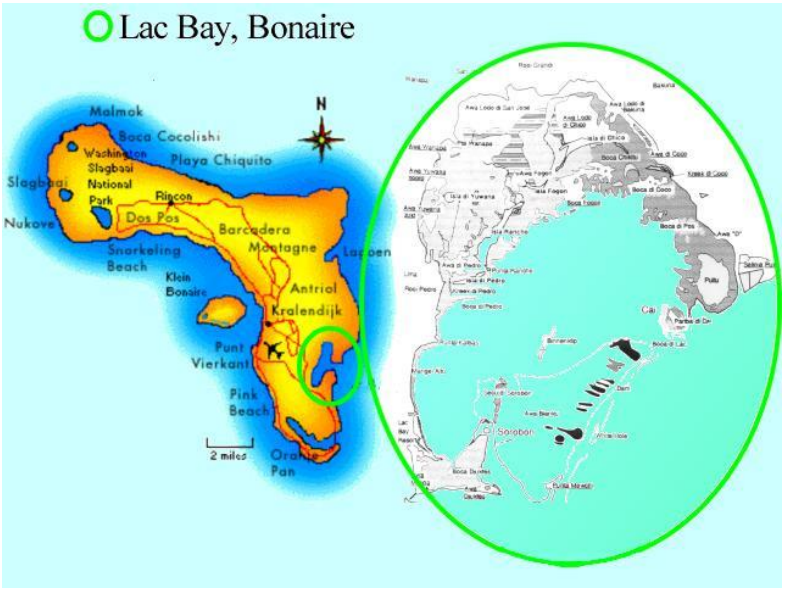

Figure 27 Overview of Lac Bay, Bonaire (Sea turtle club, 2018)

6.1 Risk assessment; method

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). By dividing these two factors, an risk quotient (RQ) can be determined.

For the estimation of PEC's, first the daily potential release of UV filters is calculated. For this multiple factors are taken into account according to the method of (Sharifan, Klein, & Morse, Environmental occurence and ecological risk assessment of organic UV filters in marine organisms from Hong Kong coastal waters., 2016); (Sharifan, Klein, & Morse, UV filters are an environmental threat in the Gulf of Mexico: a case study of Texas coastal zones, 2016); (Schaap & Slijkerman, 2018). Namely:

- The yearly amount of bathers on peak and non-peak days is used based on 100 cruise days and 200 normal beach days. Resulting in 39000 as the estimated yearly amount of bathers, which is equivalent to 325 bathers per day
- Sunscreen application rate of 1.5 times/day
- Sunscreen applied body surface area of 2.1 m^2 for males and 1.7 m^2 for females with an 87% body coverage

- Sunscreen wash off rate of 25% when entering the water
- The average content of UV filters in eco-friendly sunscreens, based on the list of products in (appendix 1). The average percentages applied in the estimations were 15% for non-nano-ZnO, 10% for nano-ZnO and 4% for nano TiO_2

The PEC is calculated by dividing the daily potential release of UV filters with the rounded volume of three different zones in Lac Bay. Namely, the bathing zone (16000 m^3), inner reef zone (826200 m^3) and the mangrove zone (5497300 m^3) (Figure 28).

Figure 28 Three zones in Lac Bay. Bathing zone (red), inner reef zone (green) and mangrove zone (yellow)

The PNEC is calculated based on the criteria set by (ECHA, 2008), including the application of an assessment factor (a kind of uncertainty factor). The number and type of available data determines the level of assessment factor to be applied on the lowest available toxicity endpoint to derive the PNEC. This is done by using the toxicity databases of appendix 5.2 and 5.3.

Finally, RQ's are determined by dividing PEC by PNEC. If this ratio is above 1, it indicates a potential risk from the UV filter towards the marine 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). In this estimation it has to be kept in mind that the RQ's are based on the worst case scenario, when all visitors in Lac Bay shift to using sunscreen products solely containing non-nano-ZnO, nano-ZnO or nano TiO_2.

6.2 Risk assessment; results & discussion

The risk assessment is performed for the UV filters non-nano-ZnO, nano-ZnO and nano TiO_2. For the other UV filters, described in (2.3.3 and 2.3.4), no or limited toxicity data was present and therefore can't be used in a risk assessment following (ECHA, 2008).

Following the described method the estimated released amounts are presented in (Table 13) for the three UV filters in kg/day and gram/day. Non-nano-ZnO has the highest potential release, because the average content is the highest (15%).

Table 13 Daily estimated release of UV filters in kg/day and gram/day

Daily potential release of UV filters		
UV filter	**Kg/day**	**Gram/day**
Non-nano-ZnO	0.20	201.3
Nano-ZnO	0.13	134
Nano TiO_2	0.05	54

PEC's for each of the three zones (bathing zone, inner reef zone and mangrove zone) are presented in (Table 14). PEC's in the bathing zone are higher than in the other zones, because with increasing volumes the dilution is a bigger factor resulting in lower concentrations.

Table 14 PEC's (µg/l) for three UV filters for three zones at Lac Bay

Predicted environmental concentration value (µg/l)			
UV filter	**Bathing zone**	**Inner-reef zone**	**Mangrove- zone**
Non-nano-ZnO	12.58	0.24	0.05
Nano-ZnO	8.39	0.16	0.04
Nano TiO_2	3.36	0.06	0.01

The PNEC's per UV filter are presented in (Table 15). From the toxicity database the corresponding data coverage criteria from (ECHA, 2008) are described, resulting in a certain assessment factor (AF). Furthermore the lowest LOEC/NOEC is described including the species and type of effect. PNEC estimation included both freshwater and marine data, as marine toxicity data was limiting.

Table 15 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 (µg/L)**
Non-nano-ZnO	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) 50 µg/l (Heinlaan, Ivask, Blinova, Dubourguier, & Kahry, 2008)	0.10
Nano-ZnO	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 T. , Miller, Lenihan, & Bielmyer, 2013)	0.20
Nano TiO_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

RQ's based on PEC/PNEC's are presented in (Table 16). RQ's for non-nano-ZnO are 125.8 in the bathing zone and 2.4 in the inner reef zone. This suggests that when everyone shifts to using

sunscreen products containing non-nano-ZnO, these two zones in Lac Bay could face a potential environmental risk. The mangrove zone has no risk for non-nano-ZnO.

RQ's for nano-ZnO are 42 in the bathing zone. This suggests that when everyone shifts to using sunscreen products containing nano-ZnO, the bathing zone in Lac Bay faces a potential environmental risk. The other zones have no risk for nano-ZnO.

Comparing the RQ's of these two UV filters, non-nano-ZnO forms a higher risk than nano-ZnO, even though the toxicity database and literature suggest that nano-ZnO is more toxic. This can be explained because the RQ's are based on estimations. In addition, the available toxicity data for non-nano-ZnO is minimal, resulting in the application of a higher AF. Nevertheless, with the current knowledge of these substances, this method is the best to predict environmental risks. Measuring the field concentrations to derive a realistic PEC would largely improve the overall risk assessment.

RQ's for nano TiO_2 are all <1, which suggests that Lac Bay doesn't face a potential environmental risk. This can be explained by an the average low amount of TiO_2 (4%) used in sunscreen products, resulting in lower potential release and thus lower PEC's. Adding the fact that nano TiO_2 shows lower toxicity than both ZnO UV filters, resulting in lower PNEC's, explains why RQ's of nano TiO_2 are lower than both ZnO UV filters.

Table 16 Risk quotients per UV filter, derived from PEC (Table 5) and PNEC (Table 6) at three locations at Lac Bay

Risk quotients			
UV filter	Bathing zone	Inner-reef zone	Mangrove- zone
Non-nano-ZnO	125.8	2.4	0.5
Nano-ZnO	42	0.8	0.2
Nano TiO_2	0.2	0.003	0.0005

7 Conclusion & recommendation

7.1 Conclusion

After performing literature research, bioassays and a risk assessment the research questions can be answered successfully.

"What are eco-friendly sunscreen products?"

Products that claim to be reef safe, eco-friendly, oxybenzone free, biodegradable, etc. Which most of the time contain the UV filter non-nano ZnO or other mineral based UV filters. But also quite a few products contain organic UV filters, of which still some products contain oxybenzone, see chapter 3.

"What are the known effects of (eco-friendly) UV filters for the marine environment?"

In general the most common effects that are shown are mortality, growth inhibition, developmental toxicity and malformations. But also genotoxicity, DNA damage, coral bleaching, bioaccumulation and other types of effects are shown in some cases, see chapter 2.3.

"What are the effect concentrations?"

The general pattern that is observed from the toxicity data is that the organic UV filters oxybenzone and octocrylene show the strongest toxicity effects, as was expected. However, both nano and non-nano ZnO also show similar toxicity effects. Furthermore, both nano and non-nano TiO_2 show weak toxicity effects, especially when compared with both ZnO UV filters. Keeping in mind that for both non-nano ZnO and TiO_2 UV filters relatively less toxicity data is reported that for nano ZnO and TiO_2. This was also the case for new organic UV filters, which most of the time show relatively weak toxicity effects, only show toxicity effects exceeding the highest tested concentration and only toxicity data for freshwater species was reported, see chapter 2.3.

"Do toxicity effects occur in a variety of laboratory tests?"
Yes, toxicity effects were observed in all the performed bioassays. From the 7 testes products, product A, B, C and F relatively show stronger toxicity effects than the eco-friendly products D, E and G, see chapter 5.4.

Product A, which contains oxybenzone, shows the highest score, followed by product F. Which is remarkable, as product F contains similar active ingredients as the eco-friendly product G and the ingredients are not reported to show strong effects on aquatic organisms in the toxicity data.

Furthermore, product B, containing organic UV filters excluding oxybenzone, and product C containing nano-ZnO, both have a relatively higher score than the eco-friendly products.

In addition, product E shows no relative toxicity effects for any of the bioassays, compared to the other products. This can be explained by the fact that product E only contains non-nano TiO_2 as UV filters, for which very weak toxicity effects are reported in the toxicity data. Compared to product D, which also contains non-nano ZnO, for which stronger toxicity effect are reported in the toxicity data, resulting in a higher score than product E.

"Are these effects field relevant?"

Comparing the reported effects of the bioassays with the toxicity database, it is observed that in most cases the reported toxicity differs, see chapter 5.3. In general the toxicity effects reported in the toxicity data are stronger than the effects observed in the bioassays. A possible explanation for these differences is that the UV filters in sunscreen products are less bioavailable because of

sunscreens being a complex mixture of many substances, while the chemical on itself shows much stronger toxicity effects. Even though, some studies used in the toxicity database state that there observed toxicity effects are field relevant. However, all bioassays, including these bioassays, are performed in a laboratory environment. Therefore it can't directly be translated to the field. However the toxicity of oxybenzone and octocrylene is compared with field measured concentrations by (Schaap & Slijkerman, 2018), which resulted in a similarity between toxicity data and field concentrations. Nevertheless it is still very hard, or impossible to measure ZnO, TiO_2 or new organic UV filter concentrations in the field, so as of yet it is unsure if these toxicity effects are 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 estimations, the daily average emissions of non-nano ZnO will be 201.3 gram/day, 134 gram/day for nano-ZnO and 54 gram/day for nano-TiO_2 for the case of Lac Bay, Bonaire, when everyone in the case changes to using products containing these UV filters.

By means of the risk assessment performed in chapter 6.2 non-nano ZnO relatively forms the highest environmental risk in the case, with risk quotients of 125.8 and 2.4 at two different zones in the case. Nano-ZnO also forms an environmental risk for the case with a risk quotient of 42. However nano-TiO_2 forms no environmental risk based on the risk assessment. Keeping in mind that these are estimations of the worst case scenario, when everyone changes to products containing these UV filters. Furthermore only risk quotients could be derived for these 3 UV filters, because toxicity data was limited for the other UV filters to perform a risk assessment.

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

The main question can be answered in three ways for the 3 different parts of this research. The toxicity data shows that for the UV filters generally used in eco-friendly sunscreen products, both nano and non-nano ZnO do show toxicity effects similar to some organic UV filters like oxybenzone and octocrylene. However both nano and non-nano TiO_2 and new organic UV filters show very weak toxicity effects, especially when compared with both ZnO UV filters. So from the toxicity data it can be concluded that products containing both forms of ZnO as UV filter, are potentially harmful for the marine environment. Keeping in mind that the toxicity data represents the toxicity of the individual chemical, not the toxicity in a sunscreen product.

The bioassays show a clear pattern in which products that claim to be eco-friendly, show relatively weak to no toxicity effects compared to the products which don't claim to be eco-friendly, which show relatively strong toxicity effects. So from the bioassays it can be concluded that eco-friendly products are not harmful for the marine environment, especially when compared to products which don't claim to be eco-friendly.

The risk assessment shows a potential environmental risk for both nano and non-nano ZnO, but for nano-TiO_2 no potential environmental risk is observed. This outcome is similar to the outcome of the toxicity data, that both ZnO UV filters are potentially harmful for the marine environment, but other UV filters used in eco-friendly sunscreen products are not potentially harmful for the marine environment.

7.2 Recommendation

For a continuation of this research it is recommended to perform more experiments on sunscreen products, to better describe their potential effects on marine organisms.

With this research only 4 bioassays with 3 different marine organisms were performed, which gave a general idea of the toxicity of eco-friendly sunscreen products. However, performing other bioassays on other marine species will give even more information on the toxicity of eco-friendly sunscreen products for the marine environment. In the case of tropic destinations like the risk assessment of Lac Bay, research on the toxicity on several coral species is recommended. Furthermore when the Rotoxkit M chronic test is finalised, it is recommended to perform this again for more accurate results.

For the general principle of performing bioassays with sunscreen products, some changes in the principle might give other outcomes. It is recommended to do experiments with glass test plates instead of plastic to exclude the hypothesis that plastic might have an effect on the toxicity or bioavailability of the substances in sunscreen products. Furthermore, to take away the fat/oils layers that are formed in the stock solutions and the test concentrations, making concentration series based on a water accommodated fraction (WAF) method is recommended. With this method the oil/fat layer is separated from the water column and can potentially result in other outcomes.

During the range finding tests growth stimulation was observed in some cases for the algae growth inhibition test. Even though this wasn't observed in the final tests it is recommended to do more experimental research on the, potentially, stimulating effects of sunscreen UV filters on the marine environment, as is described in theory by (Jongen, 2017).

With the algae growth inhibition test both a 1 day and 4 days old stock solution was tested, because in theory it was expected that substances used in sunscreen products become more or less bioavailable after time. This was indeed the case, which is a very interesting observation. Therefore it is recommended to do a lot more testing on the increase or decrease in bioavailability of sunscreen ingredients.

For oxybenzone and octocrylene field samples have been taken to indicate the environmental concentrations (Schaap & Slijkerman, 2018). For UV filters present in eco-friendly sunscreen products environmental concentrations are as of yet unknown. To better translate the toxicity found in laboratory testing to field relevant effects, it is recommended to perform experiments on collecting field samples to get to know the environmental concentrations of these UV filters.

Continuing on this, as of yet there is very minimal information on the chemistry composition of UV filters in sunscreen products. There is a high possibility that the toxicity of the UV filters is higher or lower than the individual chemical, as sunscreens are complex mixtures of many chemicals. Therefore it is recommended to perform chemical analysis's on sunscreen products to get a better understanding of their chemical composition, toxicity and bioavailability.

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Appendices

1 Overview eco-friendly sunscreen products

Page 2 till 5 show the overview table of all 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 in the tables.

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%								
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%	
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%								
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%									

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
SmartShield, kids sunscreen lotion	30	Eco-friendly	Biodegradable					2%		1%	3%		8%	7%
SmartShield, sunscreen lotion	15	Eco-friendly	Biodegradable					2%		1%	3.00%		7.50%	
SmartShield, sunscreen lotion	30	Eco-friendly	Biodegradable								2.50%	5%	7.50%	7%
SPF Rx	30	Reef-safe	Mineral based, natural		7%		3%							
Stream2Sea	30	Reef-safe	Biodegradable, tested and proven reef safe, mineral based, organic			8.80%								
Stream2Sea	20	Reef-safe	Biodegradable, tested and proven reef safe, mineral based, organic			6.60%								
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	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%	
Tropical seas, reef safe	30	Reef-safe	Biodegradable, non toxic to sea-life, bait safe								5%	4%	7.50%	
Tropical seas, reef safe	15	Reef-safe	Biodegradable, non toxic to sea-life, bait safe								3%	2%	7.00%	
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%										

Protocol concentration series sunscreen products

By Martijn Keur 19-2-2018

Introduction

To make a concentration series with the sunscreen products a standard protocol is used. The protocol is for making a stock solution of the highest concentration used in lab experiments. This is necessary because 1ml of sunscreen product is not the same as 1 gram. Therefore this protocol is made, to obtain the right concentrations for testing with sunscreen products. The highest concentration that can be used with this method is 200µl/l, because it was found out that at this point the maximum solubility is reached. Higher concentrations could negatively influence the concentration of the series.

Weighing process

To obtain the right concentrations, calculations are made to translate ml of product to grams. This is done by weighing 10ml of the product in a 25ml beaker on a scale. After that the amount of grams is noted in the excel file. This process is done at least 3 times to get an average weight of the product. Because products have different weights, this process is done for all products individually.

Excel calculations

The average amount of grams measured is used in an excel file. The excel file works out the grams/10ml to grams/10µl by dividing with factor 1000. The same is done for concentrations 20, 50, 100, etc. only with an extra step that the number gets multiplied with 2, 5, 10, etc. respectively.

But the concentration series used is for µl/l. So to achieve the same concentration, all numbers are divided by 5 to obtain the right concentrations for µl/200ml. Table 17 shows an example of the excel file.

Table 17 Example excel file

	Weight (gram/10ml)				To be weighted for initial concentrations (grams/200ml)							
Product:	Weight 1	Weight 2	Weight 3	Average weight:	10 µl/l	20 µl/l	25 µl/l	50 µl/l	100 µl/l	125 µl/l	150 µl/l	200 µl/l
Product A	9.53	9.36	10.05	9.65	0.0019	0.0039	0.0048	0.0096	0.0193	0.0241	0.0289	0.0386
Product B	9.73	10.03	9.85	9.87	0.0020	0.0039	0.0049	0.0099	0.0197	0.0247	0.0296	0.0395
Product C	10.59	10.20	10.35	10.38	0.0021	0.0042	0.0052	0.0104	0.0208	0.0260	0.0311	0.0415
Product D	10.45	10.13	10.51	10.36	0.0021	0.0041	0.0052	0.0104	0.0207	0.0259	0.0311	0.0415
Product E	10.40	11.85	11.26	11.17	0.0022	0.0045	0.0056	0.0112	0.0223	0.0279	0.0335	0.0447
Product F	9.20	9.37	9.95	9.51	0.0019	0.0038	0.0048	0.0095	0.0190	0.0238	0.0285	0.0380
Product G (gram/5ml)	5.03	4.85	5.09	4.99	0.0020	0.0040	0.0050	0.0100	0.0200	0.0250	0.0299	0.0399

Preparing the concentration series

The output of the excel file can now be weighted on a scale that can weigh 0,1mg. With a spoon the amount of product is weighted in a beaker of 25ml. This is dissolved with 0,45µm seawater and transferred to a 250ml Erlenmeyer. This process needs to be repeated to the point where no product is left in the 25ml beaker. Afterwards, the 250ml Erlenmeyer is filled to the 200ml mark with 0,45µm

seawater. The Erlenmeyer's need to be marked with the product name or number and the concentration.

After that, stirring beans are added to all Erlenmeyer's and they are covered with parafilm to minimise the evaporation. Furthermore, the Erlenmeyer's are placed on magnetic stirring plates to dissolve the product and to obtain a homogeneous solution. The products need to be dissolved on the stirring plates for +/- 24 hours prior to the start of the test. Additionally this process needs to take place in a dark environment, replicating the environment of a sunscreen bottle, which is also dark.

3 Range finding test results

3.1 Micro toxicity

To obtain the right concentration series for the final test a range finding experiment is performed for the micro toxicity test. This is done according to the method described in paragraph 4.3. The concentrations used in this experiment are; 11.25, 22.50, 45.00 and 90.00µl/l. Based on the concentrations used by (Corinaldesi, et al., 2017), including a more extreme highest concentration. The test included a reference toxicant, of which the outcome is according to the standard, and 4 sunscreen products; product A, B, F and G. Where product B, F and G are tested with samples coming from the stock solution and samples which are centrifuged. This is done because these 3 products don't completely dissolve, which could influence the concentration in the series. So this is taken care of by centrifuging the samples before testing.

The outcome of the test can be seen in Table 18. Where all the products are shown with the results for 5,15 and 30 minutes. Here the 50% effect concentration (EC50) is shown. For some values the EC50 couldn't be calculated, because the effect didn't reach 50%. For these values the highest effect concentration in percentage is shown.

Table 18 EC50/highest effect% calculation micro toxicity range finding test

	EC50 (µl/l) / highest effect %		
Product:	5 min	15 min	30 min
Product A	**118.44**	**26%**	**4.99%**
Product B	**18.73%**	**3.17%**	**-5.67%**
Product B (centri)	**226.4**	**287**	**591.6**
Product F	**8.202**	**32.92**	**85.82**
Product F (centri)	**28.08**	**315.4**	**22.76%**
Product G	**222.8**	**187.7**	**176.48**
Product G (centri)	**15.59%**	**6.52%**	**-4.68%**

Overall for all products, apart from product G, the EC50 concentration increases over time or the highest effect concentration decreases over time. So it can be said that there is a decrease in effect over time. A possible explanation for this can be that at the start of the exposure the bacteria are stressed or weakened and do therefore not emit very much light. But over time the bacteria heal or get used to the environment and with that do emit more light.

Furthermore it can be seen that for all products, apart from product F both normal and centrifuged, the effects are too low to calculate a EC50 concentration or the EC50 is reaching concentrations that are no longer field relevant, keeping in mind that the highest concentration of 90µl/l is already quite extreme. So it can be concluded that these products do not have a significant toxicity effect to the bacteria *Vibrio fischeri*.

Nevertheless product F is the only product that does show a significant effect, especially the not centrifuged sample and at the start of the exposure. Even though the effect is very strong at the start

of the exposure (8µl/l and 28µl/l), the effects decrease very strong over time, resulting in effects not to be significant anymore.

Concluding this range finding test the concentration series used is sufficient for the final test. Furthermore, a the stock solution of 200µl/l is used. A higher concentration can't be made because maximum solubility is already reached. Moreover, higher concentrations wouldn't be field relevant anyway. For a continuation on this range finding test, the final test will also include the 3 products that aren't tested with this test, using the same concentration series. Furthermore, both normal and centrifuged samples will be tested. The reason for this is that the hypothesis is that small particles that don't dissolve can highly influence the luminescence measured, so can affect the results. Therefore centrifuged samples will also be tested. Finally, all products will be tested again, but now with the stock solutions placed in a dark environment before used, as this wasn't the case with this test.

3.2 Algae growth inhibition

To obtain the right concentration series for the final test a range finding experiment is performed for the algae growth inhibition test. This is done according to the method described in paragraph 4.4. The concentrations used in this experiment are; 6.3, 12.6, 25.1, 50.1 and 100µl/l. Based on the concentrations used by (Corinaldesi, et al., 2017), including a more extreme highest concentration. For the concentration series, the protocol described in appendix 2 is used. For the test a reference toxicant is tested, of which the outcome is according to the standard, and apart from product D, all the to be tested sunscreen products are tested.

Table 19 EC50/highest effect % calculation algae growth inhibition range finding test

	EC50 (µl/l) / highest effect %		
Product:	24h	48h	72h
Product A	**8.49**	**10.2**	**12.1**
Product B	**50.4**	**53.2**	**56.4**
Product C	**29.2**	**29.3**	**29**
Product E	**2%**	**19%**	**32%**
Product F	**47.8**	**80.1**	**77.7**
Product G	**2%**	**7%**	**20%**

The results can be seen in Table 19. Here the EC50, or highest effect concentration in percentage is shown for all the 6 tested sunscreen products at the measurement times of 24, 48 and 72 hours.

Overall a clear division can be observed between products that claim to be eco-friendly and products who don't. Product E and G claim to be eco-friendly and these 2 products are the ones where the *S. constratum* algae shows minimal growth inhibition effects. Moreover a very interesting observation has been observed. Apart from the highest concentration, a growth stimulation of 1-9% instead of a growth inhibition is observed. A possible explanation for this can be that due to the minimal growth inhibition effect and the fact that some sunscreen ingredients have nitrogen and/or phosphorus molecules, the growth rate of the algae is positively affected, as shown by (Jongen, 2017). Keeping this in mind, the final test will also include a measurement at 96 hours, to see if the trend of growth stimulation continues or not.

Furthermore the other products, which aren't stated to be eco-friendly, do show significant growth inhibition. With product A showing the most severe effects, resulting in the lowest EC50. With the help of the conducted EC50 values and the raw growth inhibition data, the concentration series are fine tuned for all the products for the final test, which can be seen in Table 20. Furthermore the final test will also include product D. It is estimated that product D will have similar effects as product E, so therefore the same concentration series will be used.

Table 20 Concentration series for 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
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	100	50	200	200	125	200

3.3 Rotoxkit M acute

To obtain the right concentration series for the final test a range finding experiment is performed for the acute rotifer test. This is done according to the method described in paragraph 4.5. The concentrations used in this experiment are; 37.7, 53.2, 75.2, 106.2 and 150µl/l based on the concentrations used by (Corinaldesi, et al., 2017), including a more extreme highest concentration. For the concentration series, the protocol described in appendix 2 is used. For the test a reference toxicant is tested, of which the outcome is according to the standard, and all the 7 to be tested sunscreen products are tested.

The results can be seen in Table 21. Here the mortality percentages are shown for all the 7 products for the measurement time of 48 hours. Only for product A, B and F a significant effect is observed. For the other measurements the mortality effect was very minimal. Therefore there is no need to test product C, D, E and G again, so this data will be used as the final test results.

Table 21 Mortality percentages acute rotifer test range finding

Product A		Product B		Product C		Product D		Product E		Product F		Product G	
Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %	Conc. (µl/l)	Mortality %
0	**3.33**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**
37.7	**6.67**	37.7	**26.67**	37.7	**0**	37.7	**3.33**	37.7	**3.33**	37.7	**26.67**	37.7	**0**
53.2	**20**	53.2	**26.67**	53.2	**3.33**	53.2	**0**	53.2	**0**	53.2	**43.33**	53.2	**3.33**
75.2	**36.67**	75.2	**36.67**	75.2	**20**	75.2	**3.33**	75.2	**0**	75.2	**63.33**	75.2	**3.33**
106.2	**66.67**	106.2	**50**	106.2	**6.67**	106.2	**0**	106.2	**0**	106.2	**50**	106.2	**3.33**
150	**83.33**	150	**36.67**	150	**16.67**	150	**0**	150	**0**	150	**56.67**	150	**13.33**

Product A, B and F will be tested again for the final test, where the concentration series of product A will be slightly adjusted.

3.4 Rotoxkit M chronic

To obtain the right concentration series for the final test a range finding experiment is performed for the chronic rotifer test. This is done according to the method described in paragraph 4.5. The concentrations used in this experiment are made according to the outcome of the acute rotifer test described in appendix 3.3. For product A, a concentration series of 25.1-100 µl/l is used and for product B and product F a concentration series of 12.6-50 µl/l is used. And because the other 4 products (product C, D, E and G) didn't show any effect in the acute rotifer test, only the highest concentration of 150 µl/l is tested with more (40) replicates. For the concentration series, the protocol described in appendix 2 is used. For the test a reference toxicant is tested, of which the outcome was according to the standard, and all the 7 to be tested sunscreen products are tested.

The results can be seen in Table 22, where the reproduction inhibition percentages are shown for each product for the tested concentrations.

Table 22 Inhibition percentages range finding chronic rotifer test

Product A		Product B		Product C		Product D		Product E		Product F		Product G	
Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %	Conc. (µl/l)	Inhibition %
0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.1	56.09756098	12.6	86.363636	150	85.046729	150	-78.783593	150	28.485577	12.6	87.142857	150	60.169492
35.5	44.25087108	17.7	87.727273	150	58.878505	150	1.5558699	150	52.884615	17.7	94.761905	150	76.271186
50.1	70.73170732	25.1	98.636364	150	75.700935	150	-39.60396	150	26.802885	25.1	93.809524	150	59.216102
70.8	78.04878049	35.4	96.363636	150	51.935915	150	-9.9009901	150	10.576923	35.4	90.555556	150	56.779661
100	100	50	98.636364	150	46.595461	150	-49.363508	150	33.533654	50	92.222222	150	63.665254
Mean					63.631509		-35.219236		30.456731				63.220339

It can be seen that product A, B and F show very high inhibition percentages, so for the final test they will be tested again with a lower concentration series. Product C and G were tested with only one concentration, and the outcome is that there is an effect, so for the final test a second concentration will be added to make sure the effect can be shown in a better way. Product D and E are also tested with one concentration and the outcome is that no or minimal effect is observed so for the final test they will be tested in the same way.

4 Final test results data

4.1 Micro toxicity

In Table 23 an overview of all the EC50's for the micro toxicity final test can be seen. The measurement is coded as 1 or 2 for the 2 replicates of the normal samples or C1 or C2 for the 2 replicates for the centrifuged samples. K5, K15 and K30 are the 3 measurement times of 5, 15 and 30 minutes. The EC50 in µl/l and the 95% confidence interval are shown. For empty cells the EC50 or confidence interval could not be calculated due to the effect not reaching 50% or the confidence interval being too big, therefore the highest effect concentration is given in % effect.

Table 23 overview of all the results for the micro toxicity final test. measurement, EC50 and 95% confidence interval

Product	Measurement	EC50 in ul/l	95% confidence interval		Highest effect concentration in %
Product A	1K5	51.67	36	74	
Product A	1K15	65.88	30	147	
Product A	1K30	174.33	33	921	
Product A	2K5	52.47	48	57	
Product A	2K15	71.27	59	86	
Product A	2K30	101.16	30	343	
Product A	C1K5	53.67	46	63	
Product A	C1K15	74.28	58	95	
Product A	C1K30	125.73			
Product A	C2K5	53.75	33	89	
Product A	C2K15	62.16	22	179	
Product A	C2K30	88.04			
Product B	1K5	244.35	71	842	
Product B	1K15				18.29
Product B	1K30				10.11
Product B	2K5	311.13	47	2062	
Product B	2K15				18.42
Product B	2K30				8.93

Product B	C1K5	95.67	34	267	
Product B	C1K15	123.30			
Product B	C1K30	158.67			
Product B	C2K5	109.17	14	862	
Product B	C2K15	151.65			
Product B	C2K30	201.15			
Product C	1K5				9.86
Product C	1K15				-1.19
Product C	1K30				6.57
Product C	2K5				4.56
Product C	2K15				-4.89
Product C	2K30				-1.02
Product C	C1K5				4.08
Product C	C1K15				-2.44
Product C	C1K30				2.50
Product C	C2K5				1.72
Product C	C2K15				-7.24
Product C	C2K30				-2.38
Product D	1K5				-17.55
Product D	1K15				-19.59
Product D	1K30				-20.48
Product D	2K5				-8.82
Product D	2K15				-11.69
Product D	2K30				-10.82
Product D	C1K5				-4.61
Product D	C1K15				-8.36
Product D	C1K30				-12.85
Product D	C2K5				-8.79
Product D	C2K15				-10.91
Product D	C2K30				-11.92
Product E	1K5				-7.03
Product E	1K15				-11.20
Product E	1K30				-13.05
Product E	2K5				-4.88
Product E	2K15				-9.49
Product E	2K30				-11.87
Product E	C1K5				-7.55
Product E	C1K15				-9.64
Product E	C1K30				-13.83
Product E	C2K5				-5.39
Product E	C2K15				-4.58
Product E	C2K30				-10.35
Product F	1K5	2.64	2	4	
Product F	1K15	6.81	4	12	
Product F	1K30	21.92	13	36	
Product F	2K5	2.10	1	3	
Product F	2K15	6.34	5	8	
Product F	2K30	24.06	24	24	
Product F	C1K5	3.71	3	5	
Product F	C1K15	9.24	9	10	

Product F	C1K30	26.65	23	31	
Product F	C2K5	4.06	3	5	
Product F	C2K15	15.59	13	18	
Product F	C2K30	61.35	41	93	
Product G	1K5	80.28	9	739	
Product G	1K15	178.38			
Product G	1K30	288.99			
Product G	2K5	84.25	29	247	
Product G	2K15	117.81			
Product G	2K30	180.18			
Product G	C1K5	84.92			
Product G	C1K15	21.03			
Product G	C1K30	18.77			
Product G	C2K5	96.21	81	115	
Product G	C2K15	133.65	23	768	
Product G	C2K30	241.65			

4.2 Rotoxkit M acute

Table 24 and Table 25 show the measured mortality percentages for the acute rotifer test. The mortalities for both the 24h and 48h measurements are shown, for all the 7 products including the mean mortalities. For product B both the normal measured mortality and the mortality minus the physical effects is shown.

Table 24 Mortality percentages of the acute rotifer final test including mean mortalities

Product A 24h							Mean mortality		**Product A 48h**						
Concentrations	% Mortality								Concentrations	% Mortality					
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0
25.1	0	40	0	0	20	0	10	20	**25.1**	0	40	20	0	40	20
35.5	0	0	0	20	0	20	7	37	**35.5**	60	20	40	40	20	40
50.1	0	20	0	0	0	0	3	60	**50.1**	60	60	60	100	40	40
70.8	40	20	0	60	40	40	33	77	**70.8**	100	80	40	80	80	80
100	20	40	40	20	20	20	27	80	**100**	100	80	80	100	40	80
Product B 24h									**Product B 48h**						
Concentrations	% Mortality								Concentrations	% Mortality					
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0
37.7	60	0	40	40	60	60	43	57	**37.7**	80	0	60	80	60	60
53.2	80	40	40	20	60	60	50	60	**53.2**	80	60	60	40	60	60
75.2	20	60	40	0	80	60	43	53	**75.2**	20	60	40	40	80	80
106.2	40	40	40	80	40	40	47	73	**106.2**	100	40	80	80	60	80
150	20	40	20	60	20	80	40	60	**150**	60	40	60	60	60	80
Product B 24h (minus physical effect)									**Product B 48h (minus physical effect)**						
Concentrations	% Mortality								Concentrations	% Mortality					
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0
37.7	0	0	0	0	0	0	0	3	**37.7**	0	0	20	0	0	0
53.2	20	0	0	0	40	20	13	23	**53.2**	20	20	20	20	40	20
75.2	0	0	0	0	0	0	0	10	**75.2**	0	20	0	20	0	20
106.2	0	0	20	0	20	0	7	30	**106.2**	40	0	60	0	40	40
150	0	0	0	0	0	20	3	23	**150**	40	0	40	0	40	20
Produc C 24h									**Product C 48h**						
Concentrations	% Mortality								Concentrations	% Mortality					
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0
37.7	0	0	0	0	0	0	0	0	**37.7**	0	0	0	0	0	0
53.2	0	0	0	20	0	0	3	3	**53.2**	0	0	0	20	0	0
75.2	0	0	0	0	0	0	0	20	**75.2**	0	0	100	0	0	20
106.2	0	0	0	0	0	0	0	7	**106.2**	0	0	40	0	0	0
150	0	0	0	0	0	0	0	17	**150**	0	0	0	0	0	100

Table 25 Mortality percentages of the acute rotifer final test including mean mortalities

Product D 24h									**Product D 48h**							
Concentrations	% Mortality								Concentrations	% Mortality						
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	
37.7	0	0	0	20	0	0	3	3	**37.7**	0	0	0	20	0	0	
53.2	0	0	0	0	0	0	0	0	**53.2**	0	0	0	0	0	0	
75.2	0	0	0	0	0	0	0	3	**75.2**	20	0	0	0	0	0	
106.2	0	0	0	0	0	0	0	0	**106.2**	0	0	0	0	0	0	
150	0	0	0	0	0	0	0	0	**150**	0	0	0	0	0	0	
Product E 24h									**Product E 48h**							
Concentrations	% Mortality								Concentrations	% Mortality						
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	
37.7	0	0	0	0	0	20	3	3	**37.7**	0	0	0	0	0	20	
53.2	0	0	0	0	0	0	0	0	**53.2**	0	0	0	0	0	0	
75.2	0	0	0	0	0	0	0	0	**75.2**	0	0	0	0	0	0	
106.2	0	0	0	0	0	0	0	0	**106.2**	0	0	0	0	0	0	
150	0	0	0	0	0	0	0	0	**150**	0	0	0	0	0	0	
Product F 24h									**Product F 48h**							
Concentrations	% Mortality								Concentrations	% Mortality						
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	
37.7	0	0	0	0	0	0	0	0	**37.7**	0	0	0	0	0	0	
53.2	20	0	20	0	0	40	13	13	**53.2**	20	0	20	0	0	40	
75.2	0	0	0	0	20	0	3	3	**75.2**	0	0	0	0	20	0	
106.2	0	0	0	0	0	0	0	3	**106.2**	0	0	0	0	20	0	
150	0	0	0	0	0	0	0	13	**150**	20	0	0	40	0	20	
Product G 24h									**Product G 48h**							
Concentrations	% Mortality								Concentrations	% Mortality						
0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	
37.7	0	0	0	0	0	0	0	0	**37.7**	0	0	0	0	0	0	
53.2	0	0	20	0	0	0	3	3	**53.2**	0	0	20	0	0	0	
75.2	0	20	0	0	0	0	3	3	**75.2**	0	20	0	0	0	0	
106.2	20	0	0	0	0	0	3	3	**106.2**	20	0	0	0	0	0	
150	20	20	0	0	20	0	10	13	**150**	20	20	0	0	20	20	

4.3 Rotoxkit M chronic

Table 26 and Table 27 show the overview of the measurements for the chronic rotifer test. Within the block the measured rotifers are shown, empty cells are empty test wells or wells left out of the calculation. From these measurements a total value, mean, standard deviation (stdev) and growth rate (r) are calculated. From the means the reproduction inhibition is calculated in percentage.

Table 26 Measurement overview of the chronic rotifer final test, including rotifer count, mean, growth rate and inhibition

Product A													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0		9	30		2		16	31	88	17.60	12.78	0.48	0
5.5	0	52	0	23	31	28	25	26	185	23.13	16.91	0.52	-31
8.8	2	42	13	27	23	16	27	38	188	23.50	13.13	0.53	-34
13.9	28	10	27	16	0	23	23	20	147	18.38	9.46	0.49	-4
22.1	33	16	13	0	8	18	11	21	120	15.00	9.74	0.45	15
35	30	6	12	29	11	43	11	17	159	19.88	12.77	0.50	-13
Product B													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0	6	23	24	27	21	27	18	3	149	18.63	9.24	0.49	0
3.2	6	29	18	48	20	30	31	26	208	26.00	12.15	0.54	-40
5	6	22	24	17	15	13	12	18	127	15.88	5.74	0.46	15
8	10	6	20	35	19	9	18	1	118	14.75	10.61	0.45	21
12.6	3	6	6	3	2	4	17	5	46	5.75	4.77	0.29	69
20	6	9	10	21	19	4	6		75	10.71	6.68	0.40	42
Product C													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0	1	15	14	1	29	33	40	14	147	23.42	14.38	0.53	0
75	11	27	19	2	21	0	17	18	115	14.38	9.38	0.44	39
150	7	0	0	3		27	21	0	58	8.29	11.16	0.35	65
0			39		29		34	32	134	33.50	4.20	0.59	0
75	28	86	73	21	20	75	15	14	332	41.50	30.75	0.62	-77
150	5	12	7	2	0	0	13	14	53	6.63	5.80	0.32	72
Product D													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0		24	28	38				25	115	33.44	6.40	0.58	0
150	20	25	36	0	30	21	32	41	205	25.63	12.61	0.54	23
150	42	34	40	17	36	0	37	48	254	31.75	15.65	0.58	5
150	12		33	0	25	26	34	23	153	21.86	12.08	0.51	35
150	52	35	60	35	24	65	38	21	330	41.25	16.16	0.62	-23
0	28	52		36	43			27	186	37.20	10.52	0.60	0

Table 27 Measurement overview of the chronic rotifer final test, including rotifer count, mean, growth rate and inhibition

Product E													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0	34	25	19	55	36		26	9	204	28.60	14.58	0.56	0
150	31	32	44	9	38	24	28	23	229	28.63	10.56	0.56	0
150	9	34	16	41	21	33	21	25	200	25.00	10.49	0.54	13
150	12	18	0	29	21	0	27	26	133	16.63	11.61	0.47	42
150	17	35	26	10	22	32	21	0	163	20.38	11.45	0.50	29
0	27	12	29	28	45	34	24	26	225	28.13	9.28	0.56	0
Product F													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0	22	29	34			27		6	118	23.60	10.74	0.53	0
3.8	7	0	0	0	16	0	37	17	77	9.63	13.21	0.38	59
5.3	0	9	2	13	40	25	0	0	89	11.13	14.58	0.40	53
7.5	0	23	0	2	5	3	0	0	33	4.13	7.85	0.24	83
10.6	4	2	24	4	0	18	0	2	54	6.75	9.07	0.32	71
15	0	0	3	11	13	14	0	10	51	6.38	6.21	0.31	73
Product G													
Conc.									**Total**	**Mean**	**Stdev**	**r**	**Inhibition %**
0	41			20	9		33	31	134	28.77	12.46	0.56	0
75	19	17	18	29	0	11	23	35	152	19.00	10.70	0.49	34
150	11	33	12		7	4	11	18	96	13.71	9.55	0.44	52
0	18	62	23	29	26	10	29	43	240	30.00	16.05	0.57	0
75	17	36	34	25	4	13	27	30	186	23.25	11.06	0.52	19
150	12	21		21	19	22	13	34	142	20.29	7.25	0.50	29

4.4 Algae growth inhibition

Table 28 and Table 29 show the growth inhibition overview of the algae final test in percentage inhibition, for measurement times 24, 48, 72 and 96 hours. In addition, if possible, the EC50's are shown including the 95% confidence intervals and with the standard deviations of the dataset (stdev).

Table 28 Growth inhibition overview of the algae final test, including EC50's, confidence intervals and standard deviations

	Product A; 1 day				Product A; 4 days			
ul/l	**T=24**	**T=48**	**T=72**	**T=96**	**T=24**	**T=48**	**T=72**	**T=96**
0	-3%	0%	0%	0%	0%	0%	0%	0%
4	85%	24%	11%	9%	-3%	0%	1%	2%
6.3	75%	24%	11%	9%	-1%	2%	4%	4%
10	24%	10%	5%	5%	10%	6%	6%	6%
15.8	35%	59%	45%	32%	46%	35%	20%	16%
25	85%	92%	95%	97%	57%	62%	58%	55%
Time (hr)	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**
24					20.1	18.1	22.3	16
48	13.6	11.0	16.8	32	20.8	19.8	21.7	9
72	16.3	15.4	17.2	11	22.9	22.2	23.6	5
96	16.1	10.9	23.6	7	27.5	16.6	45.5	4
	Product B; 1 day				**Product B; 4 days**			
ul/l	**T=24**	**T=48**	**T=72**	**T=96**	**T=24**	**T=48**	**T=72**	**T=96**
0	0%	0%	0%	0%	0%	0%	0%	0%
10	0%	0%	0%	0%	-2%	1%	1%	1%
17.8	-1%	1%	1%	1%	-5%	-1%	0%	0%
31.6	-8%	-2%	-1%	0%	-3%	-2%	-1%	0%
56.2	36%	36%	20%	17%	5%	0%	0%	1%
100	69%	82%	88%	89%	27%	38%	40%	37%
Time (hr)	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**
24	74.4	69.6	79.5	15				19
48	64.4	64.7	68.1	5				9
72	71.3	70.5	72.2	2				5
96	69.9	54.9	88.9	2				4
	Product C; 1 day				**Product C; 4 days**			
ul/l	**T=24**	**T=48**	**T=72**	**T=96**	**T=24**	**T=48**	**T=72**	**T=96**
0	0%	0%	0%	0%	0%	0%	0%	0%
12.6	3%	1%	2%	2%	4%	0%	2%	2%
17.7	18%	17%	15%	14%	30%	37%	40%	41%
25.1	64%	77%	82%	84%	73%	86%	91%	94%
35.4	100%	100%	100%	100%	100%	100%	100%	100%
50	100%	100%	100%	100%	100%	100%	100%	100%
Time (hr)	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**
24	22.7	22.3	23.2	18	20.7	20.1	21.2	14
48	21.6	21.4	21.9	7	19.2	18.9	19.6	7
72	21.4	21.2	21.6	4	18.6	18.4	18.8	4
96	21.5	18	25.6	3	18.9	16.7	21.3	3
	Product D; 1 day				**Product D; 4 days**			
ul/l	**T=24**	**T=48**	**T=72**	**T=96**	**T=24**	**T=48**	**T=72**	**T=96**
0	0%	0%	0%	0%	0%	0%	0%	0%
31.7	-1%	0%	0%	1%	-5%	-1%	0%	0%
50.2	-4%	-1%	0%	1%	1%	0%	1%	1%
79.6	-11%	-2%	0%	1%	13%	9%	9%	9%
126.2	23%	41%	45%	44%	73%	86%	91%	92%
200	96%	98%	100%	100%	100%	100%	100%	100%
Time (hr)	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**	**EC50 (ul/l)**	**95% LC**	**95% UC**	**Stdev (%)**
24	142.5	132.0	153.9	19.9	107.6	103.4	111.9	13
48	130.3	127.6	132.9	5.9	102.8	100.7	105.0	8
72	128.4	127.3	129.5	2.8	100.0	98.2	101.9	5
96	127.0	111.9	144.1	2.1	94.6	74.1	120.7	3

Table 29 Growth inhibition overview of the algae final test, including EC50's, confidence intervals and standard deviations

	Product E; 1 day				Product E; 4 days			
ul/l	T=24	T=48	T=72	T=96	T=24	T=48	T=72	T=96
0	0%	0%	0%	0%	0%	0%	0%	0%
31.7	-10%	-2%	-2%	-1%	-1%	-1%	-1%	-1%
50.2	-2%	1%	1%	1%	-5%	-3%	-2%	-2%
79.6	-11%	-3%	-1%	-1%	-7%	-4%	-2%	-2%
126.2	-9%	0%	1%	0%	-7%	-2%	0%	0%
200	5%	43%	45%	47%	8%	33%	37%	38%
Time (hr)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)
24				37				10
48				13				3
72				8				2
96				6				1
	Product F; 1 day				Product F; 4 days			
ul/l	T=24	T=48	T=72	T=96	T=24	T=48	T=72	T=96
0	0%	0%	0%	0%	0%	0%	0%	0%
12.5	-9%	-3%	-3%	-3%	-4%	-2%	-2%	-2%
22.2	-11%	-3%	-3%	-3%	-6%	-4%	-3%	-2%
39.5	-12%	-4%	-4%	-3%	-12%	-5%	-3%	-3%
70.3	3%	2%	2%	-1%	-10%	5%	6%	6%
125	36%	56%	58%	58%	41%	62%	65%	66%
Time (hr)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)
24				17				33
48				5				14
72				3				7
96				2				6
	Product G; 1 day				Product G; 4 days			
ul/l	T=24	T=48	T=72	T=96	T=24	T=48	T=72	T=96
0	0%	0%	0%	0%	0%	0%	0%	0%
31.7	-4%	-2%	-2%	-2%	-2%	-2%	-1%	0%
50.2	-1%	0%	0%	0%	-8%	-3%	-1%	-1%
79.6	-3%	-1%	-1%	-1%	-7%	-3%	-1%	0%
126.2	-2%	0%	0%	-8%	-7%	-1%	-1%	0%
200	10%	19%	19%	12%	34%	54%	56%	58%
Time (hr)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)	EC50 (ul/l)	95% LC	95% UC	Stdev (%)
24				23				28
48				8				11
72				5				5
96				4				4

5 Overview toxicity data

5.1 Toxicity data oxybenzone and octocrylene

Table 30 shows the toxicity database of oxybenzone and Table 31 shows the database of octocrylene.

Table 30 Toxicity database oxybenzone

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 31 Toxicity database of 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	

5.2 Toxicity data nano-ZnO and TiO_2

Table 32 shows the toxicity database of nano-ZnO and Table 33 shows the toxicity database of nano TiO_2.

Table 32 Toxicity database nano-ZnO

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 33 Toxicity database nano TiO2

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

5.3 Toxicity data non-nano-ZnO and TiO_2

Table 35 shows the toxicity database of non-nano-ZnO and Table 34 shows the toxicity database of non-nano TiO_2.

Table 35 Toxicity database non-nano-ZnO

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 34 Toxicity database non-nano TiO2

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

5.4 Toxicity database new organic UV filters

Table 36, Table 37, Table 38 and Table 39 show the toxicity databases of Tinosorb M, ensulizole, Uvinul A plus and Uvinul T150.

Table 36 Toxicity database Tinosorb M

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 39 Toxicity database ensulizole

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 38 Toxicity database Uvinul A plus

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 37 Toxicity database Uvinul T150

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