Inhibition Growth Effect of Sunscreen UV Filters on the Freshwater Microalga *Scenedesmus acutus*

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ABSTRACT

As the use of personal cosmetic care products (PCCPs) with organic ultraviolet (UV) filters are increasing, so is the exposure risk of these compounds to aquatic ecosystems. This study focuses on the inhibition growth effect of 4 common UV filters found in PCCPs on the freshwater microalga, *Scenedesmus acutus.* Fluorescence of chlorophyll was used as a measure of growth during a 96-h exposure period, and growth inhibition was utilized as the endpoint. All UV filters inhibited growth with increasing concentration, except for avobenzone and octisalate, which did not decrease reproduction at any treatment level up to water solubility. Lowest observed effect concentrations without UV light for atrazine, oxybenzone, and homosalate were 117 µg/L, 1875 µg/L, and 100 µg/L, respectively. Homosalate was the most toxic followed by oxybenzone with avobenzone and octisalate likely to be not toxic to *S. acutus*. The results from this study indicated that emissions of these UV filters into waterways could have harmful effects on biota.

1. Introduction

Concern regarding the environmental impacts of ultraviolet (UV) filters found in personal cosmetic care products (PCCPs) in aquatic ecosystems are increasing due to recent findings of their environmental occurrence and toxicity. UV filters, commonly referred to as sunscreen compounds, can be found in lotions, shampoos, and hair products as well as in insecticides and plastic packaging (Silvia Díaz-Cruz, Llorca and Barceló, 2008, pp. 873-887; Gago-Ferrero, Díaz-Cruz and Barceló, 2012, pp. 2597-2610). As the use of sunscreen products has become more prevalent, aquatic systems are at increased risk of exposure to UV filters (Paredes et al, 2014, pp. 44-50). These substances can pollute waterways through many different mechanisms, such as direct exposure from swimmers during recreational activities or indirect point source pollution from sewage plants (Verlicchi, Al Aukidy and Zambello, 2012, pp. 123-155). Several studies have measured multiple UV filters at detectable levels at different locations ranging from ng/ L to µg/L concentrations with oxybenzone being the highest measurable UV filter at 44.0 µg/L from a river water sample (Kasprzyk-Hordern, Dinsdale and Guwy, 2009, pp. 363-380; Ramos et al, 2015, pp. 278-311). The highest concentrations of sunscreens were measured during the summer months

when human populations around waterways were highest (Bratkovics et al, 2015, pp. 370 377).

There are two types of UV filters found in sunscreen products: physical and chemical organic compounds. The most common organic chemical compounds used are benzophenone derivatives and salicylate compounds, which convert sunlight into heat. Physical compounds, such as titanium dioxide and zinc oxide, reflect and disperse the UV rays (Serpone, Dondi and Albini, 2007, pp. 794-802). Both types of sunscreens are highly lipophilic and can lead to possible bioaccumulation in both biota and human tissues (Gago-Ferrero, Díaz-Cruz, and Barceló, ,2015, pp. 518-525; Schlumpf et al, 2010, pp. 1171-1183); however, studies have focused more on organic chemical sunscreens due to their endocrine disrupting capabilities (Morohoshi et al, 2005, pp. 457-469; Suzuki et al, 2005, pp. 9-17). Park et al (2017, pp. 57-63) tested ethylhexyl methoxycinnamate, octocrylene, and avobenzone on the crustacean *Daphnia magna* and found that avobenzone was the most toxic at EC_{50} of 1950 μg/L following ethylhexyl methoxycinnamate at 2730 μg/L and octocrylene at 3180 μg/L. Another study found that oxybenzone caused coral planulae mortality at concentrations of LC_{50} of 3100 μg/L and 1680 μg/L after 8-h exposure in light and darkness, respectively (Downs et al, 2016, pp. 265-288).

Phytoplankton organisms play an essential role in aquatic ecosystems and are often used when evaluating the ecotoxicological effects of a substance. Rodil et al (2009, p. 1513) found that the UV filters EHMC, padimate O, and oxybenzone inhibited the growth of the green alga *Scenedesmus vacuolatus* ranging from 170 - 760 μg/L whereas octocrylene and 4-methylbenzylidene camphor (4MBC) did not have a toxic effect. Another study focusing on the effect of UV filters on the green alga Desmodesmus suspicatus found that growth was inhibited after 72-h exposure to oxybenzone, 3- benzylidene camphor (3BC), 4MBC, and EHMC with EC_{10} ranging from 210-560 μg/L (Sieratowicz et al, 2011, pp. 1311-1319).

To further investigate the toxic potential of UV filters in aquatic ecosystems, the freshwater green alga *Scenedesmus acutus* was used to test the inhibition growth effect of oxybenzone, avobenzone, homosalate, and octisalate. The herbicide atrazine was also used as a positive control to verify the experimental design before testing the UV filters. The purpose of this study was to test and compare the inhibition growth effect of different sunscreen compounds on microalgae in order to assess the potential risk to aquatic ecosystems. These results will provide the foundation for later testing including UV light.

2. Experimental Details

Algae Stock Culture:

Original culture of *Scenedesmus acutus* was obtained from a stock agar plate culture at Oklahoma State University. *S. acutus* cultures were maintained in 250 mL Erlenmeyer flasks filled with 200 mL of sterile media obtained from Carolina ®. Algal cultures were incubated at room temperature (28 ± 1 °C) under 24 W 6400K lighting with a 12-h light/dark

photoperiod. Flasks were aerated continuously using tubes connected to an air generator. Cultures were subcultured into fresh media every 5-7 days during logarithmic growth stage.

Preparation of Test Concentrations:

Atrazine was dissolved in acetone and diluted to testing concentrations in algae media. There was a maximum of 0.05% (v/v) of acetone in testing medium. Oxybenzone, avobenzone, homosalate and octisalate were dissolved in DMSO and followed identical dilution process. All testing concentrations were based on experiments previously conducted for this study.

Growth Inhibition Assays:

The growth inhibition assays were in accordance to OECD guidelines (OECD, 2006). Algal stock culture was centrifuged at 1,000 rpm for 10 minutes, and supernatant was poured off and replaced with fresh sterile media. Cells were diluted to an initial concentration of approximately 1 $X10^4$ cells/mL. Nominal spiking concentrations were as followed: atrazine (26.7, 40, 60, 90, 135, and 200 μg/L); oxybenzone (853, 1109, 1442, 1875, 2338, and 3169 μg/L); avobenzone, homosalate, and octisalate (100, 250, 625, 1562, 3906, and 9776 μg/L).

Assays were conducted in 5-mL tubes that were inoculated with 3500 mL algal media solution. Tubes with algae and media, media and toxicant, and media only served as control and blank solutions. Each experimental group was replicated six times making a total of 54 test tubes. Tubes were covered with a translucent and gas permeable film to avoid evaporation of solution. Tubes were incubated at the same conditions as stock cultures for a 96-h period and vortexed twice a day. Chlorophyll a measurements were collected using a spectrofluorometer in relative fluorescence units (RFU) and recorded at hours 0, 24, 48, 72, and 96. Growth response (μ) was calculated using the following equation:

$$\mu = \frac{N_2 - N_1}{N_1}$$

Relative inhibition percentages were also estimated based by normalizing each tubes growth rate to the average control growth rate for that test. Analysis of Variance with a Dunnett's mean separation test (comparing each treatment to the control) was used to identify treatment levels that had statistical significant inhibition of growth (SPSS v23). The lowest observed effective concentration (LOEC) was identified as the lowest concentration that was statistically different from the control ($p<0.05$). If significance was identified and greater than 50% inhibition was reached, IC_{50} values (substance concentration at which 50% of growth inhibition of population occurred) for each compound was determined based on a 4-compartment log-logistic model (Sigma Plot v10.0).

3. Results

Growth rates and growth inhibition percentages are summarized in Table 1, and the LOEC and IC_{50} values are reported in Table 2. The LOEC for atrazine, homosalate, and

oxybenzone was 117 µg/L, 100 µg/L, and 1875 µg/L, respectively. Inhibition of atrazine, oxybenzone, and homosalate were concentration-dependent. Atrazine growth inhibition percentages ranged from 14.2% to 80.1% after exposure to concentrations ranging from 40 µg/L to 200 µg/L (Fig 3a). Growth inhibition percentages for oxybenzone ranged from 42.2% to 98.8% at concentrations of 1875 µg/L to 3169 µg/L (Fig 3b). Homosalate growth inhibition percentages ranged from 23.5% to 71.1% after exposure to concentrations ranging from 100 µg/L to 9766 µg/L (Fig 3d). The most toxic compound was atrazine (IC_{50} 93µg/L) followed by homosalate (IC_{50} 404 µg/L) and oxybenzone (IC_{50} 1940 µg/L). Avobenzone and octisalate did not inhibit growth at very high concentrations and therefore are not likely toxic to *S. acutus* (Figure 3c and 3e). Exponential growth was observed for all substances over a 96-h exposure period (see Figure 3 for example).

Table 1. Growth rate and inhibition growth percentages of atrazine, oxybenzone, and homosalate after 96-h exposure.

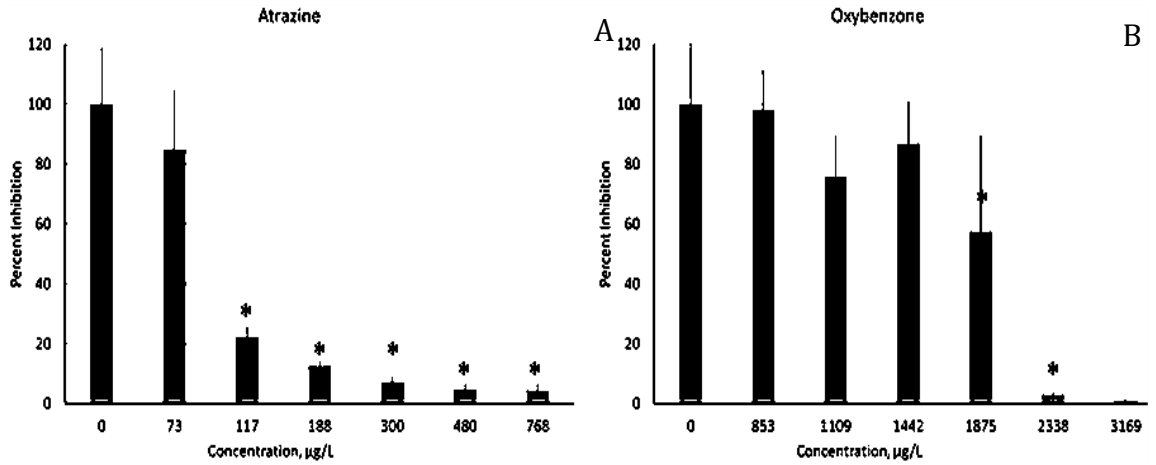
Chemical	Control	Treatment A	Treatment B	Treatment C
Atrazine				
Growth Rate (µ)	50.03	42.95	27.39	9.94
Inhibition (%)	0	14.16	45.25	80.12
Homosalate				
Growth Rate (µ)	115.79	77.17	39.65	33.42
Inhibition (%)	0	33.36	65.76	71. 14
Oxybenzone				
Growth Rate (µ)	95.58	73.45	55.05	1.15
Inhibition (%)	0	23.15	42.4	98.79

Note: Treatment A, B, and C represent atrazine (40 µg/L, 90 µg/L, and 200 µg/L), oxybenzone (1109 µg/L, 1875 µg/L, and 3169 µg/L), and homosalate (250 µg/L, 1562 µg/L, 9766 µg/L).

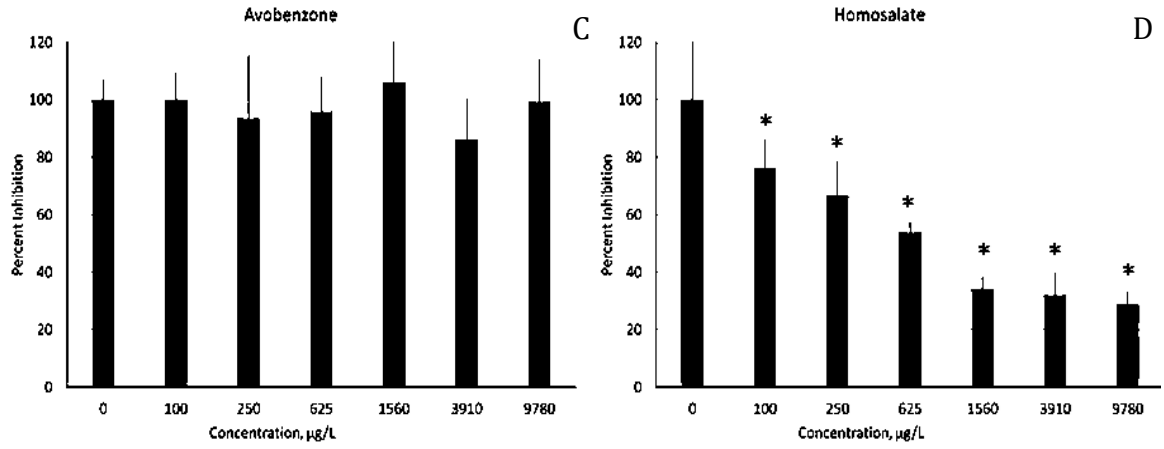
Table 2. NOEC, LOEC, and IC_{50} values of atrazine, homosalate, and oxybenzone after 96-h exposure.

Chemical	LOEC	IC_{50}
Atrazine	117	93
Homosalate	100	404
Oxybenzone	1875	1940

Note: All values reported as µg/L.


Atrazine
A
Percent Inhibition
120
100
80
60
40
20
0
0
73
117
188
300
480
768
Concentration, µg/L
Oxybenzone
B
Percent Inhibition
120
100
80
60
40
20
0
0
853
1109
1442
1875
2338
3169
Concentration, µg/L

*


Avobenzone
C
Percent Inhibition
120
100
80
60
40
20
0
0
100
250
625
1560
3910
9780
Concentration, µg/L
Homosalate
D
Percent Inhibition
120
100
80
60
40
20
0
0
100
250
625
1560
3910
9780
Concentration, µg/L

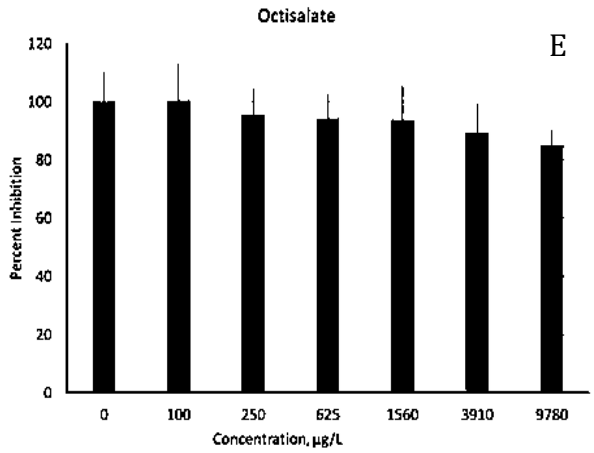


Fig 1. Percent growth inhibition (%) of various concentrations (µg/L) for atrazine, oxybenzone, avobenzone, homosalate, and octisalate. The 95% confidence intervals are depicted by error bars. (*) denotes statistical significance from the control.

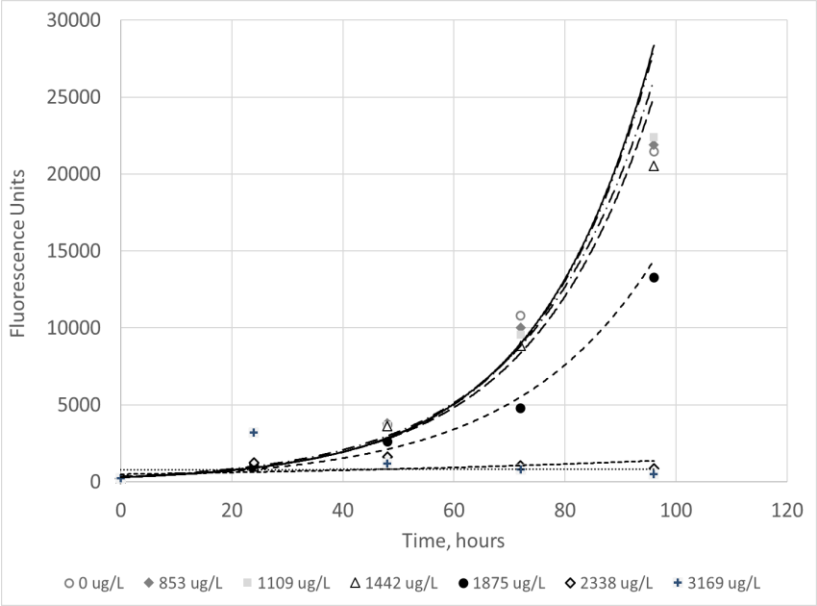


Fig 3. Example of algal growth over the 96h test by treatment concentration. Data shown for oxybenzone.

4. Discussion and Conclusions

The purpose of this study was to understand further how UV filters might be impacting aquatic environments using the freshwater green alga *S. acutus.* We were able to calculate the LOEC and IC_{50} for atrazine, homosalate, and oxybenzone, and found no inhibition growth effect for avobenzone and octisalate.

Atrazine

Growth rates were reduced at all tested concentrations (Figure 3). The results indicated that reproduction and chl-a production could be inhibited at environmentally relevant concentrations (Russo and Lagadic, 2004, pp. 303-311). Berad et al (2003, pp. 935-944) reported similar results (IC_{50} 56 µg/L) for *S. acutus*; however, a slightly higher IC_{50} was found in this study with an IC_{50} of 96 µg/L.

Oxybenzone

Oxybenzone was the least toxic out of the 3 compounds that induced inhibition with an IC_{50} of 1940 µg/L. Sieratowicz et al (2011, pp. 1311-1319) found the green alga *Desmodesmus subspicatus* to be sensitive at an IC_{50} of 9600 µg/L. Although the IC_{50} derived from this study is high, oxybenzone has been found to affect other aquatic organisms at more environmentally relevant concentrations. One study found oxybenzone had an IC_{50} of 360 µg/L on the green algae *Scenedesmus vacuolatus*. Sensitivity to oxybenzone may depend on the organism's ability to uptake the UV filter as was seen in a study comparing the bioavailability of oxybenzone between a green alga and cyanobacterium species (Mao et al, 2017, pp. 1-8).

Homosalate

The growth rate was also significantly reduced with increasing concentration of the compound; however, homosalate required slightly higher levels of the substance to induce inhibition than atrazine. The inhibition growth effect of homosalate on microalgae has not been studied yet; however, homosalate has been shown to have endocrine disrupting capabilities and has the potential to bioaccumulate due to being highly lipophilic. Mao et al (2017, pp. 1-8) found weak estrogenic activity of homosalate in human breast cells. Multiple studies have found homosalate at detectible levels and as high as 2.8 µg/L (Ramos et al, 2015, pp. 278-311; Tsui et al, 2014, pp. 55-65); however, homosalate was found to have an IC_{50} of 404 µg/L in this study. Although the IC_{50} is higher than environmentally relevant concentrations, it is still important to understand the potential harm this UV filter could have on aquatic organisms.

Avobenzone and Octisalate

Avobenzone and Octisalate did not significantly reduced growth rates at any concentration. Concentration could not go higher as the solubility of the compounds in water was reached (Benazzouz et al, 2014, pp. 101-109; Yousef, Haidar and Al-Khayat, 2013, pp. 254-258). No other studies have been conducted on the inhibition growth effect of avobenzone and octisalate; thus, no other comparisons can be made.

The tested UV filters required relatively high concentrations to inhibit growth of *S. acutus* at and did not show effects at presumed environmental levels of less than 50 µg/L. Future work will focus on photoinduced toxicity of UV light based on previous studies that have found increased toxicity after exposure to light (Downs et al, 2016, pp. 265-288;

Kasprzyk-Hordern, Dinsdale and Guwy, 2009, pp. 363-380). However, exposure to UV light has been shown to increase the degradation rate of many UV filters and thus reducing the potential toxicity (Rodil et al, 2009, pp. 1513). Thus, although the presence of UV light may result in enhanced toxicity, there is likely a trade-off towards reduced exposure.

5. Summary

The purpose of this study was to test the inhibition growth effect of 4 common UV filters found in PCCPs on the freshwater microalga, *Scenedesmus acutus.* Assays were run in 5-mL test tubes under normal growth lighting for a 96-h period. Growth inhibition was utilized as the endpoint. Homosalate was the most toxic out of the UV filters followed by oxybenzone with avobenzone and octisalate likely to be not toxic to *S. acutus*. The results from this study indicated that emissions of these UV filters into waterways could have harmful effects on biota.

6. Appendices

Papers Published

- Poster: "Inhibition Growth Effect of UV Filters on Microalgae," ITP Symposium, Stillwater, OK, Spring 2018
- Poster: "Inhibition Growth Effect of UV Filters on Microalgae," Karen L. Smith Undergraduate Research Symposium, Stillwater, OK, Spring 2018

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