Effects of oxybenzone (Benzophenone-3) on phytoplakton growth and zooplakton mortality in a fresh water environment

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Abstract

Oxybenzone, also known as Benzophenone-3, is an organic compound that, due to its UV filter properties, it can be found in plastics and personal-care products, especially sunscreens. As skin cancer diagnosis increases over the years, sunscreen sales have also being on the rise. It has been found that after oxybenzone washes off from the human body, it enters the aquatic systems affecting organisms, such as phytoplankton, algae and corals, within that environment. Therefore, the aim of this study was to determine if exposure to oxybenzone would reduce chlorophyll *a* concentrations in phytoplankton, as well as a reduction of zooplankton populations within a freshwater environment. Filtered lake water with phytoplankton was placed in two treatment groups of 20 mg/L and 220 mg/L concentrations of oxybenzone. There were 4 replicates of each treatment group; along with 2 control groups containing no oxybenzone. Cladocerans from 3 to 5mm of length where exposed to oxybenzone concentrations, from 0.01 to 20mg/L, for 48 hours. Percent mortality was recorded. Chlorophyll *a* readings showed no effects on phytoplankton, as well as Cladocerans showed no significant percent mortality when exposed to different concentrations of oxybenzone. These results show that further research should be conducted on bioaccumulation of oxybenzone in fresh water environments.

Key words: Oxybenzone, Benzophenone-3, bioaccumulation, ecotoxicity, cladocera, phytoplankton.

Introduction

In the last sixty years, UV light exposure and protection have been studied as a preventive method against skin cancers, such as melanoma or basal cell cancer. Because coastal tourism is increasing as well (Honey M., 2007), UV filter sales have grown by $75M since 2003, especially in Europe and Latin America (ACnielsen, 2004; Poigerm et al., 2004; Tovar-Sanchez et al., 2013). Although the main target of sunscreens is UV protection, studies have found negative effects to humans and the environment, caused by active ingredients in sunscreens (Poigerm et al., 2004; Tovar A., 2013; Downs, 2015; Sieratowicz, 2011).

Recent studies have introduced the idea that oxybenzone, one of the most common active ingredients, may be harmful to humans. Oxybenzone, also known as benzophenone-3 (BP-3), was detected in 76.5% of breast milk samples in 78.8% of women who reported use of product(s) containing UV filters (Schlumpf, 2008). This chemical was also sampled from plasma, with maximun concentrations of 187 ng/mL in women and 238 ng/mL in men (Jonjua, 2008). Jonjua also found that ozybenzone levels in urine were higher in men than women, with mean concentrations of 44 ng/mL and 81 ng/mL respectively.

Oxybenzone is not only harmful via direct contact; it can also have negative effects in the environments and the aquatic organisms found within these areas. Traces of oxybenzone can be detected within marine environments (Downs et al. 2015), as well as fresh water environments through direct (swimming and bathing) and indirect sources (industrial and untreated wastewaters) (Giokas et al. 2007; Balmer et al. 2005). It has been identified that corals are showing increased bleaching in response to concentrations of oxybenzone in marine waters (Downs et al. 2015; Danovaro et al. 2008). Another study found that dissolutions of different commercial sunscreens in seawater inhibit phytoplankton (*Chaetoceros gracilis*) growth (Tovar-

Sanchez et al., 2013). Oxybenzone was also found to inhibit phytoplankton (*Desmodesmus subspicatus*) growth in a freshwater environment (Sieratowicz, 2011). Sieratowicz et al. (2011) also showed no significant immobilization or reproduction effects of oxybenzone on crustacean zooplankton (*Daphnia magna)* populations. Despite efforts to understand the effects of oxybenzone, there is still little scientific literature regarding this substance and its effects on trophic levels within fresh water environments.

Considering the increased use of sunscreens in cosmetic products, their possible effects within aquatic systems, and the limited scientific literature of oxybenzone in trophic levels in fresh water, the aim of this study is to analyze the effects of oxybenzone concentrations within the first and second trophic levels of freshwater environments. Based on previous studies (Downs, 2015; Sieratowicz, 2011; Tovar-Sanchez et al., 2013), I hypothesize that the oxybenzone concentrations will reduce not only chlorophyll *a* concentrations but also zooplankton populations within a freshwater environment.

Methodology

Sampling Site

Sampling took place in the remote south region of the University of Notre Dame Environmental Research Center (UNDERC), between Wisconsin and Michigan. Fresh water pumped from Tenderfoot Lake, was used to conduct water quality analysis; zooplankton samples were also collected from this lake. Tenderfoot Lake has public access, yet swimming is not allowed.

Experimental Design

I. Phytoplankton

Concentrations of 220*mg/L* and 20*mg/L* of oxybezone were added to 2L aquariums (Downs, et al. 2015), along with a control containing no ozybenzone. There were four replicates of each treatment of oxybenzone, along with two control aquariums to ensure equal environmental conditions. Weekly measurements were taken of dissolved oxygen, temperature, and pH, using a dissolved oxygen and temperature meter (YSI, Yellow springs, Ohio 45387 USA) and pH meter (Eco-testr pH1, Singapore). Treatments were left undisturbed for three weeks. Physical changes of phytoplankton cells, such as size, abundance, and structure, were observed at end of the experiment.

To determine changes in phytoplankton abundance, solutions of lake water and oxybenzone were homogenized, and 100ml subsamples were vacuum filtered with Whatman GF/F glass fiber filters. Filters containing phytoplankton were placed in dark canisters with 25 ml of methanol to extract the pigments from collected cells. Samples were refrigerated in the dark for 24 hours. Chlorophyll *a* fluorescence of each sample was measured using a Trilogy fluorometer (Turner Designs, Sunnyvale, CA).

II. Cladocerans

Using integrated tow nets, zooplankton were collected at a depth of 7m. Cladocerans of between 3 and 5mm length were sorted out from the samples. 10 individuals were placed in jars with 25ml treatments of 20*mg/L*, *0.1mg/L*, or *0.01mg/L* of oxybenzone, and two control groups containing no oxybenzone (Downs, 2015; Sieratowicz A., 2011). The experiment was done with three replicates of each treatment level over a period of 48 hours (Sieratowicz A., 2011). At the end of the 48 hours, live/dead counts, as well as noticeable physical/ behavioral changes, such as size and movement, were recorded.

Statistical Analysis

A one-way Analysis of Variance (ANOVA) test was used to determine the significance between Chlorophyll *a* concentrations and high/low oxybenzone concentrations, as well as to determine if there was a significant difference of pH, temperature, or dissolved oxygen between the treatment groups. A one-way ANOVA test was also used to analyze the relationships between percent mortality of cladocerans and oxybenzone concentrations. All statistical analyses were completed using Systat (version 13.0).

Results

Oxybenzone showed no significant effect on chlorophyll *a* concentrations (n=10 $p>0.439$; df 2, 7; f=0.928). Replications within both high and low treatments were similar (Figure 1). No significant changes were found in water quality, with average temperatures of 21.1 °C, and DO of 4.94 mg/L; pH remained constant with an average of 8.1 (Table 1).

This study also found there was no significant relationship between oxybenzone and percent mortality of cladocerans (n=11 $p>0.258$; df 3, 7; f=1.674). 0.01mg/L and 20mg/L concentrations showed the greatest mortality (17%) and decreased with lower oxybenzone concentrations (7%, 0.01 mg/L; 0%, control) (Figure 2).

The hypothesis, which stated that oxybenzone concentrations will reduce chlorophyll *a* and zooplankton populations, was not supported.

Discussion

The objective of this experiment was determine if there was a direct relationship between phytoplankton abundance, zooplankton mortality and concentrations of oxybenzone.

Phytoplankton

When examining the samples after filtration, treatment groups were noticeably greener in appearance compared to control samples. In contrast, chlorophyll readings showed there was not a significant difference between treated and control samples. However, there was a slight decrease of dissolved oxygen in the treatment groups, which suggests a potential increase in productivity. However, treatments with higher concentration of this compound had a mean pH of 8.0, slightly lower than the control group which measured 8.4. Even though oxybenzone has a pH of 7.8, a decrease of pH suggests that higher concentrations of this compound could reduce pH in alkaline aquatic systems, as well as an increment of decomposition processes. Prolonged studies should be conducted to more adequately investigate the effects of oxybenzone on water quality parameters.

The methods used during this study examined the chlorophyll fluorescence within water samples with oxybenzone. While it can be assumed than oxybenzone's photosensitizing abilities would not obstruct fluorometric readings, other pigments or particles present could have caused interference. Water obtained for the tests was pumped through an aquatic lab, which could have reduced the initial amount of plankton present in the samples due to filtration, as well as introduced potential copper contamination due to transportation through pipes. Further studies should be conducted to better understand how oxybenzone could potentially affect phytoplankton chlorophyll *a* production, as well as phytoplankton populations.

Zooplankton

This study showed no effect of oxybenzone on zooplankton populations. A similar study using cladocerans (D. magna) examined the effects due to oxybenzone exposure with 0.005mg/L to 0.5mg/L concentrations (Sieratowicz A. 2011). Having used concentrations both higher and lower than Sieratowicz A. (2011), results with this study are consistent with previously published literature. This study found that adult cladocerans, measuring 3mm to 5mm, of length can survive concentrations of oxybenzone up to 20mg/L in fresh water environments, with the posibility of surviving higher concentrations.

Although this study did not examine higher throphic levels, Balmer et al. (2005) found that in several Swiss lakes, oxybenzone was the second most frequent UV filter detected in the tissues of large fish, such as whitefish, roach, and perch. This suggests that cladocerans could be key organisms for bioaccumulation of oxybenzone within higher trophic levels in lakes, as well as benthic organisms. Considering this relationship, future studies should be conducted to explain how does oxybenzone bioaccumulate, as well as the possibility of bioaccumulating in benthic organisms.

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Tables and Figures

Table 1: Mean values of water quality in each phytoplankton treatment group. Treatment groups with concentrations of oxybenzone show higher readings of dissolved oxygen and pH. All p values greater than 0.74.

Concentration (mg/L)	Temperature(°C)	pH	Dissolved oxygen(mg/L)
0	21.0	8.4	5.30
20	20.7	8.1	4.79
220	21.4	8.0	4.90

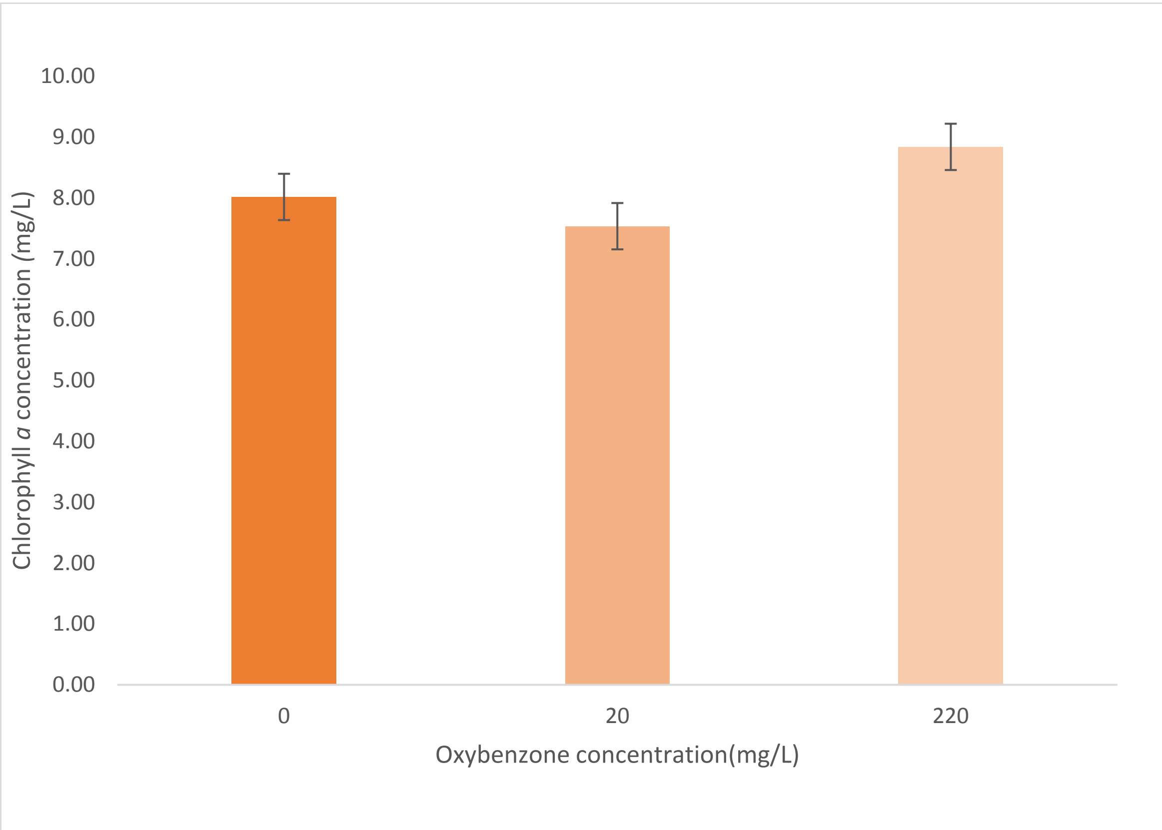

Figure 1: Chlorophyll concentrations after exposure to oxybenzone for five weeks (n=10). Bars express mean values with Standard Error. (f=0.928)

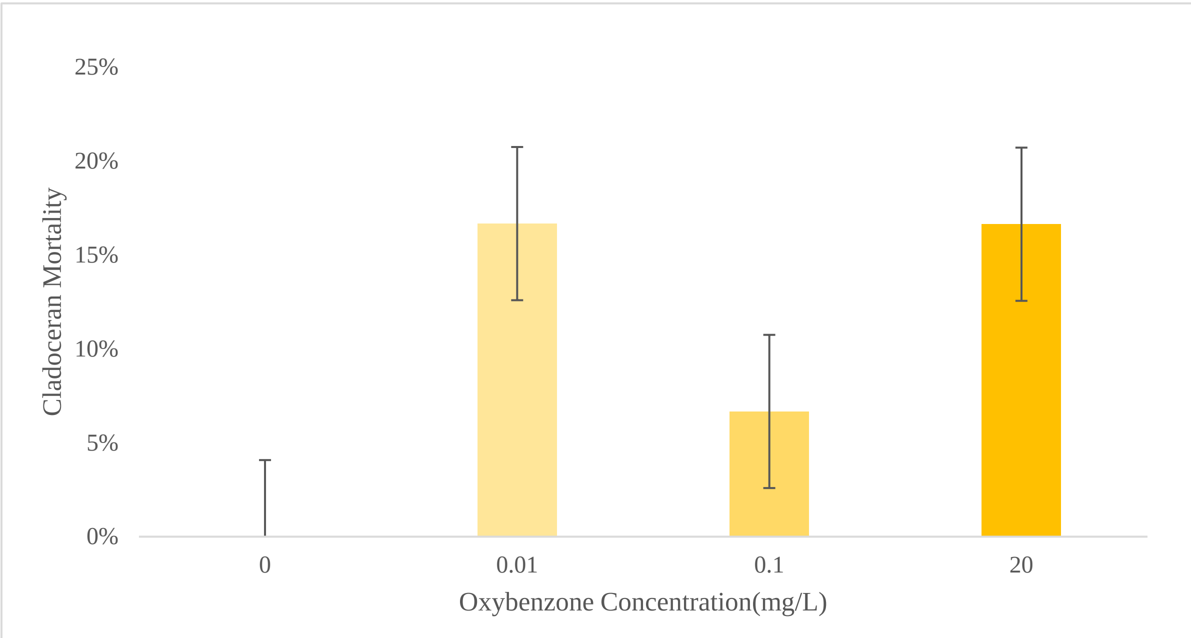

Figure 2: Mean percent mortality of cladocerans at different concentrations of oxybenzone for 48 hours. Bars express mean values with Standard Error. (p>0.258, f=1.674)