Aquatic Toxicity of Sunscreen Ingredients in Freshwater Ecosystems

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Abstract

The impact of the main inactive ingredient in sunscreens was tested on freshwater ecosystems using *Daphnia magna* as a model species. My previous experiments (2023) showed that "reef-friendly" non-nano zinc oxide caused the most harm to *Daphnia magna*, and further experimentation has been done to understand the impact of active versus main inactive sunscreen ingredients on the results. In this year's experiment, four non-nano zinc oxide sunscreens were tested, three water-based, one alcohol-based, and pure zinc oxide powder at concentrations of 1 and 2 mg/L. I hypothesized that the non-nano zinc oxide powder would have the least detrimental effects to the *Daphnia* due to the lack of inactive ingredient, while the alcohol-based sunscreen would be the most harmful due to the presence of alcohol as main inactive ingredient. The mortality of the *Daphnia magna* was recorded and contrary to my hypothesis, the results revealed that solubility of the sunscreen was the main factor contributing to mortality, regardless of the main inactive ingredient, as its difference was statistically significant compared to the control. In addition, the zinc oxide powder proved itself to be harmful, exposing the harm of the substance on freshwater environments. The supposed "environmentally friendly" UV filter was not safe to *Daphnia magna* and advertising regarding sunscreens can be misleading as the full scope of the toxicity of zinc oxide beyond coral reefs is neglected. Marketing as "environmentally friendly" should be regulated further to improve awareness of the consumer's impact on the natural world.

Keywords: **Cosmetic pollution, Environmental toxicology, Sunscreen safety**

Introduction

According to the National Cancer Institute, 30 percent of women and around 15 percent of men use sunscreen on a regular basis (NIH). While the health benefits of sunscreens have been proven, growing studies regarding impacts on the environment have led to concern over safety in certain ecosystems (Chien, 2022). Sunscreen finds its way into the environment through multiple routes. On top of washing off people's skin after application, sunscreen also gets absorbed into the skin and can be "detected in urine within only 30 minutes of application, enter[ing] sewer or septic tanks when people flush the toilet or wash off sunscreen in the shower." (Hamblen, 2022) Ultimately, "In towns near bodies of water without sophisticated sewage treatment and water management systems, sunscreen pollution is inevitable" (Hamblen). Concentrations of sunscreen in environments vary substantially; However, most existing studies have tested its impact on only saltwater ecosystems. Chemical UV filters as well as nano-zinc oxide have been found to bleach and harm coral's DNA and cause reproductive harm for other salt-water organisms due to small particle size (NOAA). Thus, non-nano zinc oxide, which has a particle size too large to enter inside of coral, has been praised as the more "environmentally friendly" type of sunscreen active ingredient and has been marketed accordingly.

Overlooking the impacts of products on freshwater environments neglects aspects of sunscreen safety. In my previous (2023) study it was determined whether these findings translated to freshwater ecosystems using *Daphnia magna*. Three types of sunscreens were tested, one with chemical active ingredients, one with nano-zinc oxide, and one with non-nano zinc oxide, and the data recorded was surprising. Instead of seeing lower mortality with non-nano zinc, that sunscreen killed the most *Daphnia* in the shortest amount of time. This finding is partially in line with the limited pre-existing research. A previous study on nano and non-nano zinc oxide found that "the toxicity in the acute tests was independent of particle size" (Wiench et al, 2009). However, some studies have still found non-nano zinc oxide to be less harmful.

Findings of high mortality due to non-nano zinc oxide led to two possible explanations.

a) modern toxicology and advertising has been neglecting the ill-effects that non-nano zinc oxide

poses to freshwater environments or b) the inactive ingredients in the sunscreen were to blame, as past experiments have been focused solely on the active ingredients of sunscreens, using the pure ingredient instead of full lotion in tests. Thus, in this experiment, I explore the effect of inactive ingredients in non-nano zinc oxide sunscreens on the mortality of freshwater invertebrates, establishing whether harm stems from the active or inactive ingredients of sunscreen lotion.

Question

How does the main inactive ingredient and concentration of non-nano zinc oxide sunscreens affect mortality of freshwater invertebrates?

Hypothesis

If exposed to water-based non-nano zinc oxide sunscreens, there will be a lower mortality for *Daphnia magna* than alcohol-based sunscreen. Furthermore, the powder zinc-oxide (no inactive ingredients) will have the lowest mortality. Finally, if two concentrations are tested, then the higher concentration will be more lethal.

Methods

Concentrations were decided based on previous studies, "lethal concentrations were determined to be in the low mg/L range" (Boyd, 2021). Stock solutions of 2mg/L for each UV filter active ingredient(s) were created by adding 32 mg of sunscreen #1, 31 mg of sunscreen #2, 37 mg of sunscreen #3, or 9 mg of ZnO powder to each Spring Water. 100mL of each stock solution were transferred

into plastic storage containers to create the higher concentration test samples (2mg/L, 3 replicates each). 50mL of each stock solution were transferred into plastic storage containers, then diluted with 50mL of spring water to create the lower concentration test samples (1mg/L, 3 replicates each). 100mL of Spring Water was added to plastic storage containers to create the control sample (0mg/L, 3 replicates). 5 similar sized *Daphnia magna* (Carolina Biological Supply) were added into each storage container using a pipette. The number living *Daphnia* was recorded over 72 hours. *Daphnia* in the samples were fed by transferring 1mL of water daily containing bacteria. The experiment was performed with a uniform type of water, sunscreen SPF, and in a climate-controlled building (77° F).

Fig. 1. Experimental set-up

Fig. 2. Sunscreens Tested

Four sunscreens, each with non-nano zinc oxide active ingredient and one zinc oxide powder were tested. Sunscreens #1, #2, and #4 were water based, while sunscreen #3's first inactive ingredient is alcohol.

Statistical Analysis

A one-way ANOVA test for each concentration was performed to compare the effect of non-nano zinc oxide sunscreen types on the mortality of *Daphnia magna* after 72 hours. The ANOVA single factor method in Microsoft Excel's analysis tool pack was used. These first ANOVA tests revealed that there was a statistically significant difference in mortality between at least two groups for both low and high concentration samples (reference table below). Additional ANOVA tests were performed, separating the sunscreens based on solubility. Comparing the insoluble sunscreens with the control revealed that there was no significant statistical difference between the results ($p>0.05$), while comparing the soluble sunscreens with the control revealed that there was a significant statistical difference between the results ($p<0.05$).

Data Analysis

The control sample resulted in the smallest number of deaths in the experiment, revealing that the deaths in the other solutions were caused directly by the sunscreens. Sunscreens 1 and 2 (insoluble) are statistically indistinguishable from the results on the control (Figure 3/ Figure 4). Their insolubility also resulted in very similar low mortality for both concentrations. Both Sunscreens 3 and 4 (which *did* fully dissolve) had very similar data, resulting in high mortality of *Daphnia*, despite their different inactive ingredients (#3's is alcohol while #4's is water) (Figure 5/ Figure 6). The higher

Results

1	2	3	4	ZnO powder
• Sunscreen did not dissolve in solution • In first trial*, babies survived • Both concentrations resulted in very similar mortality	• Sunscreen did not dissolve in solution • In first trial*, babies survived • Both concentrations resulted in very similar mortality	• Sunscreen did dissolve in solution • In first trial*, babies died • Higher concentration resulted in higher mortality	• Sunscreen did dissolve in solution • In first trial*, babies died • Higher concentration resulted in higher mortality	• Powder did not fully dissolve, Undissolved ZnO visible • In first trial*, babies survived • Higher concentration resulted in higher mortality • Bodies of the daphnia appeared very white

*Two trials of the same experiment were done since the daphnia in the first trial were at the end of their natural life cycle (and reproduced), which invalidated the first experiment

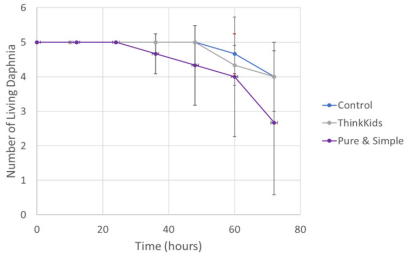

Fig. 3. Number of Living *Daphnia* in 2mg/L Insoluble Sunscreen Solution. Standard deviation error bars are present for figures 3-8

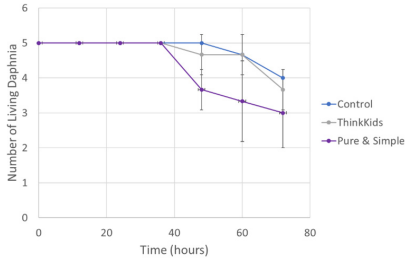

Fig. 4. Number of Living *Daphnia* in 1mg/L Insoluble Sunscreen Solution.

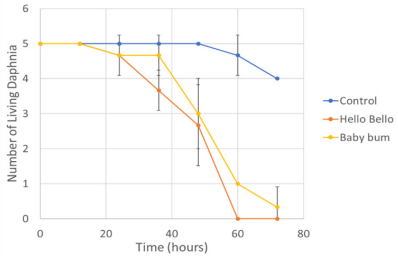

Fig. 5. Number of Living *Daphnia* in 2mg/L Soluble Sunscreen Solutions

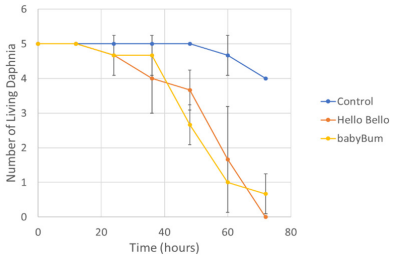

Fig. 6. Number of Living *Daphnia* in 1mg/L Soluble Sunscreen Solutions

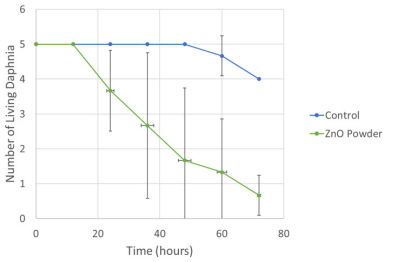

Fig. 7. Number of Living *Daphnia* in 2mg/L Sunscreen Solutions

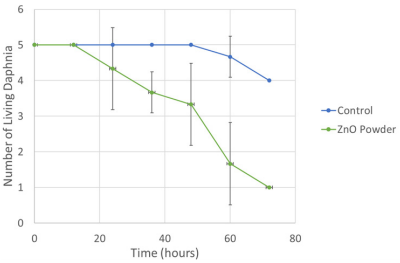

Fig. 8. Number of Living *Daphnia* in 1mg/L Sunscreen Solutions

One-way ANOVA Analysis	F	P-value
1 mg/L concentration		
All samples	31.4	0.00000171
Insoluble (#1 and #2) and control	1.75	0.251932
Soluble (#3 and #4) and control	124	0.0000132
2 mg/L concentration		
All samples	10.18889	0.000539
Insoluble (#1 and #2) and control	1	0.421875
Soluble (#3 and #4) and control	133	0.0000107

Fig. 9. Statistical Analysis

target concentrations resulted in higher mortality, since their solubility ensured a more uniform increase in concentration. Zinc Oxide powder resulted in high mortality despite not fully dissolving (Figure 7/ Figure 8). Zinc Oxide's insolubility makes sense as NIOSH has a recorded solubility of 0.0004% for ZnO. While it is currently unclear, the *Daphnia magna* may have consumed the specks of powder, a theory supported by their whitened dead bodies. More research must be done to understand the effects of pure zinc oxide on *Daphnia magna*. The higher 2mg/L concentration of Zinc Oxide powder resulted in higher mortality than the 1mg/L solution (Figure 7/ Figure 8) because despite its insolubility, there were likely more individual specks of powder present in the higher target concentration. Sunscreens 3 and 4 used identical ineffective emulsifiers unlike the natural emulsifiers used in sunscreen 1, including castor oil, and sunscreen 2, including beeswax.

Discussions/Conclusions

The data collected did not support my hypothesis of the main inactive ingredient (alcohol or water) being to blame for the detrimental effects of non-nano zinc oxide sunscreen on mortality of *Daphnia*. I originally theorized that alcohol-based sunscreen lotion would result in the highest mortality, water-based sunscreen being better, and pure zinc oxide being the best for freshwater ecosystems. The outcome of this experiment revealed that non-nano zinc oxide poses a threat to *Daphnia magna* regardless of the main inactive ingredient in the lotion. The main determiner of mortality seems to be its solubility in water, altered by the strength of emulsifiers used in the sunscreen lotion. More research must be done to determine the quantified rate of dissolution for full products including emulsifiers, as information on Ksp values is lacking in these areas.

Additionally, further studies must be performed to understand how the chemical composition of zinc oxide is toxic for *Daphnia magna*, regardless of particle size. Non-nano zinc oxide, despite being advertised as "environmentally friendly" is hazardous to freshwater environments, and the term 'reef safe' cannot be equated to "environmentally friendly". Awareness of non-nano zinc oxide's impact on freshwater environments is crucial to forming a more informed opinion on how personal choices impact the natural world. This project serves to broaden the scope of traditional toxicology studies and reveal the environmental impact of such common products as sunscreen, motivating more studies and causing companies to test products in more diverse environments.

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