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Exploring the Acute Toxic Effects of Benzophenone-3 on Global DNA Methylation in *Saccharomyces Cerevisiae* Yeast: Preliminary Findings

Abstract

The purpose of this research is to study the effects of oxybenzone on global DNA methylation in *Saccharomyces Cerevisiae* yeast. Oxybenzone (BP-3) is a common UV filter found in many brands of sunscreen lotions and aerosols and has been linked to endocrine disruption in humans. It is hypothesized that the application of oxybenzone will increase the rate of DNA methylation, activation of methylase, and the amount of methylase in yeast cells after exposure.

A solution of 1 mg/ml of BP-3 was prepared from a stock solution of oxybenzone in absolute ethanol. The cell viability after a 20 minute incubation in the 1 mg/ml oxybenzone solution was determined by using the Trypan Blue Assay. A negative control was performed by incubating the yeast for 20 minutes in ethanol and YPD broth, and a positive control for cell death was performed by boiling yeast-inoculated YPD broth for 5 minutes. Western Blot assays will be performed to determine the presumed increase of the methylase enzyme. An ELISA will be used to assess a change in the rate of global methylation. Lastly, a kinase assay will be used to confirm the presence of methylase as the protein acting on DNA.

The following conclusions are drawn from preliminary data from acute exposure experiments. The negative control, 10% ethanol exposure, produces an average cell viability of 92.81%. From this it is inferred that while 10% ethanol does kill some yeast cells, it does not have a devastating effect on cell viability. The l mg/ml oxybenzone exposure does have an adverse effect on the yeast cells. The average cell viability after exposure to the 1 mg/ml oxybenzone solution is 88.81%. These preliminary results suggest BP-3 does have an adverse effect on yeast viability at a concentration of 1 mg/ml.

1. Introduction

1.1 PPCPs and their impact on the environment

2-hydroxy-4-methoxybenzone (common name Benzophenone-3 or oxybenzone) is a UV filter found in a large percentage of sunscreen lotions and aerosols, as well as in a variety of other personal care products such as cosmetics, shampoos, and fragrances. This chemical has been linked to the disruption of the human endocrine system, specifically lowering estrogen and testosterone production, and the bleaching of coral reefs.[1] The US Centers for Disease Control and Prevention suggest that approximately 96.8% of the US population are exposed to oxybenzone.[2] Studies have indicated that humans tend to absorb 1% to as high as 10% of oxybenzone through topical applications with the highest absorption identified in human urine and breast milk.[3] In recent years, awareness of UV protection has increased and as a result sunscreen consumption has, as well. Studies have also shown that oxybenzone has been found in almost all water resources and aquatic species across the world. Benzophenone-3 enters aquatic ecosystems through human waste, water from showering and bathing, and waste from manufacturing facilities, particularly for sunscreens and cosmetics. Due to the low water solubility, high lipophilicity, and a high organic carbon-water coefficient it is particularly challenging for Wastewater Treatment Plants (WWTPs) to remove oxybenzone from the water.[4]

1.2 DNA Methylation (5-methylcytosine)

DNA methylation is involved in changing the activity of a gene, without altering its sequence. In general, methylases add a methyl group from the methyl donor, Sadenosylmetionine (SAM) to the fifth-base of the cytosine ring. The added methyl group prevents transcription, thereby silencing the gene it has been added to.

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[1]From "Systemic Absorption of the Sunscreens Benzophenone-3… ," by Janjua, N. R, 2004.
[2]Ibid,.
[3]Ibid,.
[4]From "Combined effects of benzophenone-3 and temperature in gene expression and enzymatic activity…" by Muñiz-González, 2020.

1.3 Why are we studying DNA Methylation? What is our model organism and why? What is our toxicant?

Yeast and humans share many genomic similarities. Therefore if there is an increase in the rate of DNA methylation in the yeast, it could reveal some of the adverse effects of oxybenzone on human DNA. This is amplified by the fact that yeast has been cited as a model organism in cancer research.[5] The Yeast species *Saccharomyces cerevisiae* acquired from Carolina Biological will be our model organism. Our toxicant is 2-hydroxy-4-methoxybenzone (common name oxybenzone), obtained from Sigma Aldrich.

2. Research Questions and Objectives

- **Does acute exposure of oxybenzone increase global DNA Methylation in yeast?**

3. Materials

The following materials were used for the dose-dependent assay: 2-hydroxy-4-methoxybenzone (common name oxybenzone), deionized water (dH_20), agar plates, inoculation loop, *Saccharomyces cerevisiae* Yeast, a glass flask, pipettors, Trypan Blue, a hemocytometer, a microscope, and microfuge tube.

4. Methods

The LD_{50} of *Saccharomyces cerevisiae* will be determined through a dose-dependent assay. To begin with, a stock solution of 100 mg/ml of a working solution of oxybenzone and ethanol will be prepared. A serial dilution with deionized water will be performed 3x to prepare solutions of the following concentrations of oxybenzone: 1.0 mg/ml, 0.1 mg/ml, and 0.01 mg/ml.

A cell count will be determined on the yeast culture before the Trypan Blue assay is performed. 1 ml of oxybenzone and the yeast culture will be aliquoted for a desired cell count of 5.0×10^6 yeast. The sample will be placed on a shaker for 20 minutes. The cell viability of the yeast after incubation in each solution will be determined, using the Trypan Blue Assay. Based on the cell

[5]From "DNA Methylases" by Razin, A, 2020.

viability values obtained, the LD_{50} for *Saccharomyces cerevisiae* will be determined. This solution will be used for the remainder of the experiment.

Figure A: Figure of the Titration Assay with specific concentrations of oxybenzone for each well

Western Blot Analysis will be used to determine if there is a change in the expression level of methylase.

Global Methylation Assay will be used to determine if there is a change in methylation levels following exposure to oxybenzone. A 5-mC DNA ELISA kit, obtained from Zymo Research, will be used to quantitate the amount of 5-methylcytosine in the controls and the sample. 200 ng DNA will be obtained from the yeast sample with the decided dose of oxybenzone and 100 ng DNA from the positive and negative controls supplied from the kit. The experiment will be repeated at least three times to ensure accuracy.

Time permitting, a protein kinase assay will be used to determine if there is a change in the activation of methylase following exposure to oxybenzone.

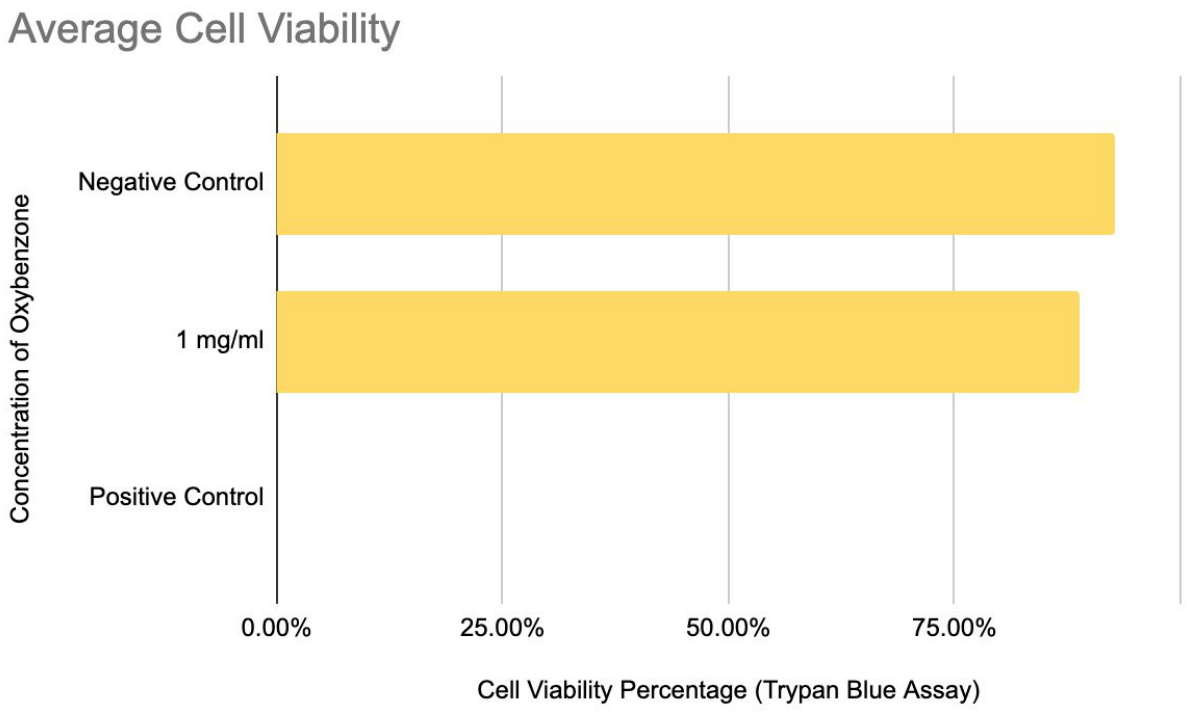
3.1 Instructions Followed

Trypan Blue Assay

In a 1.5ml microfuge tube, combine trypan blue solution and the yeast solution in a 1:1 ratio. Resuspend the mixture and pipette 10 microliters into one side of a hemocytometer. Count the unstained (viable) and stained (nonviable) cells separately in the hemocytometer.

5. Preliminary Results

The following conclusions are drawn from preliminary data from acute exposure experiments. The negative control, 10% ethanol exposure, produces an average cell viability of 92.81%. From this it is inferred that while 10% ethanol does kill some yeast cells, it does not have a devastating effect on cell viability. The l mg/ml oxybenzone exposure does have an adverse effect on the yeast cells. The average cell viability after exposure to the 1 mg/ml oxybenzone solution is 88.81%. These preliminary results suggest BP-3 does have an adverse effect on yeast viability at a concentration of 1 mg/ml.



6. Assays and Timeline

4.1 Toxicant

2-hydroxy-4-methoxybenzone (common name oxybenzone)

4.2 Assays

Assays

Trypan Blue Exclusion

5-mC DNA methylation ELISA kit

Western Blot Apparatus

Kinase Assay

4.3 Timeline

Month	**Objectives**	**Analysis**
October	- *Acquire toxicants* - *Do pilot studies*	- *Observe how the yeast cells interact with the toxicant*
November	- *Do pilot studies* - *Determine best concentration of BP-3 for assays* - *Gather preliminary data*	- *Optimize experimental design based on the properties of the toxicant*
December	- *Begin acute studies* - *Determine LD_{50}*	- *Determine the lowest concentration of BP-3 at which 50% of the yeast cells die*

		- *Assess the effects the concentration of BP-3 has on DNA methylation levels*
January	- *Western Blotting Assay* - *DNA Methylation assay*	- *Assess protein levels in yeast exposed to the* LD_{50}
February	- *Kinase Assay*	- *Confirm that methylase is the active protein*

7. References

Baccarelli, Andrea, and Valentina Bollati. (2009). Epigenetics and environmental chemicals. *Current Opinion in Pediatrics*, *Volume 21* (2) : 243-51. doi:10.1097/mop.0b013e32832925cc

Guyon, Anais et al (2018). "Effects of chronic exposure to benzophenone and diclofenac on DNA methylation levels and reproductive success in a marine copepod." *Journal of xenobiotics Volume. 8*,1 7674, doi:10.4081/xeno.2018.7674

Janjua, N. R., Mogensen, B., Andersson, A.-M., Petersen, J. H., Henriksen, M., Skakkebæk, N. E., & Wulf, H. C. (2004). Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor After Whole-Body Topical

Application and Reproductive Hormone Levels in Humans. *Journal of Investigative Dermatology*, *123*(1), 57–61. doi: 10.1111/j.0022-202x.2004.22725.x

Muñiz-González, Ana-Belén, and José-Luis Martínez-Guitarte. (2020). Combined effects of benzophenone-3 and temperature on gene expression and enzymatic activity in the aquatic larvae Chironomus riparius. *The Science of the total environment, Volume. 698* : 134292. doi:10.1016/j.scitotenv.2019.13429.

Razin, A. (1989). DNA Methylases. *Genetic Engineering*, 1–11. Doi 10.1007/978-1-4615-7084-4_1

Steffen, Kristan K et al (2009). "Measuring replicative life span in the budding yeast." *Journal of visualized experiments : JoVE* ,28 1209. doi:10.3791/1209

Zachos, E., & Rosen, E. (n.d.). "What Sunscreens Are Best for You-and the Planet?" Sunscreen, Clothing, and Other Coral Reef-Safe Ways to Protect Your Skin, National Geographic, 21 May 2019. Retrieved February 7, 2020.

Zhang, Qiuya et al. (2017). Evaluation of ecotoxicological effects of benzophenone UV filters: Luminescent bacteria toxicity, genotoxicity and hormonal activity. *Ecotoxicology and environmental safety*, *Volume 142*. pp. 338-347. doi:10.1016/j.ecoenv.2017.04.027

Zhong, Xin et al. (2019). The toxicological effects of oxybenzone, an active ingredient in suncream personal care products, on prokaryotic alga Arthrospira sp. and eukaryotic alga Chlorella sp. *Aquatic toxicology, Volume* 216. doi: 10.1016/j.aquatox.2019.105295