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Exploring the Acute Toxicological Effects of Benzophenone-3 on Global DNA Methylation in *Saccharomyces cerevisiae* Yeast Cells.

QUESTION BEING ADDRESSED:

The aim of this study is to determine the effects of the chemical Benzophenone-3 on the process of Global DNA methylation in *Saccharomyces cerevisiae* Yeast. This study seeks to answer the question "Does acute exposure of oxybenzone affect DNA methylation and methylase activity in yeast cells?"

RATIONALE

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. The US Centers for Disease Control and Prevention suggest that approximately 96.8% of the US population are exposed to oxybenzone. 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. 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.

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.

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 have been cited as a model organism in cancer research. The yeast species *Saccharomyces cerevisiae* acquired from Carolina Biological will be our model organism. Our toxicant is 2-hydroxy-4-methoxybenzone (common name oxybenzone), was obtained from Sigma Aldrich.

HYPOTHESIS/GOALS

It is hypothesized that the acute exposure of yeast to oxybenzone will increase the rate of DNA methylation, methylase activity, and the amount of methylase in yeast cells after exposure. The specific aims of this experiment are to determine the LD_{50} for oxybenzone in yeast, assay the levels of global methylation when exposed to oxybenzone, determine whether methylase activity changes after exposure to oxybenzone, and determine the amount of methylase in yeast cells after exposure.

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PROCEDURE

A 1 mg/ml solution of oxybenzone and water was made from a stock solution of 100 mg/ml of oxybenzone and 100% ethanol. A sample of the yeast culture was counted to determine the amount of yeast-inoculated YPD broth necessary for a resulting solution containing $5x10^6$ cells. A 1 ml solution of yeast and oxybenzone was made and incubated on a shaker for 20 minutes. A negative control was set up by incubating the yeast for 20 minutes on the shaker in a 10% ethanol and 90% YPD broth solution. A positive control was set up by boiling yeast-inoculated YPD broth in water for 5 minutes. The cell viability was determined using the Trypan Blue Assay. Cell viability values were then compared.

The following conclusions are drawn from **preliminary data** from acute exposure experiments. The negative control, ethanol exposure, produces an average cell viability of 92.81%. From this it is concluded that the ethanol does kill some yeast cells, but does not have as devastating of an effect on the yeast cells as the BP-3. 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%. From this result, it is concluded that the oxybenzone is somewhat deadly to the yeast cells at a concentration of 1 mg/ml.

The Western Blot Assay will be used to determine if there is a change in the amount of methylase. The Western Blotting Apparatus (and all associated materials) will be used.

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A 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 quantify the amount of 5-methylcytosine in the controls and the sample. 200 ng of DNA will be obtained from the yeast sample with the decided dose of oxybenzone and 100 ng of 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. Protein Kinase is an enzyme that aids in the transfer of a phosphate from ATP to a protein to activate it.

RISK ASSESSMENT

This team will utilize 2-hydroxy-4-methoxybenzophenone, (common name: oxybenzone) at a concentration of 1mg/ml solubilized in absolute ethanol. All stock and working dilutions will be prepared for student use by the mentor/teacher (Ileana Rios). Due to stringent safety protocols, safety training, and direct supervision, the risks to the students are minimal and may involve accidental spills. Skin contact and eye exposure are entirely minimal due to personal protective equipment; In addition, there is a shower and eye wash station in the biology lab. First, the BSL-1 prep room which houses the CO2 incubator, cell media reagents, autoclave, and biohazardous waste is secure with a combination door lock; A few instructors in the science department and maintenance are familiar with the key code; the prep room is always closed and locked unless the mentor is present in the room. Students will wear personal protective equipment consisting of lab coats, nitrile disposable gloves, goggles, and facemasks from VWR.

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All activities and protocols with BSL-1 entities are carried out in the safety hood in a BSL-1 prep room and under direct supervision by the mentor. All stock and working dilutions of oxybenzone and will be prepared by the mentor for student use. All yeast media waste is disinfected with 10% bleach and autoclaved for 20 minutes at 212°F prior to disposal in a red biohazard bag which is picked up for incineration by Sharps Compliance, Inc. All oxybenzone-treated liquid waste is collected in amber chemical waste bottles and collected by PEGEX Hazardous Waste Removal (Account NumberA-96207).

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