The Effect of Blue Light on Oxidative Stress in *C. elegans*

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a. **RATIONALE:** Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research.
   - Oxidative stress is harmful because it can cause damage to DNA bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins all of which leads to a number of diseases (cancer, cardiovascular, neurodegenerative, etc.). Oxidative stress forms as a result of more free radicals present in an organism than there are antioxidants to neutralize those free radicals. Oxidative stress in blue light-exposed *C. elegans* was assessed because American adults spend more than 11 hours per day exposed to excessive blue light, which disrupts the circadian rhythm, leads to sleep deficit, and accumulates beta amyloid in the brain. As we are entering the digital age it is important to understand the effect that so much blue light is having on the human body so that there is awareness for what needs to be done to maintain a healthy population. If blue light is found to increase oxidative stress and/or degrade locomotive ability in *C. elegans*, then further research must be conducted to identify a viable solution for decreasing oxidative stress levels.

b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?
   - If *C. elegans* are exposed to blue light, will they exhibit significantly more oxidative stress than if they were not?
     i. It is expected that *C. elegans* exposed to 450nm blue light will have significantly greater oxidative stress than *C. elegans* raised in the dark because of the high frequency of the light that will likely cause a greater production of free radicals than antioxidants.
   - If *C. elegans* are exposed to blue light, will they exhibit significantly inferior locomotive ability than if they were not?
     i. It is expected that blue light-exposed *C. elegans* will have an inferior locomotive ability because the free radicals that result from oxidative stress cause damage to cell structures needed for normal functions.

c. Describe the following in detail:
   - **Procedures:** Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others.
     - *C. elegans* age synchronization
       - *S*ubculture *C. elegans* a week prior and allowed them to age for four days before the synchronization.
       - Wash the plate with M9 buffer and transfer the worms to centrifuge tubes (1 ml per tube).
       - Centrifuge for one minute, remove the supernatant.
       - Add 200 µl of distilled water and 800 µl of bleaching solution, composed of 6.25 ml of 4M NaOH, 2.5ml of NaOCl, and 50 ml of M9 buffer, to each tube.
       - Leave the *C. elegans* for five minutes, then check every thirty seconds until about 90% of the worms have lysed.

        - Add 2 ml of M9 buffer to the embryos in the tube before incubating overnight at 20 °C.
        - In a ventilation hood, transfer *C. elegans* to a Petri dish and put in the incufridge for three days.
    - Exposure of *C. elegans* to blue light
        - Place two synchronized plates in a dark drawer for 24 hours (control), and place the other two plates under a 450nm blue light for 24 hours (experimental).
    - Measurement of oxidative stress
        - Make a 50mM dichlorofluorescein diacetate (DCF) stock solution by combining 0.0244 grams of DCF and one ml of cell culture grade DMSO.
        - One hour before the application of the DCF, dilute the 50mM stock 1,000 fold smaller with M9 buffer by serial dilution, (vortex to ensure homogenous concentration).
        - After 23 hours, wash *C. elegans* off of the petri dish with M9 buffer and transfer them into well plates.
        - Add 50 µl of *C. elegans*, 50µl of M9, and 100 µl of DCF to each well.
        - Within one hour the DCF will bind to the byproducts of oxidative stress formation to cause fluorescence. The greater the intensity of fluorescence, the greater the stress. View the wells were viewed under the zeiss fluorescence microscope and take photographs.
    - Data analysis for oxidative stress assessment
        - Use ImageJ software to determine the corrected total cell fluorescence (CTCF) in each image and calculate an average for each group using the formula CTCF = (integrated density of *C. elegans)* -(area of *C. elegans)*(mean fluorescence of background).The resulting values will be used to compare stress levels between blue light exposed and unexposed worms.
        - Use a t test to determine if the difference in oxidative stress levels in each group is significant.
    - Locomotive assessment
        - Age synchronization procedure (outlined above)
        - Exposure procedure (outlined above)
        - Record the movement of the *C. elegans* under white light with the zeiss microscope for three minutes.
    - Data analysis for locomotive assessment
        - Calculate average body bends per minute from a slow motion version of the three minute video to determine the effects that oxidative stress had on the locomotive ability of *C. elegans*.
        - Use a t test to determine if the difference in average body bends per minute in each group is significant.

- **Risk and Safety:** Identify any potential risks and safety precautions needed.
    - Dichlorofluorescein diacetate

        - can cause eye and skin burns, digestive and respiratory tract burns. Harmful if inhaled or swallowed.
        - Safety precautions: wear goggles, gloves, and clothing that covers the skin. Use in a ventilation hood.
    - *C. elegans*
        - acquired from the Caenorhabditis Genetics Center (CGC)
        - Biosafety level: 1
        - Safety precautions: There is negligible risk of exposure and infection by *C. elegans* because these organisms cannot infected vertebrate cells. Good standard laboratory practices of appropriate lab protective equipment, containment and appropriate disinfection/disposal will prevent any accidental exposure of the human skin to the *C. elegans*.
        - Disposal method: decontamination of all work surfaces daily, and appropriate chemical disinfection (1% hypochlorite) of all liquid cultures. After this treatment is complete, all instruments and plates can then be bagged and disposed of in the regular solid waste.
    - *E. coli* OP50
        - Biosafety level: 1
        - Used to grow *C. elegans*
        - Safety precautions: harmful if swallowed, avoid contact with eyes
        - Disposal method: dissolve in water and autoclave before disposal
    - 4 M NaOH
        - Potential risk: Causes severe skin burns and eye damage,
        - Safety precautions: Do not breathe mist, spray, vapors, Wash exposed skin thoroughly after handling, Wear eye protection, face protection, protective clothing, protective gloves
    - NaOCl
        - Potential risk: May be corrosive to metals, Causes serious eye damage, Causes skin irritation, Toxic to aquatic life with long lasting effects
        - Safety precautions: Wear protective gloves/protective clothing/eye protection/face protection, Wash skin thoroughly after handling, IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
    - Cell culture grade DMSO
        - Potential risk: liquid is combustible
        - Safety precautions: Keep away from heat/sparks/open flames/hot surfaces. No smoking, Wear protective gloves/ eye protection/ face protection.
        - In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
    - M9 buffer
        - Potential risk: may cause eye irritation, may cause skin irritation, harmful if inhaled or swallowed
        - Safety precautions: wear eye and skin protection when handling, if in contact with eyes or skin rinse with water, if ingested seek medical help

- **Data Analysis:** Describe the procedures you will use to analyze the data/results.
    - Calculate if the differences in each assay (oxidative stress and locomotive ability) between the control and experimental groups were statistically significant by using an independent t test.
    - Numerical values based on the averages for the control and the experimental group for each assay will be used in each t test.
    - At least 30 trials will be conducted for both the experimental and the control group to minimize the effect of possible interference.

d. **BIBLIOGRAPHY:** List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.

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Items 1–4 below are subject-specific guidelines for additional items to be included in your research plan/project summary as applicable.

1. **Human participants research: N/A**
2. **Vertebrate animal research: N/A**
3. **Potentially hazardous biological agents research: N/A**
4. **Hazardous chemicals, activities & devices:**

Dichlorofluorescein diacetate

- Describe the risk assessment process: Causes eye and skin burns. Causes digestive and respiratory tract burns. Harmful if inhaled or swallowed. Target Organs: Eyes, skin, mucous membranes.
- Supervision: research teachers of syosset high school
- Safety precautions: wear goggles, gloves, and clothing that covers the skin. Use in a ventilation hood.
- Methods of disposal: Transfer to chemical waste container.
- Include details regarding chemical concentrations and drug dosages: Dose and concentration: 100mg and >= 97%
- Material Safety Data Sheets for ALL chemicals used individually OR as part of a premande reagent.: https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=D6883&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fd6883%3Flang%3Den

4 M NaOH

- Describe the risk assessment process: Causes severe skin burns and eye damage,
- Supervision: research teachers of syosset high school
- Safety precautions: Do not breathe mist, spray, vapors, Wash exposed skin thoroughly after handling, Wear eye protection, face protection, protective clothing, protective gloves
- Methods of disposal: Dispose of contents/container to comply with local, state and federal regulations, avoid exposure to the environment
- Include details regarding chemical concentrations and drug dosages: Dose and concentration: 4M
- Material Safety Data Sheets for ALL chemicals used individually OR as part of a premande reagent.: http://www.labchem.com/tools/msds/msds/LC24430.pdf

NaOCl

- Describe the risk assessment process: May be corrosive to metals, Causes serious eye damage, Causes skin irritation, Toxic to aquatic life with long lasting effects
- Supervision: research teachers of syosset high school
- Safety precautions: Wear protective gloves/protective clothing/eye protection/face protection, Wash skin thoroughly after handling, IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

- Methods of disposal: Do not allow product to reach sewage system or open water.Neutralize with dilute acid solutions.
- Material Safety Data Sheets for ALL chemicals used individually OR as part of a premande reagent.: https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/education/regulatory-documents/sds/chemicals/chemicals-s/S25552.pdf

Cell culture grade DMSO

- Describe the risk assessment process: liquid is combustible
- Supervision: research teachers of syosset high school
- Safety precautions: Keep away from heat/sparks/open flames/hot surfaces. No smoking, Wear protective gloves/ eye protection/ face protection.
- Methods of disposal: Absorb into dry earth or sand. Transfer to a closable, labelled salvage container for disposal by an appropriate method.
- Material Safety Data Sheets for ALL chemicals used individually OR as part of a premande reagent.: http://www.apolloscientific.co.uk/downloads/msds/BID1200_msds.pdf

M9 buffer

- Describe the risk assessment process: may cause eye irritation, may cause skin irritation, harmful if inhaled or swallowed
- Supervision: research teachers of syosset high school
- Safety precautions: wear eye and skin protection when handling, if in contact with eyes or skin rinse with water, if ingested seek medical help
- Methods of disposal: Absorb into dry earth or sand. Transfer to a closable, labelled salvage container for disposal by an appropriate method.
- Material Safety Data Sheets for ALL chemicals used individually OR as part of a premande reagent.: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FSDS%2FA1374401_MTR-NALT_EN.pdf&title=QTEzNzQ0MDE=