Risk Assessment Form (3)

Must be completed before experimentation.

Student's Name(s) Kevin Carratu

Title of Project Supplementation of Antioxidants to Reduce dopaminergic Neurodegeneration and Alpha-synuclein Accumulation Associated with Parkinson's

To be completed by the Student Researcher(s) in collaboration with Designated Supervisor/Qualified Scientist:
(All questions must be answered; additional page(s) may be attached.)

1. List all hazardous chemicals, activities, or devices that will be used; identify microorganisms exempt from pre-approval (see Potentially Hazardous Biological Agent rules).

Curcumin concentrations of 25 µM, 50µM, and 100µM (150µL) dissolved in dimethyl sulfoxide will be used. Concentrations of 50µM, 100µM, and 200µM (150µL) quercetin dissolved in dimethyl sulfoxide will be used. Concentrations of 0.1mM, 1mM, and 10mM (340µL) L-glutathione dissolved in distilled water will be used. Concentrations of 10mg/L (150µL) copper sulfate dissolved in spring water will be used. M9 media buffer will also be applied to the C. elegans. 70% isopropyl alcohol (5.0mL) will be used for equipment sterilization, 20% alkaline hypochlorite (5.0mL) will be used for age synchronization, 40% ethanol (3.0mL) will be used as an anesthetic for C. elegans, and 10% bleach (5.0mL) will also be used for sterilization of work areas. Devices include the Bunsen burner, scalpel, autoclave, and centrifuge. Caenorhabditis elegans BZ555 and Caenorhabditis elegans OW13 will be used. The C. elegans will be fed E. coli OP50.

2. Identify and assess the risks involved in this project.

Curcumin can be hazardous in case of eye contact or ingestion and can be slightly hazardous in case of skin contact or inhalation. Quercetin may cause eye and skin irritation if excessive exposure. L-glutathione reduced may be harmful if swallowed, may cause eye and skin irritation.Copper Sulphate may be harmful if swallowed, may cause skin irritation and serious eye irritation, and is toxic to aquatic life. M9 buffer may be harmful in contact with skin or eyes. May cause respiratory irritation.70% isopropyl alcohol is flammable as a liquid and vapor, can cause serious eye irritation, and may cause drowsiness or dizziness. 20% alkaline hypochlorite inhalation can cause respiratory irritation, prolonged skin exposure can cause redness and irritation, eye contact could result in burning of eyes, and ingestion can cause severe corrosion of the mouth, and throat. 40% ethanol is highly flammable, and can cause damage to eyes and irritate skin. 10% bleach can cause irritation in the eyes, mouth, lungs and skin. Dimethyl sulfoxide can cause skin reactions, headaches, respiratory problems and nausea if not handled properly. The Bunsen burner can cause serious burns to skin and can ignite clothing or hair. The scalpel can cause cuts and puncture wounds that can lead to infection, especially if the blade or point is contaminated by bacteria, if not handled properly. The autoclave can potentially risk steam burns if autoclave is opened prior to depressurization. May result in burns to the hands when removing autoclaved material. The centrifuge can fail due to mechanical stress and possibly not function correctly. Centrifuge use may also result in inhalation risks of aerosolization of chemicals. Caenorhabditis elegans BZ555 (BSL-1 level and non-pathogenic) and Caenorhabditis elegans OW13 (BSL-1 level and non-pathogenic) can cause skin irritation and eye irritation. Escherichia coli OP50 (BSL-1 level and non-pathogenic) is harmful if swallowed.

3. Describe the safety precautions and procedures that will be used to reduce the risks.

Curcumin powder will not be inhaled and will be kept away from eyes and face to avoid irritation. Quercetin, copper sulfate, and L-glutathione reduced will not be inhaled or touched without personal protection. Curcumin will be stored in a closed freezer at -20°C and L-glutathione reduced will be stored in a sealed container at 2-8°C. Copper sulfate will be stored in the locked poisons cabinet. 70% isopropyl alcohol, 40% ethanol, 10% bleach solution, and quercetin (dissolved in dimethyl sulfoxide) will be stored in a tightly sealed container and stored far away from heat and sparks in a locked flammables cabinet. Student researcher will be trained sterile technique and how to properly work with, and dispose, bacteria and C. elegans. Prior to and after each experiment, the lab tables will be cleaned with a 10% bleach solution. Sterile technique will be used at all times when working with E.coli OP50 and C. elegans cultures. The contaminated cultures will be disposed of by incorporating 10% bleach solution, parafilming, and disposing in the waste removal bin. All tools will be sterilized in autoclave. Nitrile gloves, goggles and a lab apron will be worn at all times when using chemicals.While using E.coli OP50 all workspaces will be kept sterile and contaminated equipment will be disposed of in autoclave bag. Hands will be washed thoroughly after handling bacteria. The use of a Bunsen burner will be used with extreme caution and flammable objects will be kept away at all times and this activity will be supervised by an adult. While working with the Bunsen burner, loose clothing will not be worn and hair will be tied back. While using the scalpel to cut blocks of agar, extra precaution will be taken to avoid injury. Goggles will be worn and materials will be cut in a direction away from the body. Autoclave gloves will be used along with goggles and a lab apron when using the autoclave. When using the centrifuge, student will make sure the centrifuge is shut prior to start and will make sure the centrifuge has stopped spinning prior to removing the components.

4. Describe the disposal procedures that will be used (when applicable).

Curcumin, quercetin, and L-glutathione will be disposed of in the appropriate waste removal bin and a licensed disposal company will remove them. 70% isopropyl alcohol will be disposed of through an approved waste disposal plant. C. elegans will be disposed of by incorporating plates with 10% bleach and parafilming, then putting into waste removal bin. E.coli cultures will be disposed of by incorporating 10% bleach and parafilming. Copper sulfate will be disposed of in an approved waste disposal plant. All cotton tip applicators used to spread E. coli will also be exposed to 10% bleach, placed in an autoclave bag, and disposed of. The scalpel will be sterilized with 10% bleach and 70% isopropyl alcohol and covered with a sterile package. All tools will be autoclaved prior to and after use.

5. List the source(s) of safety information.

-Caenorhabditis elegans: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html
-Escherichia coli OP50:https://nemametrix.com/wp-content/uploads/2018/09/LabTIE-OP50-MSDS-v3.pdf
-Curcumin: https://www.sigmaaldrich.com/catalog/substance/curcumin3683845837117lang=en&region=US
-Quercetin: https://www.sigmaaldrich.com/catalog/product/sigma/q4951?lang=en&region=US
-L-glutathione reduced: https://www.sigmaaldrich.com/catalog/product/sial/g4251?lang=en&region=US
-70% isopropyl alcohol: https://www.hydrolabs.com/images/pdf/SDSPDF/SDS-002-SDS-Isopropyl-Alcohol-70%25-1-09-18.pdf
-20% alkaline hypochlorite: https://www.unil.ch/cig/files/live/sites/cig/files/FAQ/Safety/PDF/MSDS/Sodium%20hypochlorite-msds.pdf
-Ethanol: https://www.airgas.com/msds/001114.pdf
-M9 buffer: https://www.sigmaaldrich.com/catalog/product/sial/63011?lang=en&region=US
-Bunsen burner: https://www.airgas.com/msds/001114.pdf
-Scalpel: https://www.bio-world.com/msds/43230008/Disposable-Scalpel-w-Blade-PK.html

To be completed and signed by the Designated Supervisor (or Qualified Scientist, when applicable):
I agree with the risk assessment and safety precautions and procedures described above. I certify that I have reviewed the Research Plan/Project Summary and will provide direct supervision.

Alison Huenger	Alison Huenger	9/15/19
Designated Supervisor's Printed Name	Signature	Date of Review (mm/dd/yy)

stem research specialist - Manhasset High School	(516) 859-5254 Alison_Huenger@manhassetschools.org
Position & Institution	Phone or email contact information

Experience/Training as relates to the student's area of research: degrees in chemistry and biology - worked at Stony Brook University Biotechnology camp and past experience as a chemical engineer