Risk Assessment Form (3)

Must be completed before experimentation.

* see attached sheets for additional info.

Student's Name(s) Kaitlyn Clarke and Spyridoula Xenophontos

Title of Project Using Antioxidants to Remediate Motility, Fertility, ROS production, and ASH neuronal death in a Huntington's model of C. elegans.

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).

Ginkgo Biloba Extract(100 and 200µg 100mL)
Epigallocatechin gallate (1and 5µg 100mL)
Salidroside (50µM and 150µM 100mL)
Hydrogen Peroxide (20% 5mM 50mL)
Ethanol (40% 50mL)
M9 Broth (100mL)
Alkaline Hypochlorite(20% 50mL)
Isopropyl Alcohol (70% 100mL)
DMSO (used for solvent solution 400mL)

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

Gingko Biloba Extract: Gingko Biloba is classified as a not hazardous substance or mixture according to the Regulation EC. This should not be directly inhaled as this could be harmful.
Epigallocatechin gallate (EGCG): EGCG is classified as a non hazardous mixture or substance by the GHS. This should not be directly inhaled as this could be harmful and care should taken to avoid dust formation.

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

Gingko Biloba Extract: All handling and use of gingko biloba extract will be supervised by an experienced teacher. Goggles and nitrile gloves will be worn at all times and will be inspected prior to use. A lab apron will also be worn. If breathed in the student will be moved into fresh air and medical attention will be sought as soon as possible.

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

Gingko Biloba Extract: Any excess extract will be given to a professional waste disposal service whom will be contacted.

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

Gingko Biloba Extract: https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=NIST3247&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3DGinkgo%2Bbiloba%2Bextract%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct
EGCG:https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=03970590&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3DEGCG%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct
Salidroside: https://www.sigmaaldrich.com/MSDS/MSDS/PleaseWaitMSDSPage.do?language=&country=US&brand=SIAL&productNumber=05410590&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dsalidroside%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct
Hydrogen Peroxide: https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=216763&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dhydrogen%2Bperoxide%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

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
Designated Supervisor's Printed Name

Alison Huenger
Signature

10/01/19
Date of Review (mm/dd/yy)

(516)859-5254

Scieri Research Specialist - Manhasset High School
Position & Institution

Alison_Huenger@ManhassetSchools.org
Phone or email contact information

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

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Spyrithoula Xenophontos and Kaitlyn Clarke
Using Antioxidants to Remediate Motility, Fertility, ROS production, and ASH neuronal death in Huntington's model of *C.elegans.*

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

- Sodium Chloride
- Autoclave
- Bunsen Burner
- Microcentrifuge
- Scalpel
- E.coli OP50 (food source for C.elegans, but an exception to form 6A)
- N2, HA659, HA759 C.elegans (exception to form 6A)

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

Alkaline Hypochlorite (NaClO): Under GHS classification in cooperation with OSHA HCS, NaClO is a category 2 skin irritation, category 2A eye irritation, category 3 acute aquatic toxicity, and category 3 aquatic life toxicity. NaClO may cause skin irritation, serious eye irritation, serious eye damage, may be corrosive to metals, and have long lasting harmful effects on aquatic life.

Salidroside: Under GHS classification in cooperation with OSHA HCS, salidroside is a category 2A eye irritant. May cause acute serious eye damage and should avoid being inhaled.

Hydrogen Peroxide: Under GHS classification, Hydrogen Peroxide is a category 4 acute toxicity, category 1A skin corrosion, category 1 serious eye damage, category 3 specific organ toxicity, category 2 short term aquatic hazard, and category 3 long term aquatic hazard. May cause respiratory irritation if inhaled directly disregarding safety parameters.

Ethanol: Under GHS classification, ethanol is a category 2 flammable liquid and a category 2A eye irritant. Vapours or gases may form explosive mixtures when formed in the air. Avoid heat, flames, and sparks. Alkali metals, oxidizing agents, and peroxides are all incompatible with ethanol. May cause skin irritation or serious eye damage when in use.

M9 Broth: Under GHS classification, M9 Broth is a category 3 short term aquatic hazard. M9 Broth is harmful to aquatic life and should be avoided being released into nature. M9 should not come directly in contact with skin to avoid irritation and should not be inhaled or swallowed.

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DMSO: Under GHS classification, DMSO is a category 4 flammable liquid. DMSO should be avoid coming in contact with the skin and eyes. An individual should avoid inhaling vapors or mist that DMSO produces.
Sodium Chloride: Sodium chloride is categorized as a non-hazardous substance or mixture.

Autoclave: Autoclaves use high pressure and temperature steam to kill microorganisms and render biohazardous material inactive. Potential hazards of using an autoclave are heat and steam burns, hot fluid scalds, and injuries to hands and arms from the door. Exposure to hazardous material may occur if biohazardous waste is improperly handled.

Bunsen Burner: Bunsen burners create a fire hazard by producing an open flame and burning at a high temperature. An individual should tie back any long hair or clothing while using. The gas will be turned on once the hose is secured onto the burner, and lab personnel will be notified when turned on and off.

Microcentrifuge: Will be spinning at very high rpm's and care will be taken to make sure the centrifuge is closed fully. The science research students will not be touching centrifuge while spinning.

Scalpel: Scalpels are extremely sharp and therefore should be stored in the case at all times.

C.elegans strains: Worms are non hazardous and nitrile gloves, goggles, and aprons should be worn at all times. Students will be supervised by a lab advisor at all times.

E.coli OP50: Under GHS classification, *E.coli* is a non hazardous substance and nitrile gloves, goggles, and aprons should be worn at all times when using the bacteria.

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

Alkaline Hypochlorite: All handling and use of alkaline hypochlorite will be supervised by an experienced teacher or mentor. Exposure to the skin and eye contact will be avoided at all times. Safety goggles will be worn. Nitrile gloves will be worn. A lab apron will also be worn too. If on skin, skin will be washed with plenty of soap and water. If on eyes, eyes will be washed with water for several minutes. If skin irritation occurs medical attention will be sought out as soon as possible.

Epigallocatechin gallate (EGCG): All handling and use of EGCG will be supervised by an experienced teacher or mentor. Nitrile gloves (0.11mm) will be worn and inspected before use. A lab apron will be worn. If breathed in, the person will be moved into fresh air. In case of skin contact wash off with soap and plenty of water. In case of eye contact, flush eyes with water as a precaution for several minutes. If swallowed, never give anything by mouth to an unconscious

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person. Rinse mouth with water. Extinguishing methods include using water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Salidroside: All handling and use of salidroside will be supervised by an experienced teacher or mentor. Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU) will be worn. Nitrile gloves will be worn to prevent skin contact and gloves will be inspected before use along with being disposed appropriately. A lap apron will also be worn. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes and consult a physician immediately. In case of skin contact, wash with plenty of soap and water. If swallowed, never give anything by mouth to an unconscious person. Rinse mouth with water and consult a physician. Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide to extinguish any fires.

Hydrogen Peroxide: All handling and use of hydrogen peroxide will be supervised by an experienced teacher or mentor. Tightly fitted eye goggles will be worn. Nitrile gloves (0.11mm) will be worn and inspected before use. A lab apron will be worn If breathed in, move person into fresh air. If not breathing, give artificial respiration and consult a physician. In case of skin contact, take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. If swallowed, vomiting will not be induced. Rinse mouth with water and consult a physician. Water spray, alcohol-resistant foam, dry chemical or carbon dioxide will be sprayed to extinguish any fires created. Salidroside will be stored in a locked corrosives cabinet.

Ethanol: All handling and use of ethanol will be supervised by an experienced teacher or mentor. Nitrile gloves (0.3mm) will be worn and disposed properly. A lab apron will also be worn. Ethanol will be stored in a locked flammables cabinet when not in use. Avoid breathing vapours, mist or gas. If breathed in, move the person into fresh air. If not breathing, give artificial respiration. In made contact with skin, wash off with soap and plenty of water. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. If swallowed, do not induce vomiting and never give anything by mouth to an unconscious person. Rinse mouth with water and consult a physician. Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide to extinguish any fires created.

M9 Broth: All handling and use of M9 broth will be supervised by an experienced teacher or mentor. Face shield/eye glasses will be worn in accordance with government standards such as NIOSH (US) or EN 166(EU). Rubble gloves (0.3mm) will be worn and disposed properly. A lab apron will also be worn. If breathed in, move person into fresh air. If not breathing, give artificial respiration and consult a physician immediately. In case of eye contact, wash off with soap and

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plenty of water. In case of eye contact, flush eyes with water as a precaution. If swallowed, never give anything by mouth to an unconscious person and rinse the mouth with water. Consult a physician. Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide to extinguish any fires. Do not let product enter drains. Discharge into the environment must be avoided.

DMSO: The solution must be contained a dry and well ventilated place in a tight container. It should be kept away from any open flames or hot surfaces. If a fire were to occur, dry sand or alcohol resistant form should be used for extinction. Nitrile gloves and goggles must be worn at all times. If a person breathes in vapors or mist from the DMSO, the person would be immediately moved into a fresh open space and if unconscious artificial respiration should be performed. If a person were to ingest DMSO, vomiting should not be induced and a physician should be consulted immediately.

Sodium Chloride: A teacher will be present at all times. Sodium Chloride can cause severe skin burns and eye damage. Goggles, latex gloves, and an apron will be worn when working with chemical.

Autoclave: When working the autoclave the latches will remain closed at all times. Autoclave gloves will be worn when removing hot (and sterile) solutions and items from the autoclave to prevent burns.If the autoclave is open prior to depressurization, there is a risk of getting burned by hot steam. Once done using the autoclave, a person should make sure to wash their hands. An autoclave should be stored away from incompatible materials like flammable materials. If skin comes in contact with the autoclave, the skin should be immediately flushed with cold water. Obtain medical attention if eye irritation were to happen.

Bunsen Burner: The Bunsen burner should stored away from any light fixtures or incompatible equipment. Loose clothing should not be worn when the Bunsen burner will be used and all long hair should be tied back. The Bunsen Burner should not be touched after immediate use as the device could burn the skin.

Microcentrifuge: If the centrifuge is opened and touched prior to stopping, there is a risk of damaging fingers/hands. Will be spinning at very high rpm's and care will be taken to make sure the centrifuge is closed fully. The science research students will not be touching centrifuge while spinning. Also the centrifuge will only be opened when the machine comes to a complete stop so the solutions can be safely retrieved.

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Scalpel: Also a teacher will be supervising when using this tool for safety precautions.The scalpel will be kept in its protective case when it is not being used. A scalpel will be kept in a lab cabinet with its' case on at all times.

C.elegans strains (N2, HA759, HA659): Worms will be stored in parafilm petri dishes that are placed in well ventilated storage cabinets. Nitrile gloves, aprons, and goggles will be worn at all times when working with the *C.elegans.*

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

Alkaline Hypochlorite: A professional waste disposal service will be contacted to come and dispose of this material. Alkaline hypochlorite should not enter any drains as it is a threat to aquatic life.

Epigallocatechin gallate: Pick up and arrange disposal without creating dust. Offer surplus and non-recyclable solutions to a licensed disposal company.

Salidroside: The excess will be arranged and disposed in a well ventilated area. A licensed professional waste disposal service will be contacted to dispose of this material.

Hydrogen Peroxide: A licensed professional waste disposal service will be contacted to dispose the excess hydrogen peroxide.

Ethanol: Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

M9 Broth: A professional waste disposal service will be contacted to come and dispose of this material. M9 broth should not enter any drains as it is a threat to aquatic life.

DMSO: DMSO will be sealed in a tight container and the surplus will be handed to a licensed disposal company as a licensed professional waste company will be contacted.

Sodium Chloride: Sigma Aldrich suggest that when ready for disposal the surplus and non-recyclable solutions should be distributed to a licensed disposal company.

Autoclave: N/A

Bunsen Burner: All defective hoses should be disposed to prevent the gas from leaking.

Microcentrifuge: N/A

Scalpel: Sharp knife is placed back in case and disposed of.

C.elegan strains: Once mold sporulation has formed or the *C.elegans* have died 10% bleach will be used to spray the plates. The plates will be para filmed after and disposed of.

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

Alkaline Hypochlorite:
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=A1727&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dalkaline%2Bhypochlorite%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

Ethanol:

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https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E7023&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3DEthanol%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

M9 Broth:

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=79438&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3DM9%2Bbuffer%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

BunsenBurner:

https://ncifrederick.cancer.gov/Ehs/Media/Documents/SafetyGram_ISM-203_BunsenBurnerSafetyUseInBSC.pdf

Autoclave:

https://www.hu-friedy.com/sites/default/files/Autoclave%20Wrap%20-%20English%20SDS%20-%20Aug14.pdf