Standard Operating Procedures

Chemical: **Naphthalene**

Please fill out the form completely. Print a copy and insert into your
Laboratory Safety Manual and Chemical Hygiene Plan.
Refer to instructions for assistance.
08/2012 Edits added from original protocol provided at ehs.ucr.edu/laboratory/SOP are highlighted in red.

Department:_____ Date when SOP was written:_____

Date when SOP was approved by the lab supervisor: _____

Principal Investigator:_____

Internal Laboratory Safety Coordinator/Lab Manager:_____

Laboratory Phone:_____ Office Phone:_____

Emergency Contact:_____
(Name and Phone Number)

Location(s) covered by this SOP:_____
(Building/Room Number)

Type of SOP: ☐ Process ■ Hazardous Chemical ☐ Hazardous Class

Purpose

Testing naphthalene-induced production of current by *S. oneidensis* in bench scale reactors

Physical & Chemical Properties/Definition of Chemical Group

CAS# 91-20-3

Class: **Cal/OSHA Regulated Carcinogen/Acute & Reproductive Toxin**
Carcinogen (A4 by ACGIH)

Molecular formula: $C_{10}H_8$

Form (Physical State): White Crystalline Solid

Melting Point: 80.2°C

Potential Hazards/Toxicity

Oral LD_{50}: 490 mg/kg [Rat]

Permissible Exposure Limits (PEL): 75 mg/m^3

- Acute exposure of humans to naphthalene by inhalation, ingestion, and dermal contact is associated with hemolytic anemia, damage to the liver, and, in infants, neurological damage. Symptoms of acute exposure include headache, nausea, vomiting, diarrhea, malaise, confusion, anemia, jaundice, convulsions, and coma.

- Cataracts have been reported in humans acutely exposed to naphthalene by inhalation and ingestion. Cataracts have also been reported in animals following acute oral exposure.

- Tests involving acute exposure of rats, mice, rabbits, and guinea pigs have demonstrated naphthalene to have moderate to high acute toxicity from ingestion and low to moderate acute toxicity from dermal exposure.

- Hemolytic anemia has been reported in infants born to mothers who "sniffed" and ingested naphthalene (as mothballs) during pregnancy. The mothers themselves were anemic, but to a lesser extent than the infants. Signs of maternal toxicity (e.g., decreased body weight and lethargy) but no fetal effects were reported in rats and rabbits exposed to naphthalene via gavage. Maternal toxicity (increased mortality and reduced weight gain) and fetotoxicity (reduced number of live pups per litter) were observed in mice exposed via gavage.

- Workers occupationally exposed to vapors of naphthalene and coal tar developed laryngeal carcinomas or neoplasms of the pylorus and cecum. However, this study is inadequate because there were no controls, exposure levels were not determined, and subjects were exposed to complex mixtures containing other demonstrated carcinogens.

- Di-, tri-, and tetramethyl naphthalene contaminants of coal tar were found to be carcinogenic when applied to the skin of mice, but naphthalene alone was not. An increased number of alveolar/bronchiolar adenomas and carcinomas were reported in female mice exposed by inhalation. No carcinogenic responses were reported in rats exposed to naphthalene in their diet and by injection.

- EPA has classified naphthalene as a Group C, possible human carcinogen.

Personal Protective Equipment (PPE)

- All persons shall wear personal protective equipment when handling naphthalene. This includes wearing a lab coat, nitrile gloves, splash goggles, and closed toe shoes when working with naphthalene. Gloves should be changed frequently. Leave lab coats, gloves, and other personal protective equipment in the lab once your work is complete to prevent the spread of this or other chemicals outside of the lab.

Engineering Controls

- All operations involving naphthalene should be carried out in a certified chemical fume hood or a ducted Biosafety cabinet to keep airborne level below recommended exposure limits.

- Chemical fume hoods used as containment areas for particularly hazardous chemicals must have a face velocity of 100 feet/min, averaged over the face of the hood and must be certified annually.

- Laboratory rooms must be at negative pressure with respect to the corridors and external environment. The laboratory/room door must be kept closed at all times.

- Vacuum lines are to be protected by HEPA (high efficiency particulate air) filters or higher efficiency scrubbers.

First Aid Procedures

- *Inhalation*: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

- *Ingestion*: Do not induce vomiting. Examine the lips and mouth to ascertain whether the tissues are damaged, a possible indication that the toxic material was ingested. The absence of such signs is not conclusive. Loosen tight clothing such as a collar, tie, belt, or waistband. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention.

- *Skin Contact*: Immediately flush skin with plenty of water and non-abrasive soap for at least 15 minutes while removing contaminated clothing and shoes. Take care to clean folds, crevices, creases, and groin. Get medical attention immediately. Wash clothing before reuse. Thoroughly clean shoes before reuse. Contaminated work clothes should be laundered by individuals who have been informed of the hazards of exposure to this substance. If irritation persists, seek medical attention.

- *Eye Contact*: Check for and remove contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

Special Handling and Storage Requirements

- All work with naphthalene is to be done in the "naphthalene" designated area in order to keep contamination to a minimum. Area on bench for reactors with naphthalene will be taped off as a designated area. Peristaltic pumps (including pump heads) are not to be moved from taped-off area once naphthalene is introduced to the lab space.

- All chemicals containing naphthalene must be secondarily contained with proper signage. Containers of naphthalene and designated areas, including storage cabinets, must be labeled with a “CANCER HAZARD” warning. Any persons in this area are required to wear personal protective equipment. Safety shower and eye wash stations should be easily accessible where naphthalene is used. Reactors on benchtop will be placed in a plastic bin labeled “Naphthalene – cancer hazard” as a secondary container in case of spills.

- All laboratory equipment (such as beakers, pipettes, gel electrophoresis systems etc.) used in the "naphthalene" designated area are to be labeled as " naphthalene contaminated" and are not to be removed from the area without first being decontaminated. A designated tin will be placed in the naphthalene contaminated benchtop area for solid waste. A separated waste container will also be provided for fluid waste.

- Naphthalene stock solutions will be stored within taped-off area, and will be filter-sterilized upon injection into media vessel. Following use, syringes, needles, and filters will be stored in hazardous solid waste container within containment area.

- Store away from incompatible chemicals including the following: Oxidizers, including acids. Store locked up away from heat and sources of ignition. All equipment containing naphthalene must be grounded.

Spill and Accident Procedure

Highly flammable. Combustion products are carbon oxides. In the event of a small fire, use Carbon dioxide and/or dry fire extinguishers.

Chemical Spill Dial 911 and x59797

Spill – Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. Eliminate sources of ignition. If possible, confine the spill to a small area using a spill kit or absorbent material. Keep others from entering contaminated area (e.g., use caution tape, barriers, etc.).

Small (<1 L) – If you have training, you may assist in the clean-up effort. Use appropriate personal protective equipment and clean-up material for chemical spilled. Double bag spill waste in clear plastic bags, label and take to the next chemical waste pick-up.

Large (>1 L) – Dial **911** (or 310-825-1491 from cell phone) and EH&S at x59797 for assistance.

Chemical Spill on Body or Clothes – Remove clothing and clean body thoroughly in emergency shower with non-abrasive soap for at least 15 minutes. Seek medical attention. *Notify supervisor and EH&S at x59797 immediately.*

Chemical Splash Into Eyes – Immediately rinse eyeball and inner surface of eyelid with water for 15 minutes by forcibly holding the eye open. Seek medical attention. *Notify supervisor and EH&S at x59797 immediately.*

Medical Emergency Dial 911 or x52111

Life Threatening Emergency, After Hours, Weekends And Holidays – Dial **911** (or 310-825-1491 from cell phone) or contact the Ronald Reagan UCLA Medical Center (emergency room) directly at **x52111** (located at 757 Westwood Plaza, enter from Gayley Avenue). *Note: All serious injuries must be reported to EH&S at **x59797** within 8 hours.*

Non-Life Threatening Emergency– Go to the Occupational Health Facility (OHF), **x56771,** CHS room 67-120 (This is on the 6th floor, 7th corridor, room 120. Enter through the School of Dentistry on Tiverton Drive and proceed to the "O" elevator to the 6th floor.)Hours: M - F, 7:30 a.m. to 4:30 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All serious injuries must be reported to EH&S at x59797 within 8 hours.*

Needle stick/puncture exposure (as applicable to chemical handling procedure)– Wash the affected area with antiseptic soap and warm water for 15 minutes. For mucous membrane exposure, flush the affected area for 15 minutes using an eyewash station. Page the needle stick nurse by dialing **231** from a campus phone, enter **93333** when prompted and then enter your extension. Hours: M – F, 8:00 a.m. to 4:00 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All needle stick/puncture exposures must be reported to EH&S at x59797 within 8 hours.*

Decontamination/Waste Disposal Procedure

Waste disposal procedures

1. All solid naphthalene contaminated waste shall be disposed of into waste containers specifically designated for naphthalene waste. Examples of solid naphthalene waste material include empty containers, gloves, pipette tips, and paper towels. All tubing coming in contact will be disposed of as solid waste through EHS after naphthalene testing is complete.
2. Waste containers must be labeled with "CANCER HAZARD" warning.
3. Once the waste container is full, dispose of according the Cornell EH&S hazardous waste guidelines.
4. Liquid waste will be labeled appropriately and disposed of through the Cornell EH&S once liquid waste container is full (we will start with a 4L empty plastic ethanol container, which may be sufficient for the majority of our testing) waste material include empty containers, gloves, pipette tips, and paper towels.

Decontamination of Equipment

Equipment that needs to be decontaminated (for repair or change of location etc.) must be washed with soapy water and rinsed with copious amounts of water.

Material Safety Data Sheet (MSDS) Location

A hard copy of the MSDS can be found in the MSDS binder.

Online MSDS can be accessed at http://www.sciencelab.com/msds.php?msdsId=9927671

Protocol/Procedure

No more than 5L of 75 mM naphthalene solution will be used at a given time to be within the limits for naphthalene exposure.

- Stock solutions should be made in the fume hood in secondary containment (see Engineering Controls section) and transported to Riley Robb in secondary containment. Stock solutions should then be stored within the taped-off area, in secondary containment.
- One reactor setup will be used for all testing with naphthalene. Equipment such as peristaltic pumps (including pump heads) are not to be moved from taped-off area once naphthalene containing compounds are introduced to the lab space. After naphthalene testing-everything in the taped-off area (peristaltic pumps) will be thoroughly washed with soap and water prior to use for anything else (reference that this would be okay to do).
- See PPE equipment for requirements during handling of naphthalene.
- Between tests, all autoclaved equipment should first be washed thoroughly with soap and water prior to autoclaving to mitigate production of naphthalene fumes: Fluid from a vessel containing soap and water must be pumped through used tubing and reactors for

at least two HRTs (flow rate should be increased). Reactors must be rinsed between autoclave cycles. Rinse water must be collected as naphthalene contaminated waste in the designated container.

- All autoclaving must be done in a designated naphthalene autoclave tray, which also serves as secondary containment. This tray will be provided by the iGEM team.

Note: Any deviation from this SOP requires written approval from PI.

Documentation of Training *(signature of all users is required)*

- Prior to conducting any work with naphthalene, designated personnel must provide training to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.

- The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the naphthalene compound's MSDS provided by the manufacturer.

- The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate laboratory safety training or refresher training within the last two years.

I have read and understand the content of this SOP:

Name	Signature	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____