Standard Operating Procedures

Chemicals: **Sodium hydrogen arsenate heptahydrate**

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

Physical & Chemical Properties/Definition of Chemical Group

CAS# Sodium hydrogen arsenate heptahydrate: 10048-95-0

Class: **Cal/OSHA Regulated Carcinogen/Acute & Reproductive Toxin**
Carcinogen (IARC Group 1)

Molecular formula: HAsNa2O4.7H2O

Form (Physical State): Sodium hydrogen arsenate heptahydrate: white solid

Melting Point: Sodium hydrogen arsenate heptahydrate: 57degC

Potential Hazards/Toxicity

Permissible Exposure Limits (PEL): 10 ug/m^3

- *Inhalation*: Arsenic may cause inflammation of the mucous membranes with cough and foamy sputum, restlessness, dyspnea, cyanosis, and rales. Symptoms like those from ingestion exposure may follow. May cause pulmonary edema.

- *Ingestion*: Arsenic is highly toxic! May cause burning in esophagus, vomiting, and bloody diarrhea. Symptoms of cold and clammy skin, low blood pressure, weakness, headache, cramps, convulsions, and coma may follow. May cause damage to liver and kidneys. A suspected fetal toxin. Death may occur from circulatory failure. Estimated lethal dose 120 milligrams.

- *Skin Contact*: May cause irritation, symptoms including redness, itching, and pain.

- *Eye Contact*: May cause irritation with itching, burning, watering of eyes; may cause conjunctiva damage.

- *Chronic Exposure*: Arsenic on repeated or prolonged skin contact may cause bronzing of the skin, edema, dermatitis, and lesions. Repeated or prolonged inhalation of dust may cause damage to the nasal septum. Chronic exposure from inhalation or ingestion may cause hair and weight loss, a garlic odor to the breath and perspiration, excessive salivation and perspiration, central nervous system damage, hepatitis, gastrointestinal disturbances, cardiovascular damage, and kidney and liver damage. Arsenic compounds are known human carcinogens and may be teratogenic based on effects in laboratory animals.

Personal Protective Equipment (PPE)

- All persons shall wear personal protective equipment when handling arsenic. This includes wearing a lab coat, nitrile gloves, and closed toe shoes when working with arsenic. 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.

- A 2-glove rule applies within the arsenic taped-off area. All gloves entering area must be disposed of with the arsenic-contaminated solid waste. Under no conditions can a glove that has touched anything in the arsenic area be used to touch anything not in the arsenic area.

- All persons working with arsenic must wear a lab coat, which must be washed in the laundry prior to further use.

Engineering Controls

- All operations involving arsenic 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*: Induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention immediately.

- *Skin Contact*: Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. 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.

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

  Note to Physician: If emesis is unsuccessful after two doses of Ipecac, consider gastric lavage. Monitor urine arsenic level. Alkalization of urine may help prevent disposition of red cell breakdown products in renal tubular cells. If acute exposure is significant, maintain high urine output and monitor volume status, preferably with central venous pressure line. Abdominal X-rays should be done routinely for all ingestions. Chelation therapy with BAL, followed by n-penicillamine is recommended, but specific dosing guidelines are not clearly established.

Special Handling and Storage Requirements

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

- All chemicals containing arsenic must be secondarily contained with proper signage. Containers of arsenic 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 arsenic is used. Reactors on benchtop will be placed in a plastic bin labeled “Arsenic – cancer hazard” as a secondary container in case of spills.

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

- Arsenic stock solutions will be stored within taped-off area, and will be filter-sterilized upon injection into media vessel. Syringe and filters will be stored in hazardous solid waste container within containment area.

- Store away from incompatible chemicals including the following: Oxidizers, tannic acid, infusion cinchona and other vegetable astringent infusions and decoctions, iron solutions, rubidium carbide, chlorine trifluoride, fluorine, hydrogen fluoride, oxygen difluoride, acids, bases, sodium chlorate, zinc filings, other reactive metals and mercury. Corrosive to metals in the presence of moisture.

Spill and Accident Procedure

Toxic fumes of arsenic and arsine may be given off in a fire. In the event of fire, evacuate and bar further entry.

Chemical Spill Dial 911 and x59797

Spill – Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. Eliminate sources of ignition if the chemical is flammable. 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 rinse body thoroughly in emergency shower 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 arsenic contaminated waste shall be disposed of into waste containers specifically designated for arsenic waste. Examples of solid arsenic 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 arsenic 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)

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:
https://us.vwr.com/stibo/hi_res/AA41533-AP_02042010.pdf

Protocol/Procedure

No more than 5L of 10uM arsenite solution will be used at a given time to be within the limits for arsenic 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 arsenic. Equipment such as peristaltic pumps (including pump heads) are not to be moved from taped-off area once arsenic-containing compounds are introduced to the lab space. After arsenic 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 arsenic.
- Between tests, all autoclaved equipment should first be washed thoroughly with soap and water prior to autoclaving to mitigate production of arsenic 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 arsenic contaminated waste in the designated container.
- All autoclaving must be done in a designated arsenic 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 arsenic, 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 arsenic 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**
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____