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1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to outline proper handling procedures and methods for euthanasia of both transgenic and non-transgenic fruit flies (*Drosophila* species). Proper handling of flies used in research is needed to prevent accidental release into the environment. The SOP also includes methods for monitoring fly release, reporting requirements, and waste procedures.

2.0 HAZARDS AND CAUTIONS

Flies used in academic research may be generated with specific genetic constructs as needed for each experiment. Even if the genetic inserts could be harmless, accidental release of these transgenic flies to the environment may concern the public.

3.0 ROLES AND RESPONSIBILITIES

Each laboratory is responsible for the proper maintenance of its respective fly colonies and their ultimate disposal.

Principle Investigator (PI)

The Principal Investigator (PI) of each lab shall ensure that each lab member who handles fruit flies:

- Reads this SOP and follows it accordingly.
- Properly euthanizes all fruit flies before discarding them into biohazardous waste.

Research Personnel

Personnel who work with fruit flies as part of their laboratory research should be fully proficient in not only research involving fruit flies but also in safe ways of handling and terminating fruit flies before disposing of them into biohazard waste.

4.0 PERSONAL PROTECTIVE EQUIPMENT (PPE)

Employees must wear appropriate personal protective equipment (PPE) while engaged in fly research. PPE is selected based on a risk assessment of work to be performed. At minimum, the PPE checked in the following table must be worn and follows the [USC PPE Standards](http://ehs.usc.edu).

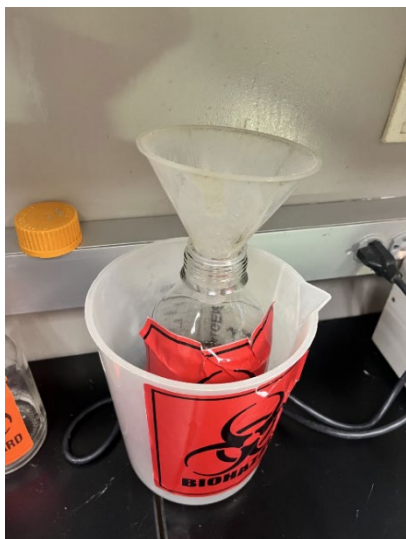
Gloves	Nitrile	X	Latex	X	Butyl			
Glasses/Goggles	Splash ¹		Safety ²	X	Face Shield ³	X		
Body	Lab Coat (e.g., FR, cotton) ⁴	X	Closed-toe footwear	X				
Other								

5.0 TOOLS/EQUIPMENT/INSTRUMENTS

- Fly Pad. A porous plastic working surface where CO₂ gas is emitted to keep the flies anesthetized.



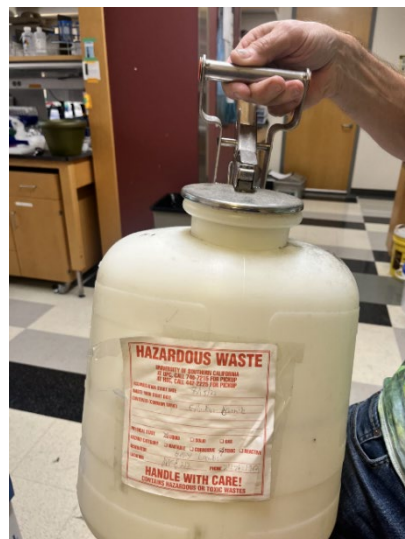
- Fly Morgue. A container of 70% ETOH which is used to euthanize the fruit flies.



Fly Morgue 1



Fly Morgue 2



Fly Morgue 3

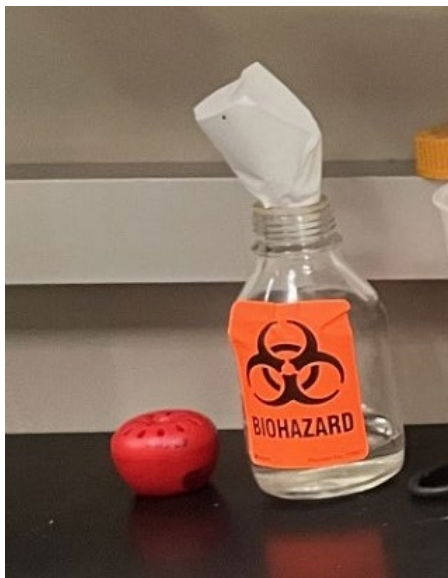
¹ Splash goggles are employed where there is the potential of splash or spray during laboratory and/or disposal procedures.

² Choose safety glasses based on a risk assessment of work to be performed.

³ To be used with safety glasses or splash goggles.

⁴ Lab coat must cover the arms. NOTE: Determine lab coat type based on the LHAT ([fillable PDF](#) or [RSS](#)). FR = Flame Resistant.

- Fly Trap. A jar or other open-mouth container which is baited with fruit, sugar source, acetic acid (vinegar) or alcohol to attract flies.



- Fly Paper. Used to catch escaped flies.

6.0 PROCEDURES

General Considerations

1. When using hazardous materials:
 - a. Wash hands before donning and doffing gloves.
 - b. Change gloves at least every 2 hours and/or when they become torn or contaminated.
 - c. Keep laboratory doors closed to outdoor hallways except for entry/exit of personnel.
2. Use secure secondary packaging (which includes a leak-proof and sift-proof container to prevent accidental release) to transport organisms.

Handling Flies

1. Flies are cultured in stoppered plastic vials and bottles and anesthetized with CO₂ gas prior to opening the containers to prevent escape.
2. During handling, flies are continuously anesthetized with CO₂ gas emitted from a porous plastic working surface ("fly pad").
3. Fly traps must be placed within the laboratory area which are designed to attract and euthanize flies that could be accidentally released (See examples in Section 5.0).

7.0 WASTE DISPOSAL

Cultures of Transgenic and Non-Transgenic Fly Lines

1. Stopper all bottles and vials containing viable flies, eggs, or larvae to prevent escape. NOTE: Do not keep the flies in this state for more than 5 days. See images A – C.
2. Place all stoppered fly bottles or culture vials with possible fly eggs, fly larvae, live or dead flies, inside a bag and seal by tying securely. See images A – C.



A. Fly Solids (larvae)



B. Storage at -20°C



C. Biohazard Bin – Disposal Ready

3. Place bags of fly waste inside a (-20°C) freezer (as in Step 1) located in or near the laboratory.
4. Freeze fly waste for a minimum of 8 hours or overnight. See images A – C.
5. Place the frozen waste – still sealed in the bag – and any other associated dry waste (dry contaminated biohazard debris) inside a biohazard bin for disposal by EH&S HazMat. See image C.
6. Dry biohazard waste shall be picked up weekly.

Liquid Waste

1. Flies on pads are disposed by knocking them into a container of 70% ETOH (see “fly morgue” in Section 5.0) which euthanizes the flies on contact.
2. The liquid fly/ETOH waste may be decanted into a marked, non-halogenated organic solvent safety container provided by EH&S for subsequent pickup – Fly Morgue 3 image. Request a chemical waste pickup through established EHSA or RSS accounts. NOTE: DO NOT place insects collected in alcohol in biohazardous waste for final disposal.

8.0 EMERGENCY/REPORTING AN EXPOSURE OR RELEASE

1. All exposures or releases of biohazardous material, including transgenic organisms or recombinant synthetic or nucleic acid molecules, must be reported to the Biosafety Officer (BSO) and reported to the IBC (biosafety@usc.edu).
2. If an incident occurs and the release is deemed reportable to the NIH Office of Biotechnology (NIH OBA), the BSO will notify the appropriate authorities and submit an incident report within thirty days.

For general emergencies:

1. Call DPS immediately: UPC (213) 742-4321, HSC (323) 442-1000.
2. Call EH&S at (323) 442-2200.
3. Report the emergency to the supervisor, lab manager, or principal investigator – see instructions at the [Emergency Notification and Incident Reporting](#) website.

Questions? Email EHS@usc.edu or dial (323) 442-2200.

9.0 TRAINING AND DOCUMENTATION

The Principal Investigator must ensure that his/her research group is trained in the application of this SOP by the principal investigator himself/herself or designee. Each user will enter his/her name, physical or electronic signature, and date below once he/she has read and understands the content of this SOP. The Principal Investigator will maintain this document (electronic or hard copy) in his/her files or central repository and will make it available upon request by EH&S during periodic or impromptu inspections. Each user will have access to a copy of the signed document.

NOTE: Users are subject to all applicable safety trainings including the General Lab Safety Course, annual laboratory safety training refresher, etc.

Name	Signature	Date

10.0 SOP REVIEW/REVISION

Date revised: 02/02/2024

By: USC EH&S Biosafety