Guidance from the Wayne State University Institutional Biosafety Committee

Preparation of Biosafety Laboratory Standard Operating Procedures (SOP) related to the use of transgenic Drosophila.

SOPs should be specific to the procedures carried out in your lab and should be considered as a framework for development of laboratory- and procedure-specific protocols that relate to safe execution of the intended experiments. The primary audience is those who will do the work. In addition to being an operational reference for experienced personnel, the SOP should be useful as part of training for new personnel. SOPs should be understandable without reference to external documents such as animal use protocols, grant applications, etc. Ideally, biosafety information should be incorporated into procedural / operational laboratory SOPs, but you may use any format if each of the categories listed below are addressed. SOPs should be free of spelling and grammatical errors.

Even if you propose the use of seemingly safe systems, carefully describe the potential risks and how you intend to address them.

Standard Laboratory Practices:

These are the standard practices to be employed by all researchers at WSU when conducting research with *Drosophila*. It is the responsibility of the PI to ensure that all research staff members under their supervision are provided with, and adhere to, these standards.

Hazards:

Describe specific procedures, materials and/or equipment associated with this protocol that may represent exposure and/or health hazards.

If a needle and syringe will be used, please state how needlestick injuries will be prevented (saying "utmost care will be taken" is not sufficient).

Personal Protective Equipment (PPE):

List PPE required when performing listed procedures, entering rooms and/or handling hazardous material. Include a statement that describes when the PPE is required. This should include a lab coat or dedicated “scrub” uniform, gloves, and eye protection. Specify glove type; if latex gloves are specified, also specify an alternative material.

Additional Special Handling:

Indicate the nature of the primary and secondary containers to be used when transporting *Drosophila* or waste materials associated with this work (e.g., fly containers). The outside of the secondary container should be clearly labeled with the following information:

- PI name
- Contact information.
- Nature of contents
- Biohazard symbol (stickers can be obtained from OEHS).

Waste Disposal:

Autoclaves:

The use of an autoclave is a standard practice for the treatment of biological material. However, to be compliant with regulatory requirements additional steps must be performed, and recorded, to verify the efficacy of this treatment method (see below for details). Only if this process is completed can waste material be disposed of in the sewer or the regular trash. The use of an autoclave in the absence of verification is a recommended intermediary practice for waste associated with the use of transgenic *Drosophila*. However, the material must be subsequently discarded in a suitable biohazard container.

Key components for autoclave validation are:
A. Appropriate use of the autoclave to decontaminate biological waste.

- Minimal parameters are 121oC at 15 psi for 15 min.
- Time may need to be increased for larger loads and larger volumes of fluid.
- Items should be loaded in a manner that ensures that steam can penetrate packages and test tubes.

B. Recordkeeping – There should be a log or notebook adjacent to the autoclave to indicate:

- Date
- Time
- User name and contact number.
- Type of load (liquids, hard goods, etc.)
- Items autoclaved (media, waste, pipettes, etc.)

Ideally, any autoclave paper tape would be kept with the waste log to verify autoclave parameters.

C. Performance verification

- If the autoclave has paper tape to record performance, this should be checked prior to opening the door to be sure all temperature, pressure, and/or time parameters were met.
- Autoclave indicator tape should be clearly visible on each item placed in the autoclave (one per rack of tubes, one per beaker, one on a bag of used plates, etc.).

- The person in charge of the autoclave operation or a designated safety officer should conduct a monthly performance verification using a biological thermophilic spore former, such as Bacillus stearothermophilus ATCC 7953. There are several different verification methods that employ this organism. One is the Sterikon Plus Bioindicator ampule system, a rapid and easy-to-use method for verifying steam sterilization. Indicators consist of an ampule containing nutrient broth, sugar, a pH indicator, and 2 mL of spores from the apathogenic organism Bacillus stearothermophilus ATCC 7953. Simply place ampules in the autoclave along with the batch to be sterilized and incubate afterwards. A color change of the ampule contents clearly indicates whether sterilization was successful. This testing should be documented monthly and readily available for inspection.

D. Annual calibration and maintenance

An outside maintenance person familiar with the operation of autoclaves should perform this service.

Waste containers:

1. Requests for 40-gallon biohazardous waste containers can be submitted through the OEHS website – Biological waste.
2. Researchers may set up additional smaller biohazardous waste containers for accumulation of biohazardous waste throughout the laboratory. These containers must be hard sided, leak proof, puncture resistant, and have a lid. The container should be lined with a suitable red biohazard autoclave bag. The outside of the container must be labeled with a biohazard symbol. Cardboard is not a suitable material for these containers.
3. Requests for 5-gallon liquid carboys for chemical waste accumulation can be submitted through the OEHS website – Chemical waste.

Appropriate disposal methods:

Fly containers

- Reusable containers must be autoclaved (minimal parameters- 121°C at 15 psi for 15 min. – verification required (see above)) or frozen (-15°C to -20°C for 24 hours) before the contents can be discarded as regular waste.
- Disposable containers must be autoclaved (minimal parameters- 121°C at 15 psi for 15 min.) or frozen (-15°C to -20°C for 24 hours) before being disposed of as biohazardous waste (non-validated autoclave) or regular trash (validated autoclave or frozen).

Please ensure that the materials being used are suitable for autoclaving. Researchers wishing to employ other disposal methods should contact the WSU Biosafety Officer to discuss the suitability of these methods.

Contents of fly traps

Fly traps should be changed regularly (every 2 weeks), and the contents treated in the same manner as the fly containers (described above).

Fly morgues

There are several acceptable methods for the disposal of the contents of fly morgues and the appropriate disposal route depends upon the liquid component being employed. At WSU, there are two main types being employed:

1. Ethanol: The ethanol/fly mixture should be collected in a carboy and tagged as chemical waste. These containers will be marked as “incinerate only”. Once full, a waste pick-up request should be promptly submitted. The waste will then be collected by an OEHS representative.
2. Soap and Water: As soapy water does not constitute a chemical waste, there are several viable options for disposal:
    a. The contents from these morgues can be treated in the same manner as the ethanol traps, with the waste carboy being tagged as chemical waste and marked “for incineration only”.
    b. The contents may be autoclaved and then strained, with the soapy water being disposed of down the drain and the flies being disposed of in a biohazardous waste container.

Please contact the WSU Biosafety Officer to discuss additional options if required (rjpearson@wayne.edu or (313) 993-7597).

Sharps

Sharps must be disposed of in a hard sided sharps container. Any sharps that are contaminated with either chemical or radiological material must be disposed of in separate sharps containers and labeled appropriately. All other sharps must be disposed of in biohazard sharps containers.

Needles remain one of the most common causes of laboratory accidents. Reinforce the correct manner for safe handling and disposal of these items with your research staff.

Accidental Release Response

In the event of an accidental release the PI should be immediately notified. Adjacent doors must be closed, and additional fly traps placed within the workspace. In the event of a significant loss of containment, the WSU Biosafety Officer (rjpearson@wayne.edu or (313) 993-7597) must be notified.