**Standard Operating Protocol**

**Sinnhuber Aquatic Research Laboratory (SARL)**

**Handling and disposal of chemicals tested for bioactivity in the developmental zebrafish**

**Purpose:** To direct SARL personnel in the safe and responsible handling of **all** chemicals and associated wastes used in testing chemical bioactivity in the developmental zebrafish.

**Personnel qualifications – NO EXCEPTIONS to 1 or 2:**

Handling of chemicals and chemical waste at the SARL requires that:

1. The individual shall be fully trained in the Oregon State University Environmental Health & Safety regulations and procedures prior to any lab chemical handling, *or*, if a visiting scientist, the individual shall have completed similar training at their Institution.
2. The individual shall first be fully instructed by a member of the **SARL permanent staff** in specifics of EH&S approved chemical handling, waste disposal and personal protective equipment procedures at the SARL.

**Types of chemicals tested routinely at the SARL:**

Pharmaceuticals

Flame retardants

Current use (USA and EU) pesticides

Current use (USA and EU) herbicides

Organic compounds used in plastics manufacturing

Organic compounds used in petrochemical refining and manufacturing

Polycyclic aromatic hydrocarbons

Nanomaterials

Mycotoxins

**Overview of procedure for chemical testing in the developmental zebrafish at the SARL**



**Typical chemical quantities handled and disposed of with each test:**

Up to approximately 0.5 mg of a chemical is used in tests when the maximum test concentration exceeds 100 uM, but testing at the SARL typically targets more toxicologically relevant concentrations of 64 uM or less. Thus, maximum chemical usage per test is generally 0.3 mg or less.

**Specifics of procedure for chemical bioactivity testing in the developmental zebrafish:**

1. *Procurement of test chemicals*. Procurement is generally by:
2. Direct purchase by SARL personnel through an approved OSU vendor in which case chemical identity and structure are known. Supporting physical property documentation and complete MSDS information is provided with the purchased chemical.
3. Client-provided, where for reasons of intellectual property (IP) protection, or to avoid test bias, SARL personnel may be blinded to chemical identity.

Condition 2 (blinded) still necessitates that any personnel involved with testing of those chemicals be made fully aware of the potential health hazards and the physical properties of those chemicals to the best of the Client’s knowledge, regardless of IP. This requires a Trade Secret MSDS sheet from the Client. The Client must also complete and return to SARL personnel, the SARL Chemical Hazard Disclosure form (Appendix 1) prior to SARL personnel commencing any work with that chemical. The hazard disclosure serves to identify whether known chemicals classified (US EPA) as high hazard concern are present necessitating special waste handling measures by SARL personnel.

1. *Chemical form*

Chemicals in solution. In solution is the preferred mode of chemical procurement because it lowers the potential hazard rating (i.e., for carcinogens) and obviates the need for restrictive dry powder chemical handling precautions.

Dry powder chemicals. Every effort should be made prior to procurement to specify that chemicals delivered as a dry powder be packaged in a septum capped vial. The septum cap allows easy solvent introduction via needle tipped syringe without the need for SARL personnel to open the vial of powder. *NOTE: handling (weighing and transfer) of known and probable carcinogens* ([listed here](http://www.cancer.org/cancer/cancercauses/othercarcinogens/generalinformationaboutcarcinogens/known-and-probable-human-carcinogens)) *in powder form requires the use of a glove box isolator. ABSOLUTELY NO EXCEPTIONS are granted for dry powder carcinogen transfer quantity or the immediate availability of an isolator.*

1. *Chemical dissolution*. Dimethyl sulfoxide (DMSO) or aqueous vehicle are the solvents for test chemicals at the SARL. DMSO is used for chemicals with very low or unknown water solubility. The typical stock concentration for a chemical depends on its solubility in the chosen solvent and is typically 1 mM to 20 mM, or roughly 0.1 mg/mL to 20 mg/mL (varies with molecular weight) for most compounds tested. Standard operating procedure at the SARL is to use amber glass vials with septum caps for all test chemical solution storage.

For aromatic compounds, like higher molecular weight PAHs and some flame retardants, where solubility in DMSO may be less than optimal, *a 10 - 20 minute bath sonication step of the test chemical stock vial, just prior to administration to zebrafish, is standard operating procedure at the SARL.*

1. *Chemical delivery to the experimental chamber*. The SARL uses a Hewlett Packard D300 digital dispenser for essentially all chemical administrations to the zebrafish experimental chamber, which is typically the individual wells of a 96 well polystyrene or polypropylene microtitre plate.

Use of the D300 dispenser is outlined here, in brief: Upon creation of a dispensing protocol in the D300 software, the user commences a run and is prompted to load a volume (typically from 2 to 12 uL) of test compound into a disposable D300 cassette. The cassette functions as an inkjet cartridge and rapidly jets 13 picolitre droplets into the plate wells to achieve the desired test concentrations. *Because of the potential for aerosolization of more volatile chemicals during the jetting, the fume hood enclosing the D300 must be running during any dispensing*.

*After delivery of the chemical to the zebrafish test plate, the test chemicals are returned to their proper storage location and the D300 spent cassettes, containing residual test chemical (approximately 0.1 to 1 uL of ~20mM conc.) are removed to the* ***closed solid waste container****.*

1. *Handling the assay plate/experimental chamber.*

The standard SARL zebrafish developmental assay for chemical bioactivity spans 5 days during which time handling of the 96 well plates is minimal and no material for disposal is removed from the assay plate. *A lab coat and nitrile exam gloves must be worn to handle any assay plates once the test chemical is delivered.*

1. Termination of assay, disposal of liquid contents and disposal of the assay plate.

At the end of the 5 day assay period, the plates are opened by the investigator and visually assessed under the microscope for developmental effects in the zebrafish larvae from the chemical exposure. Once this assessment is made and recorded, the plate is washed in a BioTek plate washer to collect the liquid contents. Briefly, the BioTek instrument flushes water into the wells while aspirating the contents to a 5 L vacuum trap which serves as the waste collection point.

*When the 5 L trap is full the contents are emptied into an OSU EH&S provided liquid chemical waste barrel at the SARL.* The air exhausted from the BioTek during the aspiration step is passed through an activated carbon filter (VapLock EF100V) to prevent any chemical vapors from escaping to the room. *This filter MUST be changed out with a new one every 2 - 3 weeks or vapor breakthrough begins to occur.*

The washed and now empty plate goes to the regular lab trash receptacle.

**Appendix 1**

**SARL Chemical Hazard Disclosure form**

Client contact information: (*To be completed by SARL personnel before sending to Client*)

SARL contact information: (*To be completed by SARL personnel before sending to Client*)

Please indicate whether any components of the samples to be tested consist of:

* Formulations containing tri-, tetra-, or pentachlorophenol or formulations containing compounds derived from these chlorophenols
* Tetra-, penta-or hexachlorobenzenes
* Chlorinated dioxins, chlorinated dibenzofurans or chlorinated biphenyls

If any of the above are present in the samples to be tested, please indicate which ones and the amount.

Client representative signature \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_