**Standard Operating Protocol**

**Sinnhuber Aquatic Research Laboratory (SARL)**

**Ensuring experimental rigor in testing bioactivity in the developmental zebrafish**

**Purpose:** To emphasize several key best practices for generating high quality chemical bioactivity data.

**SARL SOPs you must have prior familiarity with:**

1. Chemical Preparation and Dilution
2. Exposure set up - static and daily renew
3. Safe Handling and Disposal of Test Chemicals

**Key best practices of experimental rigor:**

1. *Animal replication and plate controls*
2. Best practice is that every plate contain 1 row (12 wells) or 2 columns (16 wells) of a given chemical concentration with 2 plate replication to ensure n=24 and 32 animals per concentration, respectively. Layout always includes the control treatment.
3. The row layout accommodates 7 concentrations plus control in rows A - H. The column layout accommodates 5 concentrations plus control in columns 1 – 6 with the layout replicated in columns 7 -12.
4. Row layout (n=24) is best suited to range finding experiments where more concentrations favor identifying a putative EC80 or a putative LEL. The column layout, with more replication is best suited to definitive testing of either of these tox parameters.
5. The column layout (n=32) provides sufficient animals for statistically valid 24 hpf and 120 hpf photomotor behavior analysis. The row layout must be expanded to 3 plates (n=36) for valid photomotor behavior analysis.
6. *Normalization of DMSO solvent.*
7. DMSO is nearly ubiquitous in exposures at SARL, both as the most common water-miscible solvent for lipophilic compounds, and as a necessary wetting agent for the HP D300 print head reservoirs. There is evidence in the zebrafish literature that exposure to 1% DMSO during development is an oxidative stressor. Our own empirical evidence at SARL supports the assertion that 1% DMSO is bioactive on larval zebrafish photomotor behavior and on the cognitive performance of adult zebrafish that were developmentally exposed to 1% DMSO. We have not observed these effects using 0.64 % DMSO, so that has become the SARL maximum exposure concentration. Whenever possible, 0.1% DMSO is preferred. M. Simonich and L. Truong are available to advise SARL investigators on the most appropriate DMSO concentration for their test compounds.
8. A consequence of potential bioactivity from DMSO and the best practice of eliminating non-target variable where possible is the need to normalize the DMSO concentration in all wells of the experiment to equal the DMSO in the highest concentration group.

For example, if your test chemical working stock conc. is 10 mM dissolved in 100% DMSO and the highest concentration you will test is 100 uM, the bioprinter will dispense 1 ul of the 10 mM stock into each of the 100 uM group wells. Because the assay volume is 100 uL (mostly EM), that will result in 1% DMSO in the 100 uM wells. The 1% is not ideal, as mentioned, but could be unavoidable with a highly insoluble (in EM) compound. To avoid confounding the high conc. test chemical results with a DMSO effect, you must have 1% DMSO present over the entire conc. range, including the untreated control animals.

1. *Exposure data acceptance criterion*

The threshold for a valid developmental toxicology experiment at the SARL is ≤ 20% of the untreated control animals dead or malformed, and ≥ 80% of the external positive control animals (0.2 uM Ziram, separate plate, run daily) must display the specific notochord malformation. You may be responsible for scoring mortality/malformation incidences for your own experimental plates, in which case you are also responsible for rejecting the experiment based on the internal negative control data. The external positive control animals (separate plate) are always exposed and scored by a regular member of the screen team. You will be informed if the positive control animals did not respond normally to the Ziram on a given experiment day. Certain stressors, unknown to the SARL, may occasionally render development more resistant to chemical insult. This is exceedingly rare, but it has occurred over a 2-3 day period, several times in the last 5 yrs.