Toxicity test protocol

# Selecting types

Select 2 types randomly from sheet per habitat. Include duplicates in habitats with only 2 lines. The two types are the two replicates per habitat.

Pick two mothers from each type to increase chances of getting 5 neonates per type per habitat.

# Culturing first broods

Put the two mother in two different vials. Allow to reproduce and allow neonates to grow up into adults (~10 days).

2 duplicates \* 2 types \* 16 habitats = 64 vials

Need 6.4 L FLAMES

Get a total of 6 adults. Put in separate vials and allow neonates to be made. Need a total of 15 neonates to be made for each type per habitat (15 neonates \*2 types \* 16 habitats = 480 neonates). (~10 days)

6 adults \* 2 types \* 16 habitats = 192 vials

Need 19.2 L FLAMES

# Running assay

2 types \* 3 copper conc \* 16 habitats = 96 vials

Need 9.6 L FLAMES

Spike 3.2 L with concentration of 15 ug/L and 3.2 L with 30 ug/L.

Transfer 5 neonates into each vial. Count mortality after 48 hours.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number of neonates surviving after 48 hours (out of 5) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | Habitats | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | | 12 | | 13 | | 14 | | 15 | | 16 | |
| Cu | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 30 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |