Dosing Protocol

Safety and PPE: Wash hands before beginning, wear gloves

Protocol

1. Morning of dosing: check egg water, make a new batch if there is not enough (<80 mL).
2. Collect eggs at 9:30 AM
3. At 4:30 PM (~30 minutes before dosing), sonicate nanoplastics for 60 seconds in the Gosse Lab
   1. Nanoplastics located in the refrigerator below the seahorse, must be refrigerated when not in use, transport in a styrofoam cooler
4. Immediately before dosing, remove chemicals from the freezer (in container marked ‘Nanoplastics Summer 2019’)
5. Label 8 GLASS petri dishes with date of fertilization, dosing chemical, dosing amount, and presence of nanoplastics
6. Fill each petri dish with 10 mL of egg water
7. Add dosing chemical (see chart below)
8. For doses with nanoplastics, add 10 uL of nanoplastic solution.
9. Add 10 zebrafish embryos to each dose using a pipette. Make sure to add as little egg water as possible. If there are multiple dishes of embryos, make sure to take embryos from each dish for each dose.
10. Swirl dishes/pipette to mix, place in incubator.
11. Each morning, remove any dead embryos from dosing dishes and note the number removed and from which dish they were removed.
12. Analyze 5 days post fertilization.

Dosing Chart (Bolded values indicate how much to add to dish from aliquoted tube in freezer, nonbolded indicate actual measured concentration of chemical)

|  |  |  |  |
| --- | --- | --- | --- |
| Chemical | High Dose | Medium Dose | Low Dose |
| FCCP | 15 uM (**15 uL from stock**) | 5 uM (**5 uL from stock**) | 2.5 uM (**2.5 uL from stock**) |
| Rotenone | 5 ug/L (**10 uL from H**) | 0.5 ug/L (**1 uL from H**) | 0.05 ug/L (**10 uL from M**) |
| TDCPP | 1 uM (**10 uL from M**) | 0.1 uM (**1 uL from M**) | 0.01 uM (**10 uL from L**) |
| Toxaphene | 20 mg/L (**2 uL from H/M**) | 10 mg/L (**1 uL from H/M**) | 5 mg/L (**5 uL from M/L**) |
| Glyphosate | 10 mg/L (**1 uL from H**) | 1 mg/L (**10 uL from M**) | 0.1 mg/L (**1 uL from M**) |
| Roundup | 10 mg/L (**1 uL from H**) | 1 mg/L (**10 uL from M**) | 0.1 mg/L (**1 uL from M**) |
| PFAS |  |  |  |
| Paraquat | 100 mg/L (**10 uL from H**) | 10 mg/L (**1 uL from H**) | 1 mg/L (**10 uL from M**) |