AQUATIC TEST

1. Plan your experiment and make media preps such as K, OP50, Clorox solution, SDS, dH_20, etc.; synchronize worms using previously described techniques (Dauer and egg preparations).

2. Prepare aquatic solution to be tested and aliquot 1 ml into each cell well (Costar 3512 plates). Store in 20℃ incubator for same day use (prepare solutions with NP as close to testing as possible).

3. Prepare nematodes for test. Once an age-synchronized plate has incubated for 2 days, the worms are at the L3 stage and ready for testing (it is better not to wait until the nematodes reach L4 stage to avoid matricidal hatching during the test):

a. Holding at an angle, rinse the plate with K- medium.
b. Using a pipette, transfer the worm solution into a 15 ml centrifuge tube, and allow gravity to settle.
c. Repeat the rinsing 3 times with K-medium.
d. When working with Ag-NP, K-medium cannot be used due to high ionic strength of the K-medium and instead, solution with lower ionic strength, Moderately Hard Reconstituted water (Recon), is used for the test. To reduce the osmotic stress, it is necessary to acclimate the nematodes before transferring them directly into Recon.
e. For acclimatization, after rinsing the plates with 100% K-medium, continue rinsing the nematodes in the 15 ml tube with mixture of K-medium and Recon: 1st rinse – 25% of Recon, 2^{nd}- 50%, 3^{rd}- 75% and 4^{th} – 100% of Recon. Let the nematodes to settle for 30 min between each acclimatization step.
f. After last rinse (100% Recon) remove enough Recon so that about 2 ml of solution is remaining.
g. Agitate the worm solution gently to re-suspend the pellet, and transfer it into a small Petri dish (6 cm) for transfer into wells. You can also add more Recon to the dish if necessary.

4. Transfer* 9-11 nematodes to the appropriate well of the 24-well plate (Costar) with 3-4 replicates per treatment, wrap the plate with the parafilm, and incubate at 20°C for 24 hours. **Remember to begin adding worms to lowest concentrations and rinse pipette between wells to avoid contaminating worm stock). You can also transfer the nematodes into separate plates for each treatment and use different capillary tubes.**

*Capillary tubes on a micropipette set at 20µl seem to be most effective method of transfer:

a. Using a capillary tube cutter cut a piece of tube ~ 3 cm in length.
b. Heat each end of the tube at the edge of a Bunsen burner flame for about 10-15 seconds until the ends are well rounded.

c. Cut ~1 cm of the micropipette tip off so that the modified capillary tube fits snugly into it. Fit the tube into the micropipette while one end is still hot to establish an airtight seal.

5. After 24hrs, do lethality counts under microscope. Check nematodes first under low power, and if all worms are not found, go to high power. If some nematode appears to be dead, probe gently using a glass capillary probe to verify that they are dead.

Note: when working with Au-NP, 50%K-medium in H_2O works well for the control and test solution.

MEDIA PREPARATIONS

K-Medium (pH 5.82)

2.36g KCl		3.54g KCl		7.08g KCl
3.00g NaCl	OR	4.50g NaCl	OR	9.00g NaCl
1000ml dH_2O		1500 ml dH_2O		3000ml dH_2O

Combine in flask, cover with double-thick foil, autoclave.

Moderately Hard Reconstituted Water

Per 1000ml of dH_2O, add the following:	(i.e., for 8L carboy:
96.0mg $NaHCO_3$	768mg $NaHCO_3$
60.0mg $CaSO_4 \cdot 2H_2O$	480mg $CaSO_4 \cdot 2H_2O$
60.0mg $MgSO_4 \cdot 7H_2O$	480mg $MgSO_4 \cdot 7H_2O$
4.0mg KCl	32mg KCl

Correct for hydrate forms:

$$\text{i.e., } \frac{X}{60} = \frac{MgSO_4 \cdot 7H_2O}{MgSO_4} = \frac{246.48}{120.38}$$

(etc.)

Aerate for at least 24 h before use (to avoid contamination instead of aeration, prepare about 0.5L of the solution in 1 ml bottle and mix it well by shaking)

When ready: pH=7.4-7.8
Hardness=80-100
Alkalinity=60-70