Accumulation/Depuration Experiment

1) Set up the exposure solution by combining 10 mL of your gold nanoparticle solution with 90 mL of the moderately hard water.

   *We will have a separate beaker with unexposed daphnids for comparison

2) Transfer three adult *Daphnia magna* from the mass culture to the exposure solution then place the beaker in the incubator until the next lab period.
3) After ~24 hours remove the beaker from the incubator.
4) Remove one daphind from the exposure solution and examine its gut tract

   *You are welcome to use a dissection microscope to take a closer look.
   *Notice how dark their guts appear compared to a control daphnid.

5) Transfer one of the remaining daphnids to a 100 mL MHW solution that contains algae and the other daphnid to a 100 mL MHW solution without algae.
6) Every twenty minutes, remove the daphnid from the water, look at it quickly under the dissection microscope and return it to its depuration solution.

   *Again pay attention to the color of the gut tract and take note of the differences in speed of elimination between the daphnids depuration with algae and those without.

Toxicity Test

We do not know the concentration of the nanoparticle solution that you created however we can assume that the concentration is in the ppm range. We will run a standard 48 hour *D. magna* toxicity test with three concentrations plus a control, 5 neonates per rep, 3 reps per concentration to determine the toxic potency of our nanomaterials.

1) Create 3 stock concentrations of gold NPs, 2 concentrations of a positive control ($K_2Cr_2O_7$), and 1 control using the table below for guidance.
2) Record the pH, conductivity, temperature and dissolved oxygen of each stock exposure solution on the data sheet.
3) Fill three beakers per concentration with 9.5 mL of the appropriate exposure solution.
4) Add 5 <24 hr old neonates to each exposure solution using the 0.1 mL pipet then place in the incubator.
5) After 24 hours remove the beakers from the incubator and count the number of surviving neonates. Record in the data sheet and place the beakers back in the incubator.

6) After another 24 hours remove the beakers from the incubator again and count the number of surviving neonates in each replicate. Measure the final pH, conductivity and temperature. Record the measurements and the neonate survival in the data sheet.

Exposure Solution	**Volume of Contaminant (mL)**	**Volume of Freshwater (mL)**
Control	0	40
50% AuNP	20	18
10% AuNP	4	34
1% AuNP	0.4	37.6
3.2 mg/L $K_2Cr_2O_7$	4 (from 32 mg/L stock)	34
1.0 mg/L $K_2Cr_2O_7$	4 (from 10 mg/L stock)	34