Receiving Shipments and Storing IQ-Tox Products

1) IQ Substrate, IQ Chambers and preliminary preparations:

a) Dry IQ Substrate will keep for many years. Avoid humid conditions, and it could last forever. Prepared (hydrated) IQ Substrate should be kept for no more than 24 hours, refrigerated.

b) Speckles and cosmetic imperfections in IQ Chamber plastic are normal, as they must be produced without any chemical releasing agents or sprays. Chambers should always be rinsed well with DI, distilled, control or dilution water before tests. Do not use soap or detergents on IQ Chambers. Remember that for every set of simultaneous tests, you need one control set composed of three cells (1/2 chamber), and ½ chamber for each test sample. For 5 simultaneous tests, you need three exposure chambers. For EC50's, each test or replicate requires one entire IQ Chamber.

c) Figure out where you will have enough darkness to score your tests using the UV Lights – A closet perhaps? We have found turning off the lights and drawing the shades to be insufficient. Hiding under a large thick cloth or coat will work ok in a pinch, but can be awkward or even a safety hazard.

d) Prepare a form for recording your data. It should contain whatever sample identifiers or other information pertains to your specific use, and space for recording how many organisms are adversely affected. For standard IQ-Tox tests, you will be recording how many out of each 18 daphnia (3 cells) per test sample or control are adversely affected. For EC50's, you will be counting per each individual cell. It is also advisable to include fields to record the start time for each test, plus one hour to indicate when IQ Substrate is added, and plus an additional 15 minutes for quick reference of when to score the test. (see scoring information at the end each test direction section below)

2) Daphnia magna and Control/Dilution Water Requirements and preparations:

a) Each cell of each test requires 6 fasted Daphnia magna juveniles.

One Standard IQ Tox test: ½ IQ Chamber = 3 cells = 18 D. magna
One " " " Control: ½ IQ Chamber = 3 cells = 18 D. magna
One EC50 (per replicate): Full Chamber = 6 cells = 36 D. magna

b) 5-day old D. magna are an ideal. They are of a size that is easy to see when scoring the test, while young enough to be sensitive. 3 – 7 days of age is the accepted range (provided they are not yet carrying young that are obvious to the naked eye, and certainly no neonates have been produced). The range of ages of daphnia used should be no more than 2 days (e.g., 3-4 days old, or 5-6 days old, but not 3-5) so as not to cause uneven distribution of sensitivity.

c) D. magna should be fasted for a minimum of 4 hours before testing. Transfer from culture into dishes containing fresh culture water at least four hours before scheduled testing and retain under normal room lighting and culture temperature conditions. Our low plastic "Daphnia dishes" or any similar design in glass or surfactant-free plastic will work well for fasting Daphnia, and it is easy to transfer Daphnia from these dishes directly into the IQ Chambers. Do not use soap or detergents to wash dishes used for this purpose, simply rinse very well and drain between uses. Discard any remaining Daphnia at the end of the day's testing; once fasted, daphnia should not be retained overnight for testing use.

d) Your control/ dilution water is fresh culture media, free and pure of food additives.

e) Store any control or dilution water, or toxicant solutions at the same or similar temperature as the Daphnia, so that their temperatures will not need to be modified before use. For Standard tests, tap water (which may contain chlorine or chloramines, or cationic metals) is treated with Sodium Thiosulfate and/or EDTA and warmed or cooled to within the range of 15.5 – 26.5 ºC (60-80 ºF) before being tested.

Performing the IQ-Tox Test (Standard)

1) Prepare IQ Substrate:

a) Uncap vial and add 5 mL of Control or Dilution Water. (Use syringe, or your choice of implement)

b) Cap securely, and sonicate for about a minute in water bath below cap level, *or* shake very well until liquid is a bit milky. (It is ok if a small amount of undissolved powder or small clumps remain, as long as the liquid is whitish).

2) Prepare IQ Chambers and supplies:

a) Rinse IQ Exposure Chambers with DI, distilled, or control water.

b) Label chambers according to your purposes (Sharpies work well). Remember that you will be scoring these in the dark, so it helps to see your markings clearly or it's just too easy to score the tests backwards.

c) Get ready to roll: lay out all supplies, samples, implements.

3) Warm or cool samples if necessary. If samples are collected much earlier than testing, store them near the Daphnia in order to moderate their temperature. Always check that they are within the range of 15.5 – 26.5 ºC (60-80 ºF) before proceeding.

4) Handling Daphnia:

a) Transfer Daphnia to IQ Exposure chambers. Use a wide bore pipette, or implement of your choice, to put 6 Daphnia into each of the six cells in each chamber. Be careful not to crush or maim them as you go.

b) There should be less than 1 mL water transferred with the Daphnia into each cell: either transfer as little water as possible with the Daphnia, or, easier for beginners, remove excess with a pipette (a calibrated pipette works great for this, with the tip on the bottom of the cell, so as not to suck up the Daphnia too).

c) Be sure to leave the Daphnia at least ½ mL to swim in!

5) Add control and sample waters.

a) Use a large capacity pipette to add control water to the three control cells up to the 10 mL lines on the Exposure Chamber.

b) Use a large capacity pipette to add sample water to the first test set of three cells, to the 10 mL lines. Use a new Large Capacity pipette for each additional test sample.

c) Record your start time and wait 1 hour.

6) Shake up prepared IQ Substrate again (by hand, no need to re-sonicate). Using a calibrated pipette (or any pipette that easily dispenses small drops), add 3 drops to each cell. Adding more will not affect the results, as the effect of IQ Substrate plateaus at a low level. Do not shake or stir; let sit for 15 minutes.

7) Score test.

a) 15 minutes after Substrate has been added, go to a dark place – not before, some light is needed while incubating with the substrate.

b) Don UV Protective glasses, shine the UV light on the chamber. Observe the brightness of the control Daphnia. Any Daphnia in other cells fluorescing at or near that level of brightness are considered non-adversely affected. Any Daphnia that do not fluoresce or appear very dim compared to the controls are considered adversely affected.

c) For every control or test sample (18 organisms total per each), count any adversely affected organisms and record the data.

Test instructions continued on page 3

8) Interpret Results. For a standard toxicity test, four or more adversely affected organisms out of 18 indicates toxicity. Remember that for use on finished (tap) water by water utilities, metals and chlorine/chloramines are neutralized with EDTA and Sodium Thiosulfate before testing samples, in order to exclude toxicity that is not an immediate threat to humans.

9) Discard any remaining Daphnia at the end of the day's testing; once fasted, daphnia should not be retained overnight for testing use.

Using IQ-Tox for one-hour *D. magna* EC50 (Steps differing from standard assay are highlighted)

1) Prepare IQ Substrate:

a) Uncap vial and add 5 mL of Control or Dilution Water. (Use syringe, or your choice of implement)
b) Cap securely, and sonicate for about a minute in water bath below cap level, *or* shake very well until liquid is a bit milky. (It is ok if a small amount of undissolved powder or small clumps remain, as long as the liquid is whitish).

2) Prepare IQ Chambers and supplies:

a) Rinse IQ Exposure Chambers with DI, distilled, or control water.
b) Label chambers according to your purposes (Sharpies work well). Remember that you will be scoring these in the dark, so it helps to see your markings clearly or it's just too easy to score the tests backwards.
c) Get ready to roll: lay out all supplies, samples, implements.

3) Toxicant solution must be prepared using control/dilution water as diluent. If all cells do not have consistent base medium, the data values will not be exclusive to the concentration of toxin.

4) As with regular sample assay, dilution water and toxicant solution must be within 15.5 – 26.5 ºC (60-80 ºF).

5) Handling Daphnia:

a) Transfer Daphnia to IQ Exposure chambers. Use a wide bore pipette, or implement of your choice, to put 6 Daphnia into each of the six cells in each chamber. Be careful not to crush or maim them as you go.
b) There should be less than 1 mL water transferred with the Daphnia into each cell: either transfer as little water as possible with the Daphnia, or, easier for beginners, remove excess with a pipette (a calibrated pipette works great for this, with the tip on the bottom of the cell, so as not to suck up the Daphnia too).
c) Be sure to leave the Daphnia at least ½ mL to swim in!

6) Add toxicant solution and dilution water.

a) Use a large capacity pipette to add dilution water to the five cells marked "DW". Fill precisely to "DW" lines on Exposure Chambers.
b) Use a large capacity pipette to add toxicant solution to the lines marked "TS". Be careful not to exceed the "TS" lines, as you cannot correct this with a pipette without altering the concentration.
c) Record your start time and wait 1 hour.

7) Shake up prepared IQ Substrate again (by hand, no need to re-sonicate). Using a calibrated pipette (or any pipette that easily dispenses small drops), add 3 drops to each cell. Adding more will not affect the results, as the effect of IQ Substrate plateaus at a low level. Do not shake or stir; let sit for 15 minutes.

8) Score test.

a) 15 minutes after Substrate has been added, go to a dark place – not before, they need some light while they incubate with the substrate.
b) Don UV Protective glasses, shine the UV light on the chamber. Observe the brightness of the control (0% toxic solution) Daphnia. Any Daphnia in other cells fluorescing at or near that level of brightness are considered non-adversely affected. Any Daphnia that do not fluoresce or appear very dim compared to the controls (0% toxic solution) are considered adversely affected.
c) When scoring the test, adversely affected organisms are counted for *each cell* (out of six, rather than out of 18). Some statistical programs used to fit the EC50 curve ask you to enter the number of non-adversely affected organisms; you can count these instead or simply deduce.

EC50 Instructions continued on next page

9) Discard any remaining Daphnia at the end of the day’s testing; once fasted, daphnia should not be retained overnight for testing use.

End of instructions