

Uptake From Water; Se-75

Brine Shrimp Kinetics Study

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1. Acclimate artemia for a minimum of 48 hours to test media (for example 100 g/L and 160 g/L GSL media) in 1-L tripour beakers containing ~ 800 mls media.

Transfer approximately 50 adult artemia from culture tank to acclimation beaker by gently netting with a fine mesh fish net, and feed 1 ml of algae food daily. Brine shrimp food is made by adding 1 g of Wardley Premium Algae Discs per 20 mls of deionized water and blending thoroughly. It is kept refrigerated.

2. Prepare Se-75 stock solution in a 1.5-ml micro-centrifuge tube 24 hours prior to test initiation to allow for complete equilibration. Se-75 stock is made with Se-75 isotope, unlabelled ("cold") Se and DI water in a ratio to provide the desired Specific Activity and volume necessary for test beaker spikes and determination of radioactivity.

Keep frozen (-20°C) until ready for use to inhibit microbial activity.

3. Immediately prior to test initiation, remove 20 artemia from acclimation beaker individually with a plastic transfer pipette and place in 80 mls fresh test media in a 100-ml beaker (this is done to minimize the introduction of fouled water from the acclimation beaker into the test beaker).

4. Add 25 mls of fresh test media to a clean 50-ml beaker.

5. Carefully add 20 of the rinsed individual artemia to the beaker using a plastic transfer pipette, minimizing the amount of liquid transferred with each artemia.

This density is similar to the density of the artemia in the main cultures.
6. Wait 10 minutes for the artemia to recover from handling and to acclimate to the test beaker.
7. Spike the test beaker with the appropriate volume of Se-75 stock solution to reach desired concentration. The small volume of the Se spike (i.e. 20 μ l) does not significantly alter the water chemistry, including pH of the test beaker.
8. Gently aerate the beaker with capillary tubing to ensure mixing and full air saturation and cover with a glass Petri dish (Figures 1a and 1b).
9. 10 minutes after isotope addition take an initial water sample (100 μ l) for determination of Se-75.
10. After 24 hours of exposure take a final water sample (100 μ L) for determination of Se-75. Preliminary experiments have shown that in this experimental setup, the amount of radioactivity, and therefore the [Se], remains constant during the 24-hour exposure (Figure 2).
11. Carefully remove individual artemia with plastic transfer pipette and transfer them (individually) through a series of 3 rinses (10-15 mls each) of fresh media in a 6-well plate (Figure 3). This procedure has been tested and has revealed no remaining isotope contamination after the 2nd rinse (Figure 4).
12. After rinsing, carefully blot individuals dry on a paper towel, determine mass on weighing paper to nearest 10 μ g, then place into plastic culture tube for gamma counter.

13. Rinse and weigh 10 artemia, take another final water sample (100 μ L), then rinse and weigh 5 more artemia for a total of 15 individuals.
14. Dilute the Se-75 stock as appropriate to measure cold Se on the GFAAS; then take three 10- μ L samples of this diluted stock to be read on gamma counter.
15. Determine CPMs of all samples on gamma counter: blank, initial water sample, blank, final water sample, 10 individuals, final water sample #2, 5 individuals, and diluted Se-75 stock.
16. Measure cold Se on GFAAS and determine specific activity of Se-75 stock by dividing: $(\text{cpm/L}) / (\mu\text{g Se/L}) = \text{cpm}/\mu\text{g Se}$.
17. Calculate Se uptake according to: $(\text{cpm}/\text{individual}) / (\text{cpm}/\mu\text{g}) = \mu\text{g Se}/\text{individual}$.

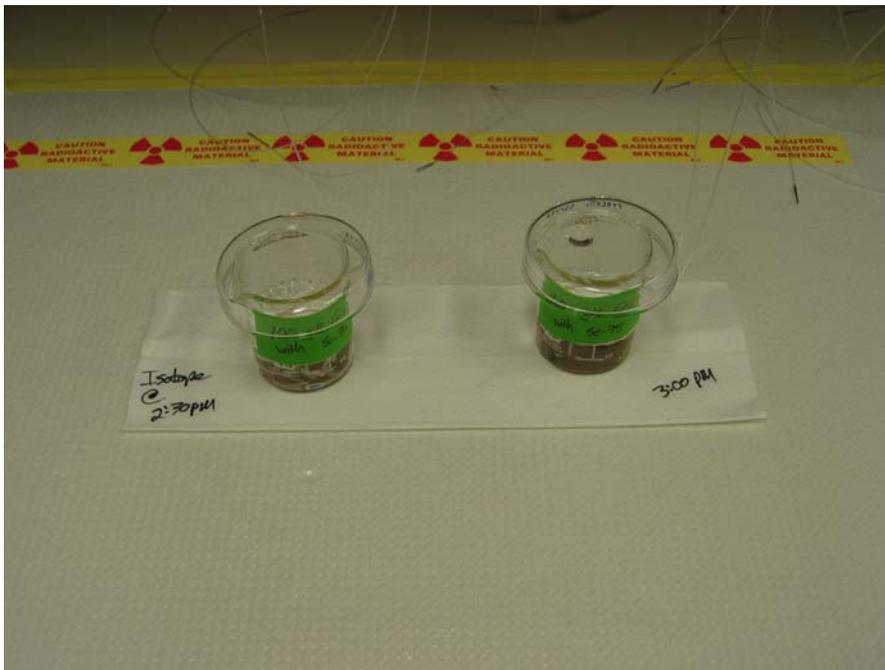


Figure 1a.

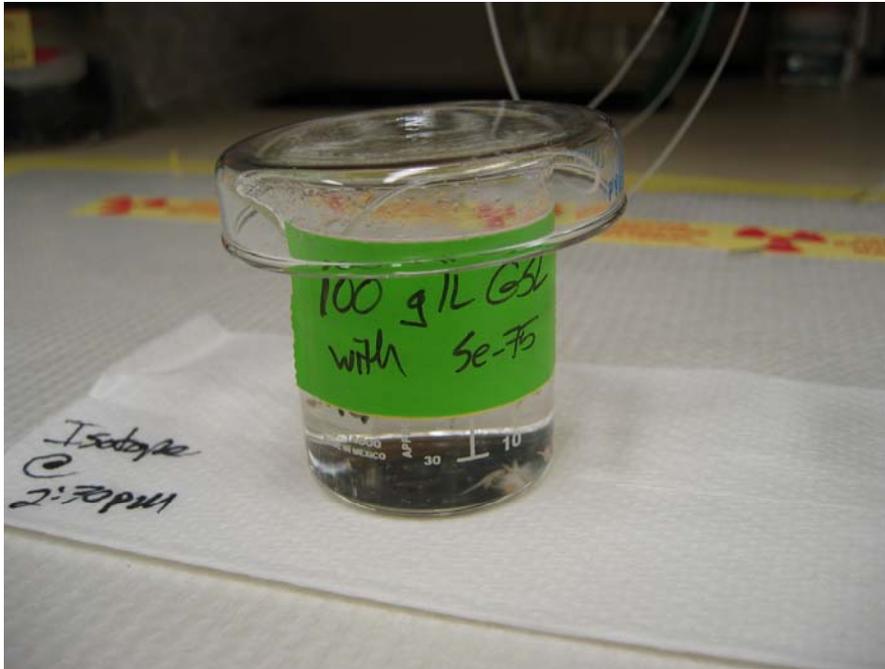


Figure 1b.

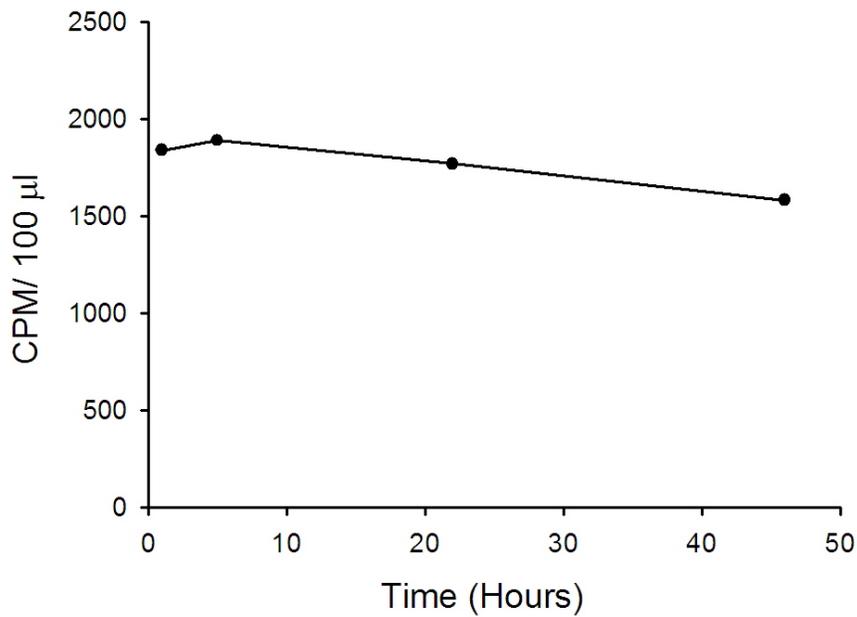


Figure 2. Radioactivity in 100-ml samples of Se-75 exposure water sampled at 1, 5, 22 and 46 hours during preliminary experiments. Radioactivity, and therefore [Se], remains relatively constant over time.



Figure 3.

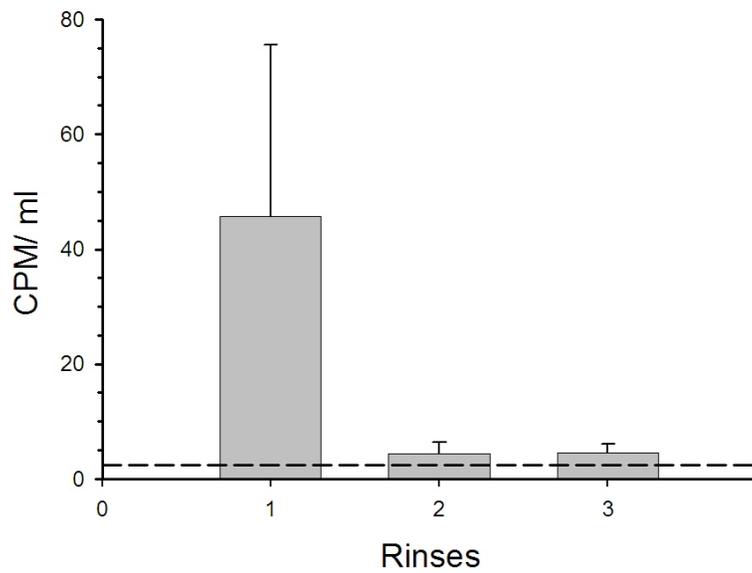


Figure 4. Radioactivity in the three rinse waters. Radioactivity in the second and third rinse waters is not significantly different from background levels (dotted line). For these experiments a total volume of 1 ml was counted for Se-75 activity.