

# Determination of Dietary Selenium Intake and Assimilation Efficiency

## Brine Shrimp Kinetics Study

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DATE: July 5, 2007

1. Grow *Dunaliella viridis* in algae media as outlined in the Scope of Work for 20 days under constant Se-75 labeled selenium concentrations (nominal 3, 15 and 50 µg/l)
2. Remove adult, size-matched *Artemia* from main culture tank and rinse in fresh media in a 200-ml beaker. (*Artemia* from the same hatch date and culture tank are very similar in size.)
3. Add 30 mls of fresh 100-g/L GSL media to 50-ml centrifuge tubes.
4. Carefully transfer 30 *Artemia* to each tube with a plastic transfer pipette, minimizing the amount of liquid transferred with each *Artemia*.
5. Gently aerate the tube with capillary tubing to ensure even mixing and full air saturation and allow *Artemia* a minimum of 10 min to recover from handling.
6. Obtain a sample of *Dunaliella viridis* from the radioactive (Se-75) culture.
7. Centrifuge the sample of *Dunaliella viridis* at 8000 RPM in a microcentrifuge tube for 2 min.
8. Discard the radioactive supernatant.
9. Re-suspend the radioactive *Dunaliella viridis* in non-radioactive algae media.

10. Repeat steps 7-9.
11. Add an appropriate density<sup>1</sup> of *Dunaliella viridis* grown in presence of Se-75 labeled selenium for 20 days.
12. Take sub-sample of the *Dunaliella viridis* culture to measure selenium concentration in the *Dunaliella viridis* at the time of feeding.
13. Obtain sample of the *Dunaliella viridis* culture for accurate determination of cell density in the feeding experiment.
14. Obtain water samples from the feeding media at regular intervals to determine feeding rate.
15. After thorough mixing to avoid problems with settling, measure the absorbance of all water samples on a spectrophotometer at 750 nm.
16. After 60 minutes<sup>2</sup>, remove *Artemia* from feeding media and place them in individual gamma counting vials in 3 ml of GSL media.
17. Pass the vials through the gamma counter to determine Se-75 radioactivity in the newly fed *Artemia*.
18. After gamma counting, transfer *Artemia* to individual 15-ml falcon tubes containing 10 ml GLS media (100 g/l).
19. Feed the *Artemia* a Se-75-free diet and allow them to deplete fecal matter overnight.
20. Collect the *Artemia* from the 15-ml falcon tubes and recount individual *Artemia* for Se-75 as in steps 16 and 17.
21. Determine the wet weight of the individual *Artemia* and dispose.

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<sup>1</sup> Algae cell density will be chosen to allow for sufficient Se-75 accumulation for accurate detection from preliminary experiments with the goal of feeding at densities as close to GSL algae densities as possible. Higher than desired algae cell density may have to be applied to allow for sufficient Se-75 uptake. So far our experiments have employed a cell density of approximately 2 mill cells/ml. Additional pilot experiments will determine the suited cell density for these experiments.

<sup>2</sup> 30 and 60 min were used for the initial experiments - exact feeding time to be determined in additional pilot experiments.

22. Allow fecal matter in the 15-ml falcon tubes to settle; then siphon off 7 of the 10 mls of media.
23. Vortex the falcon tube now containing 3 ml of GSL media and fecal matter and rapidly transfer contents to a gamma counting vial.
24. Count these vials to determine the Se-75 content in the fecal matter.
25. Calculate dietary selenium intake from the specific Se-75 activity of the original algae culture medium and the initial Se-75 radioactivity in the *Artemia*.
26. Calculate the selenium assimilation efficiency from the Se-75 activity in the *Artemia* at the first and the second Se-75 determination. The difference equals the amount lost with fecal matter.
27. Calculate the dietary selenium uptake from the specific Se-75 activity of the original algae culture medium and the second Se-75 radioactivity measurement in the *Artemia*.
28. Validate the assimilation efficiency measurements by comparing the Se-75 lost between the initial and the final Se-75 activity measurements in the *Artemia* to the values detected in the fecal matter.

## Standard Procedures for Feeding Rate Experiment

1. Remove ~100 adult, age-matched artemia from main culture tank and rinse in fresh media in a 200-ml beaker. (Artemia from the same hatch date and culture tank are very similar in size.)
2. Add 30 mls of fresh 100 g/L and 160 g/L GSL media to 50 ml centrifuge tubes.
3. Carefully transfer 15 artemia to each tube with a plastic transfer pipette, minimizing the amount of liquid transferred with each artemia.
4. Gently aerate the tube with capillary tubing to ensure even mixing and full air saturation and allow artemia a minimum of 10 min to recover from handling.
5. Add 2 mls of *Dunaliella viridis* concentrate to each tube (save sample of algae to perform cell counts for algae density).
6. Immediately take an initial water sample of 1 ml, and then take 1 ml sample every 10 minutes up to 60 minutes.
7. After thorough mixing to avoid problems with settling, measure the absorbance of all water samples on a spectrophotometer at 750 nm.
8. Plot absorbance over time and perform a linear regression on the decrease in absorbance to obtain the slope (change in absorbance per minute).
9. Divide slope by number of individuals per tube and express feeding rate as change in absorbance/ minute/ individual.

1.

Culture *Dunaliella viridis* in the presence of 1  $\mu\text{g}$  Se/L (non-radioactive selenium) for

- 20 days. (This culture was prepared at the same time as the 1 µg/L Se-75 algae culture used in the dietary uptake experiment. Se spikes and dilutions made in the radioactive culture in order to maintain exposure concentrations were mirrored with unlabelled Se stock in the non-radioactive culture.)
2. Remove ~70 adult, age-matched artemia from main culture tank and rinse in fresh media in a 200-ml beaker.
  3. Add 1 L of 100-g/L GSL media to each of two 1-L tripour beakers.
  4. Carefully transfer 30 artemia to each beaker with a plastic transfer pipette, minimizing the amount of liquid transferred with each artemia.
  5. Gently aerate each beaker with capillary tubing to ensure even mixing and full air saturation.
  6. Feed each beaker equal amounts (normalized by absorbance at 750 nm to account for differences in culture density) of either non-radioactive Se-loaded *D. viridis* or normal *D. viridis* (not cultured in the presence of Se) daily for 2 weeks.
  7. Remove 25 artemia from each beaker and transfer to beakers containing 4 L of 100-g/L GSL media.
  8. **Follow Standard Procedures for Determination of Dietary Selenium Intake, steps 5-17.**