Activity 7:
Nano Silver

Core Concept:
Nanotoxicologists can experiment with simple organisms to determine whether nanoparticles may be harmful to humans or the environment.

Class time required:
Approximately 3-4 forty minute class periods.
- 1-2 periods to set up experiment
- 2-3 days for seed germination
- 1 period to collect and graph data from the experiment
- 1 period for students to present results of the experiment

Important: The results of this activity do NOT lead to a conclusion that nanoparticles affect seed germination. If you want to adjust the teacher preparation to have the nanoparticles inhibit seed germination, we suggest that you substitute a saturated table salt solution (colored faintly gray with black food color) for the “20 ppm Nanosilver” solution.

Differentiating this activity:
This activity is designed as a scaffolded/guided experiment for students with little experience with designing and conducting experiments.

For students who have prior experience with designing and conducting experiments, you may consider deleting Part 1 and portions of Parts 2 and 3 as appropriate for your students experience level. This will allow students to apply their skills to design and conduct their own experiment. If you do this, you should ask students to write a detailed procedure for their experiment.

Teacher Provides:
- A copy of student handout “Nano Silver” for each student
- A copy of “Glossary – Experimental Design” for each student
- A copy of “Experiment Report” printed on transparency film.
- Transparency marker for each team of students.
  - For a less expensive alternative order Hot Tub Silver. Approx. 16 ounces (32 tablespoons) of Hot Tub Silver ordered from www.purestcolloids.com. Mix 1 tablespoon of Hot Tub Silver with 1 liter of water and label this solution “20 ppm Nanosilver.”
Or, if you cannot afford the real nanosilver, you might “cheat” and add a few drops of black food coloring to a liter of tap water to simulate nanosilver solutions and label this solution “20 ppm Nanosilver."

If you want to adjust the teacher preparation to have the nanoparticles inhibit seed germination, we suggest that you substitute a saturated table salt solution (colored faintly gray with black food color) for the “20 ppm Nanosilver” solution.

- 5 white plastic cups for mixing nanoparticle solutions - 10 ounce or 12 ounce size
- Container with least 100 seeds. Radish seeds are recommended because they germinate rapidly. Lettuce seeds or grass seeds may be substituted but allow several additional days for germination. May be purchased locally or purchased from a scientific supply company.
- 50 mL of tap water
- 5 clear zip-lock sandwich bags (the cheaper, thin ones work better for this)
- Magnifying glass (optional)
- 5 sheets of paper towel
- Scissors for cutting paper towel
- 1 graduated plastic droppers (1 ml)
- 1 permanent marker
- 1 graduated cylinder (10 mL) or plastic medicine cup (30 cc)
- 10 strips of masking tape (distribute as students are working to set up their experiment)
- Tweezers or forceps

Suggested Class Procedure:
Class 1 (one or two periods)

- Distribute copies of the student handout entitled “NANO Silver” to each student.
- Read the information in the box aloud to the class.
- Ask students to read the information at the bottom of page 1.
- Group students in teams of 2-4 students.
- Distribute following materials for the lab activity to each team of students:
  - 50 mL of silver nanoparticle solution (20 ppm)
  - 5 white plastic cups for mixing nanoparticle solutions
  - Container with least 100 seeds
  - 50 mL of tap water
  - 5 plastic zip-lock bags sandwich bags for growing seeds
- 5 sheets of paper towel—perforated rolls of white kitchen paper towel such as Bounty work well.
- Magnifying glass (optional)
- Large plastic dropper for moving seeds
- 1 permanent marker
- 1 graduated cylinder (10 mL)
- 1 graduated measuring dropper (1 mL)
- 10 strips of masking tape

- Distribute a copy of “Glossary - Experimental Design” to each student.

- Students complete Part 1. Arrange for an area of the classroom for storing student experiments. Students will store their experiments in this area for 2 days.

- Students complete Part 2. This may be done in class or as homework.

Class 2 (one period after waiting 2-3 days)

- Students complete Part 3 and prepare for their class presentations.

- Distribute a copy of “Experiment Report” printed on transparency film and a transparency marker to each team. They can use this to prepare for their class report.

Class 3 (one period)

- Students present and peer review their reports. They use the transparencies to present their work. Pay particularly close attention to proper construction of data tables and graphs. Consider having students make posters to show their work. These posters should include the research question, the hypothesis, a summary of the procedure, a data table, a graph, and the conclusions.

- The students’ results will vary widely. There is no clear-cut answer based on the individual experiments. Time should be provided to discuss possible sources of error and the importance of repeated trials. Consider combining students’ data to create class data and a class graph.

Extensions:

- Pool and create a data table and graph for the class data to emphasize the importance of repeating experiments multiple times and averaging the data from multiple experiments.

- Students visit [www.purestcolloids.com](http://www.purestcolloids.com) to explore the advertising claims about silver nanoparticles at this website. Then they explore other Internet sites to see what other websites say about the use and safety of nanoparticles.
• For more advanced students, suggest that they read Silver Nanotechnologies and the Environment Old Problems or New Challenges? www.nanotechproject.org/publications/archive/silver/

• If time permits, have students do an internet search for information on FDA or EPA regulations for silver nanoparticles in consumer products and in the environment.

This project was generously funded by Science Education Partnership Award R25RR023285 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.
### Glossary - Experimental Design

<table>
<thead>
<tr>
<th><strong>Research question</strong></th>
<th>What are you trying to find out?</th>
<th><em>For example: “Does fertilizer make tomato plants grow larger?”</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title of experiment</strong></td>
<td>A statement of what is being investigated which includes: the independent variable, the dependent variable, and the organisms being studied.</td>
<td><em>For example: “The Effect of Fertilizer on Growth of Tomato Plants”</em></td>
</tr>
<tr>
<td><strong>Hypothesis</strong></td>
<td>What do you predict will happen during the experiment? The hypothesis should be expressed in the form of an “If……….., then …………….” statement.</td>
<td><em>For example: “If the amount of fertilizer is increased, then the tomato plants will grow taller.”</em></td>
</tr>
<tr>
<td><strong>Independent variable</strong> (include units of measurement)</td>
<td>The independent variable is the one you purposefully changed in the experiment. What are you changing in your experiment? What are your units of measurement?</td>
<td><em>For example: “Fertilizer Concentration (%)</em></td>
</tr>
<tr>
<td><strong>Dependent variable</strong> (include units of measurement)</td>
<td>The results you will measure or the data you will collect.</td>
<td><em>For example: “Height of the tomato plant (cm)”</em></td>
</tr>
<tr>
<td><strong>Controlled factors</strong></td>
<td>Things that it would be important to keep the same in the control group and experimental groups so that it will be a fair test of your hypothesis.</td>
<td><em>For example:</em></td>
</tr>
<tr>
<td></td>
<td>• Light – “Plants will be placed in the same sunny location”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Water – “Plants will be given the same amount of water each day”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Temperature – “Plants will be placed next to each other”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Soil – “Plants will be planted in the same type of soil”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Growing container – “Plants will be planted in containers of the same size and shape”</td>
<td></td>
</tr>
<tr>
<td><strong>Control group</strong></td>
<td>The control group is the group that is NOT given the treatment.</td>
<td><em>For example: “The control group is the plants that were given pure water with no fertilizer.”</em></td>
</tr>
<tr>
<td><strong>Experimental group</strong></td>
<td>The experimental group is the group that IS given the treatment.</td>
<td><em>For example: “The experimental group is the plants that were given fertilizer.”</em></td>
</tr>
</tbody>
</table>
Experiment Report

Title: ____________________________________________________________

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions:
Nano Silver

Silver nanoparticles are rapidly becoming a part of our daily life in the form of cosmetics, food packaging, wound dressings, detergents, and antimicrobial coatings. Ultimately, the nanoparticles in these products end up in the environment during waste disposal.

Little is known about the effects of silver nanoparticles on humans and the environment. Scientists have begun to express concerns about the safety of silver nanoparticles in consumer products.

**Nanotoxicology** is the study of the toxicity of nanomaterials. Nanotoxicologists do research to determine whether nanoparticles pose a threat to the environment or to humans.

To screen for possible hazards involved in exposure to nanoparticles, scientists may use simple plants or animals, rather than humans, as test subjects. This type of screening test enables scientists to identify nanoparticles that might result in environmental or human health problems.

In this activity you will conduct an experiment to study the effects of different concentrations of silver nanoparticles on the germination of seeds.

**Germination** is the process in which a plant begins to grow and emerge from a seed.
You will have the following materials to use in your experiment:

- 50 mL of silver nanoparticle solution. The concentration of this silver nanoparticle solution is 20 parts per million (ppm)
- 5 white plastic cups for mixing nanoparticle solutions
- Container with least 100 seeds
- 50 mL of tap water
- 5 clear plastic bags for growing seeds
- Magnifying glass
- 1 permanent marker
- 1 graduated cylinder (10 mL)
- 1 graduated measuring dropper (1 mL)
- 10 strips of masking tape
- Tweezers or forceps

**ppm = parts per million**

A unit of concentration often used when measuring very low levels of pollutants in air, water, body fluids, etc.

1 ppm is 1 part per million.

The unit of concentration, **milligrams/liter**, is the same thing as ppm.

Four drops of ink in a 55-gallon barrel of water would produce an "ink concentration" of 1 ppm.
Part 1: Procedure for setting up your experiment

1. Use the information in the data table below to:
   - Label 5 white plastic cups with the concentrations of nanoparticles shown in Column A of data table below (0, 2, 5, 10, and 20 ppm).
   - Fill each of the cups with the appropriate concentration of nanoparticles using the information in Column B and Column C.

<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticle Concentration (ppm)</td>
<td>Tap Water (mL)</td>
<td>20 ppm Nanoparticle Solution (mL)</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>20 ml</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: Instead of planting your seeds in soil, you will plant them in a plastic bag.

2. Write your initials and the concentration (0 (control), 2, 5, 10, and 20 ppm) of the nanoparticle solutions you are testing on 5 clear plastic bags. You will use a different bag for each nanoparticle solution.

3. Fold a paper towel and place it in the bag as shown in the diagram.
4. Pour the contents of the cups into the appropriately labeled bags. For example, pour the nanoparticle solution from the “0 control” cup into the bag labeled “0 control.”

5. Place 20 seeds in a horizontal row in the middle of the paper towel in each of the bags. Be certain to put the seeds on the outside of the paper towel so that you can see them. You may find it easier to use forceps (tweezers) to move the seeds onto the paper towel.

6. Seal the bags and use masking tape to attach the bags to a wall or a cabinet door in the area indicated by your teacher.

7. After three days, you will count and record the number of seeds that germinated.
Part 2: Analyzing the design of your experiment

Refer to the glossary when you answer the following questions.

1. What is the research question for your experiment?
   
   *Do nanoparticles affect seed germination.*

2. What is the hypothesis that you will be testing?
   State your hypothesis as an *If……., then……. statement.*
   
   *If the concentration of nanoparticles increases, then the number of seeds that germinate will decrease (or remain the same or increase).*

3. What is the independent variable in your experiment?
   *The concentration of nanoparticles*

4. What is the dependent variable in your experiment?
   *The number of seeds that germinate.*

5. What are the experimental groups for your experiment?
   *The seeds that are exposed to different concentrations of nanoparticles.*

6. What is the control group for in your experiment?
   *The seeds that are not exposed to nanoparticles.*

7. Why is it important to include a control in the experiment?
   *The control serves as a basis of comparison OR the control shows that other factors such as the water, light, or other factors are not responsible for the effects observed.*

8. What are four controlled factors (things that are kept the same in all of the bags) for your experiment?
   *The number and kinds of seeds, the amount of liquid, the kind of bag, the kind of paper towel, the amount of light, the temperature.*

9. Why is important to keep the controlled factors the same in each of the bags?
   *So that you know that only the independent variable (nanoparticle concentration) caused any observed differences in seed germination.*
10. Why was it important to use more than one seed in each bag?

*So that you have “repeated trials” or so that you can be sure that there was not something wrong with the seed.*

11. Use the grid below to design a data table that you will use to collect data from your experiment.

- The data table should have a title that includes the independent variable, the dependent variable, and the organism studied.
- The independent variable (with units of measurement) is written in the left-hand column—arranged in increasing order from top to bottom.
- The dependent variable (with units of measurement) is written in the right-hand column. You will collect data later to complete the right-hand column.
- Be sure to include units of measurement for each variable.

**Title:** ___The Effect of Silver Nanoparticle Concentration on Seed Germination___

<table>
<thead>
<tr>
<th>Nanoparticle Concentration (ppm)</th>
<th>Number (or Percentage) of Seeds Germinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Student answers will vary</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
Part 3: Analyzing the Results of Your Experiment

1. Observe the seeds in each of the bags and count the number of germinated seeds. Record the number of germinated seeds in the data table on the previous page.

2. Summarize the data you have collected in graph form.
   - The graph should have a title that includes the independent variable, the dependent variable, and the organisms studied.
   - Each axis should be clearly labeled with the variable and the units of measurement—put the independent variable on the horizontal axis and the dependent variable on the vertical axis.
   - Mark a scale (even intervals) on each axis.
   - Use the data from your data table to create a line (not bar) graph.

Title: __The Effect of Silver Nanoparticle Concentration on Seed Germination____
3. Look at the information represented in your graph. What conclusions can you draw from the data you collected? Describe any patterns or trends you see in the data. Are there any exceptions to these patterns or trends?

   *Student answers will vary. Look at their data table and graphs.*

4. Does your data support or refute (disprove) your hypothesis? Explain.

   *Student answers will vary. Look at their initial hypothesis, data table and graphs.*

5. Based on the results of your experiment, do you think the silver nanoparticles you were testing were harmful? At what concentrations? Explain your answer.

   *Student answers will vary. Look at their data table and graphs.*

6. A good experiment is one that gives approximately the same results if it is replicated (repeated) by others. List at least two ways you could improve your experiment to be certain that it could be replicated (repeated) by others to give the same results?

   *Include more seeds or more bags of seeds at each nanoparticle concentration. Make sure the directions are followed exactly. Make sure bags are exposed to the same amount of light. Make sure the bags are exposed to the same temperature.*

7. During the next class period, you and your team members should be prepared to present your research findings to the class. You should prepare visuals (transparencies or PowerPoint slides) that show your:
   
   - Data table
   - Graph
   - Conclusions

   Be prepared to answer questions from your classmates and your teacher.