Activity 6:

Nano Toxicology

Core Concept:

Many different kinds of nanotoxicology studies are done to examine the health risks associated with nanoparticle exposure. These studies may include epidemiology studies, human studies, animal studies, and *in vitro* studies.

Class time required:

Two 40-minute class periods

Teacher Provides:

- A copy of student handout, **"Nano Toxicology**,**"** for each student.
- Materials for Part 1: Two transparencies - "Heart Attack Data" and "$PM_{2.5}$ Data" (see page 3). Ideally, these should be printed in color.
- Materials for Part 2:
  - Small test tube (or microtube) of simulated blood labeled "Nanoparticles Blood," containing at least 1.5 ml of a saturated calcium chloride solution with enough red food color added to simulate blood.
  - Small test tube (or microtube) of simulated blood labeled "Filtered Air (control) Blood" containing at least 1.5 mL of a **1:10** saturated calcium chloride solution (1 part saturated calcium chloride and 9 parts water) with enough red food color added to simulate blood.
  - Small test tube (or microtube) labeled "Thrombin" that contains saturated sodium bicarbonate (baking soda) solution, colored faintly yellow with food coloring.
  - 3 plastic disposable droppers labeled: "Nanoparticles," "Filtered Air," and "Thrombin".
  - *Blood Clotting Test Strip* printed on transparency film (see page 4).
  - Piece of black construction paper, at least the size of the Blood Clotting Test Strip.
- Materials for Part 4:
  - Small test tube or 2 mL microtube labeled "White Blood Cells" containing at least 2 mL of water. Optional: You may add a small amount of fine white sand or glitter to simulate white blood cells.
  - Small test tube or 1.5 mL microtube labeled "Nanoparticles," containing at least 1 mL of pH 5 buffer or white vinegar, colored VERY slightly with black food coloring or ink to be a very faint grayish color.
  - Test tube or 1.5 mL microtube labeled "Water," containing at least 1 mL of pH 9 buffer or 1:20 dilution of household ammonia.

- Test tube or 1.5 mL microtube labeled “Free Radical Indicator,” containing at least 1 mL of 0.04% Bromothymol blue.
- Two small cups (1 oz. or smaller). Label one cup “Carbon Nanoparticles” and the other cup “Water (control)”.
- 4 plastic disposable droppers labeled: “Water,” “White Blood Cells,” “Nanoparticles,” and “Free Radical Indicator”.
- 2 plastic toothpicks (stirrers).
- 1 color copy of *Color Chart - Free Radical Concentration* (see page 5).

Suggested Class Procedure:

- Distribute copies of the student handout entitled **“Nano Toxicology?”** to each student.
- Students read the Background Information on the first page and answer the related questions. This may be done for homework or during class.
- Distribute materials needed for Parts 1, 2, 3 and 4 to each team of students.
- Student teams complete Parts 1, 2, 3 and 4.
- Read the “More Research Needed!” in Part 5 aloud to the class.
- Students write and then share their answers to the question in Part 5.
- Reinforce the concept that while nanotoxicology research indicates that nanoparticles affect body functions, much more research is needed!

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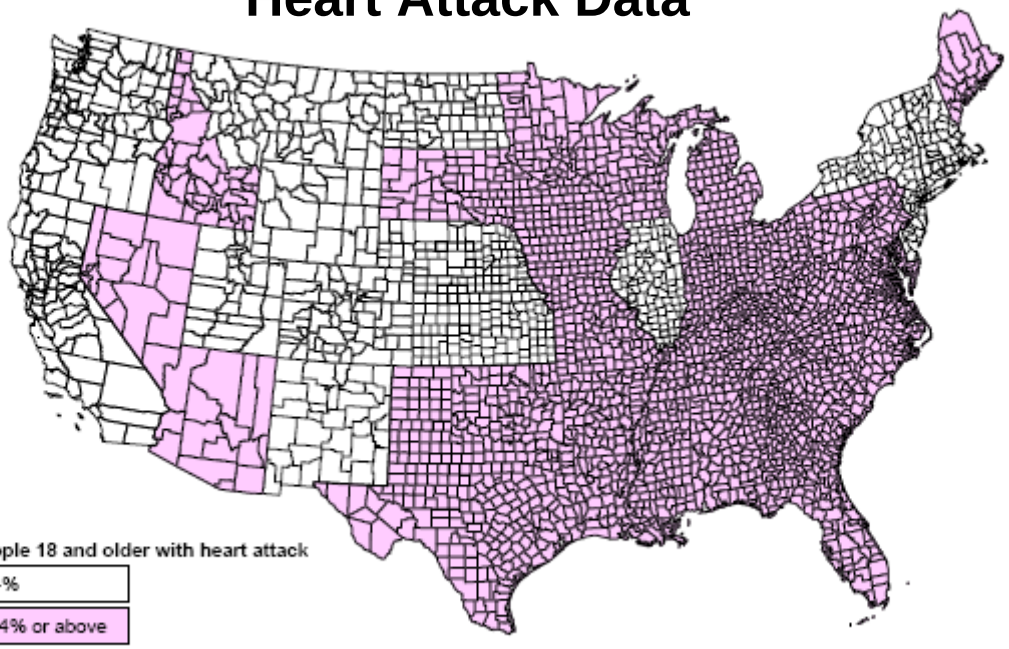
Heart Attack Data



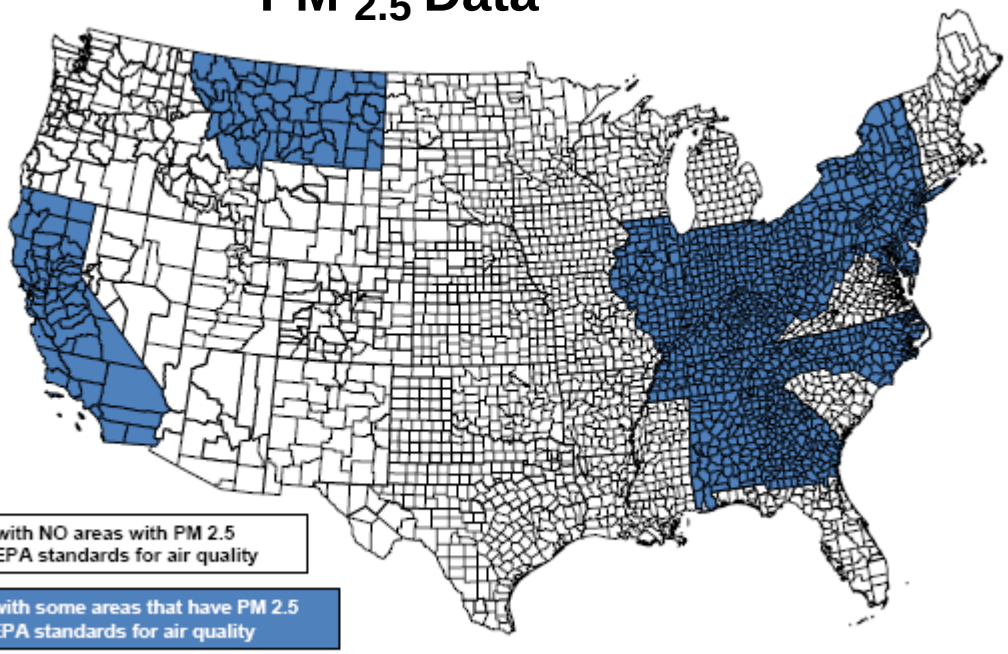
% of people 18 and older with heart attack

Below 4%

Above 4% or above

Source: http://www.msnbc.msn.com/id/17175486/ns/health-heart_health

PM $_{2.5}$ Data

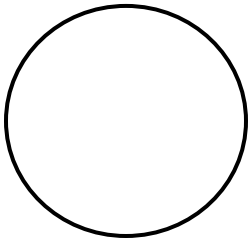


States with NO areas with PM 2.5 above EPA standards for air quality

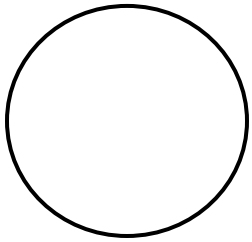
States with some areas that have PM 2.5 above EPA standards for air quality

Source:
http://www.fhwa.dot.gov/environment/aqfactbk/img/aq09figa.jpg

Blood Clotting Test Strip

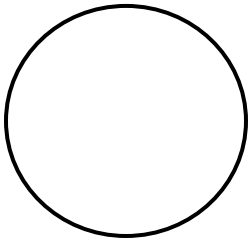


Blood from Person Exposed to FILTERED AIR (control)

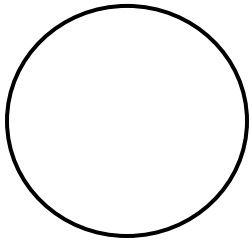


Blood from Person Exposed to NANOPARTICLES

Blood Clotting Test Strip

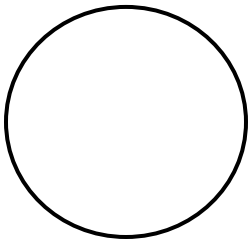


Blood from Person Exposed to FILTERED AIR (control)

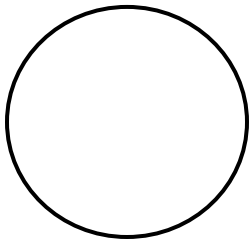


Blood from Person Exposed to NANOPARTICLES

Blood Clotting Test Strip

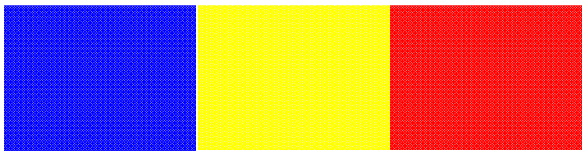


Blood from Person Exposed to FILTERED AIR (control)



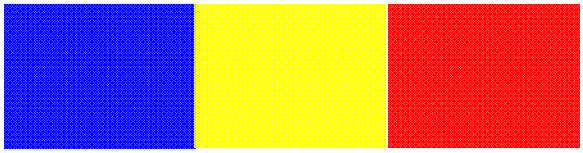
Blood from Person Exposed to NANOPARTICLES

Color Chart – Free Radical Concentration



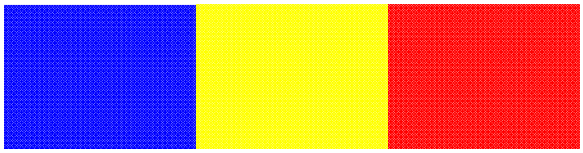
Low (normal) **Medium** **High**

Color Chart – Free Radical Concentration



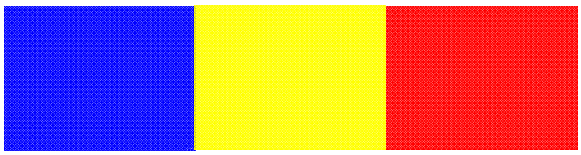
Low (normal) **Medium** **Very High**

Color Chart – Free Radical Concentration



Low (normal) **Medium** **Very High**

Color Chart – Free Radical Concentration



Low (normal) **Medium** **Very High**

Nano Toxicology

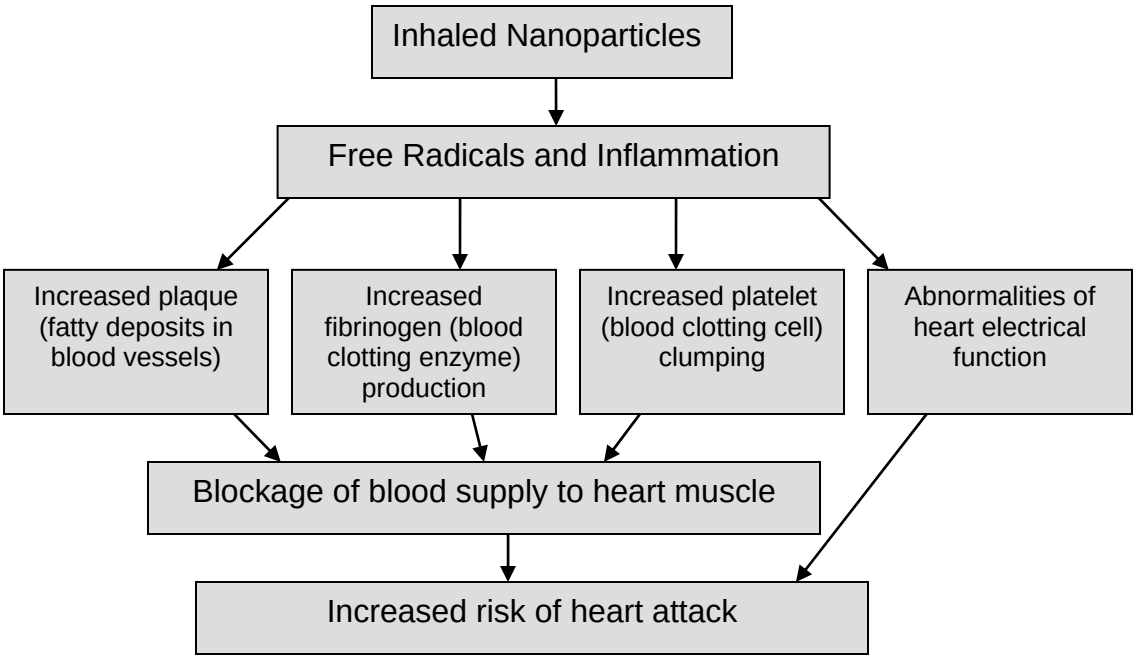
Background Information

A **nanotoxicologist** is a scientist who studies the effects of nanoparticles on human health. Some nanotoxicologists have hypothesized that inhaled nanoparticles in polluted air may lead to **inflammation** and **free radical formation** in cells and tissues. Inflammation and free radicals in the circulatory system may lead to changes in that increase the risk for heart attacks.

The graphic below illustrates some mechanisms that could explain how nanoparticles lead to increased risk for heart attacks.

What is inflammation? Inflammation removes invading pathogens or damaged tissue. It brings plasma proteins and white blood cells to the injured area to begin the process of tissue repair.

What are free radicals? Cell respiration produces ATP energy for life activities. It also produces highly reactive molecules called free radicals. Excess free radicals damage essential proteins, nucleic acids, and cell membranes.



1. Why are nanotoxicologists concerned about the effects of nanoparticles on human health?

   Some toxicologists have hypothesized that inhaled nanoparticles may lead to changes that increase the risk for heart attacks.

2. Describe two changes which might be observed in the blood or body tissues, if nanoparticles caused an increase in free radicals and inflammation?

   There may be increases, blood clot formation, increased plaque formation, fibrinogen, white blood cells, plasma proteins or platelets.

3. Explain how these changes could lead to a heart attack.

   They lead to blockage in the blood supply to heart muscle.

Nanotoxicology Research

There are many different kinds of nanotoxicology research that can be used to determine if nanoparticles lead to increased risk for heart attacks. These experiments include:

- Epidemiology research
- *In vitro* human research
- *In vitro* animals research
- *In vivo* research

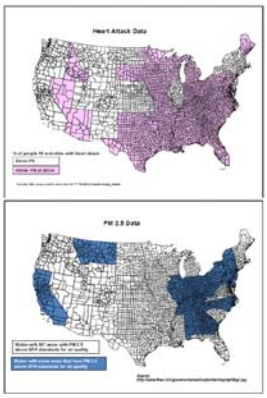
What you will do: In this lab activity, you will conduct simulated and very simplified experiments that illustrate the different types of research that toxicologists use to study the effects of inhaled nanoparticles on the circulatory system.

Part 1: Epidemiology Studies

Epidemiology studies involve statistical research on human populations in an attempt to link human health effects to a specific cause. **Epidemiologists** want to determine whether the rate of heart attacks is higher in people who live in regions that have higher concentrations of particles in the air.

Epidemiologists collected and mapped data on:

- heart attacks
- particle concentrations in the air ($PM_{2.5}$ particles that are less than 2.5 micrometers)



Note: Currently data on nanoparticle ($PM_{0.1}$) concentrations in the air is not available. For this study, the scientists used $PM_{2.5}$ data because it is a measure of particles that are less than 2.5 micrometers (including nanoparticles).

Simplified (simulated) maps representing data from this type of experiment are shown on the two colored maps.

1. Place the "Heart Attack" map on top of the "$PM_{2.5}$ Concentration" map. Hold the overlapped maps up to the light. Areas that are purple indicate areas where there is a high incidence of heart attacks AND a high $PM_{2.5}$ concentration.

2. Does the data on the maps support the hypothesis that inhaled nanoparticles increase the risk for heart attacks? Support your answer with data from the maps.

   No, because most areas with high concentrations of $PM_{2.5}$ do not have high heart attack rates. OR
   No, scientists would need to collect data on the nanoparticle concentrations and map this data.

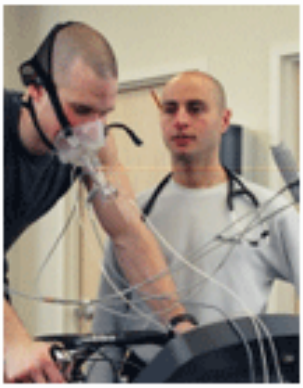
3. List three other factors could account for the different rates of heart attacks observed in different parts of the country?

   Student answers will vary but may include other types of pollution, obesity, climate, ethnic distribution, etc.

Part 2: *In Vivo* Human Studies

The term, *in vivo* comes from the Latin term "in life" and refers to studies that are performed using living organisms. ***In vivo* human research** studies are expensive and involve ethical concerns about the risks to the human research subjects. Typically, *in vivo* human studies are used when there are questions that can only be fully answered by using human research subjects.

The air near busy highways contains a mixture of different types of nanoparticles. Researchers would like to use human subjects for *in vivo* study to determine whether inhalation of these nanoparticles results in changes in the blood that could lead to heart attacks.



In this study they looked for changes in the amount of **fibrinogen**, a clotting protein, in samples of blood taken from the research subjects. An increase in the amount of fibrinogen in the blood is one change that could lead to heart attacks. An excess of fibrinogen can make blood form clots inside of blood vessels. If these clots break loose and travel to the heart, they may trigger a heart attack.

Here are the steps in their experiment:

Day 1: One human subject inhales air that had been filtered to remove most of the nanoparticles.

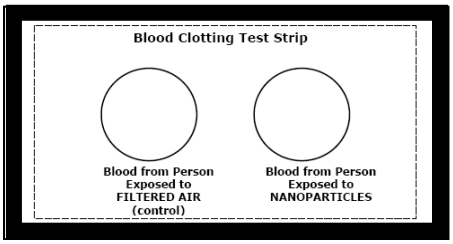
Day 2: Collect blood sample 24 hours later and test for fibrinogen level.

Day 6: The same human subject inhales air that contains a concentration of nanoparticles typical of the polluted air near a highway.

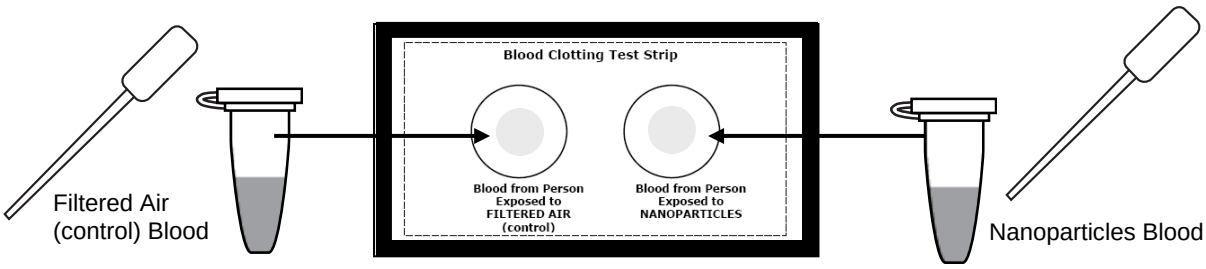
Day 7: Collect blood sample 24 hours later and test fibrinogen level.

The researchers have asked you to analyze the blood samples that they collected. Follow these instructions to compare the amount of fibrinogen in the blood from the two research subjects.

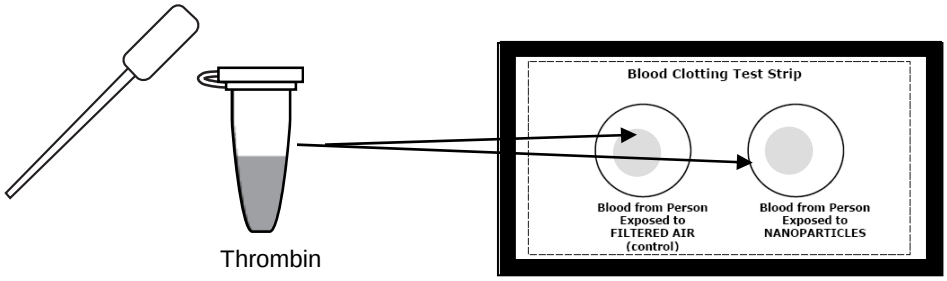
1. Place the Blood Clotting Test Strip on the sheet of black paper.



2. Transfer 2 drops of the "Filtered Air (control) Blood" and the "Nanoparticles Blood" samples to the appropriate circle on the Blood Clotting Test Strip.



3. Add two drops of thrombin to each of the circles. Thrombin is an enzyme that triggers blood clotting by converting fibrinogen into fibrin threads that form a blood clot.



4. Observe the amount of clotting in each of the circles. Record your observations in the table below.

Air inhaled by research subject	**Relative Amount of Blood "Clotting" (a lot, some, none)**
Filtered Air (control)	***some***
Nanoparticles	***a lot***

5. Does this simulated *in vivo* experiment suggest that inhalation of nanoparticles increases human risks for heart attacks? Support your answer with the data that you collected in this experiment.

There was more clotting in the blood of the person who was exposed to nanoparticles. This indicates more risk for blood clots that can lead to heart attacks.

Part 3: *In Vivo* Animal Studies

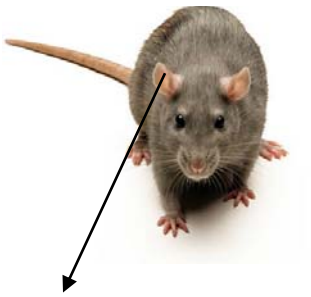
***In vivo* <u>animal</u> research** is done using living animals, such as rats or mice, as research subjects. *In vivo* animal studies are less expensive and involve fewer ethical concerns than research using human subjects. *In vivo* animal studies are typically done when researchers wish to avoid risks to human subjects.

Scientists hypothesized that exposing rats to inhaled carbon nanoparticles results in blood clots forming in the veins of the rats. If a blood clot breaks free and is carried into the heart, it may lead to a heart attack.

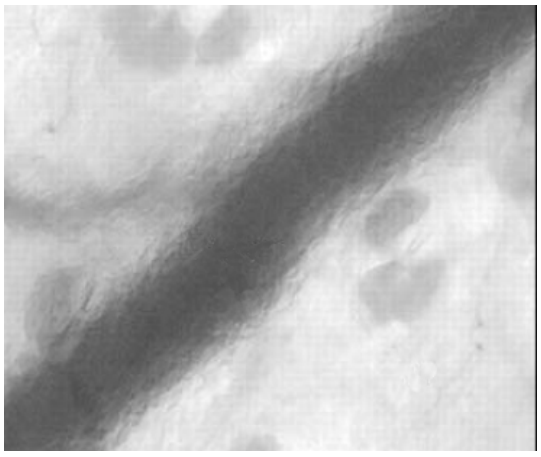
To test their hypothesis, the scientists used two rats—one that was not exposed to nanoparticles (the control) and one that was exposed to nanoparticles. Scientists shone a laser light beam onto the rats' ears to damage the veins in a tiny portion of the ears. Two minutes later, scientists used a microscope to observe the ear vein to see if a blood clot had formed.
Note: The rats did not experience any discomfort from this experiment because the rats were anesthetized and this procedure is non-invasive.

The photos below show the results of the ear vein experiment.

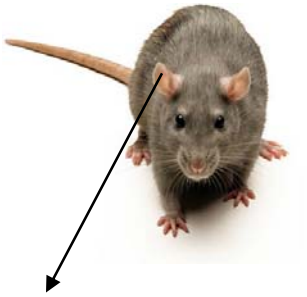
Did NOT Inhale Carbon Nanoparticles



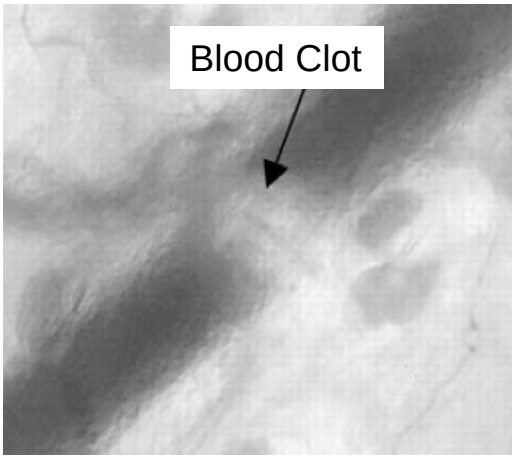
Ear vein 2 minutes after laser damage



Inhaled Carbon Nanoparticles



Ear vein 2 minutes after laser damage



Photos courtesy of Dr. Alison Elder. University of Rochester

1. Observe the photos of rat ear veins. Record your observations in the data table below.

Rat that:	Observations of blood clot in ear vein
Did NOT Inhale Carbon Nanoparticles	***No blood clot***
Inhaled Carbon Nanoparticles	***Blood clot***

2. Does this simulated experiment suggest that inhalation of carbon nanoparticles increases human risks for heart attacks? Support your answer with the data that you collected in this experiment.

   A blood clot formed in the vein of the rat exposed to nanoparticles but not in the control rat. If a blood clot breaks free and is carried into the heart, it may lead to a heart attack. Some students may point out that further research should be done using humans.

3. Why was this *in vivo* research done using rats instead of humans?

   Students may indicate that animal research is less expensive or that it avoids potential harm for humans.

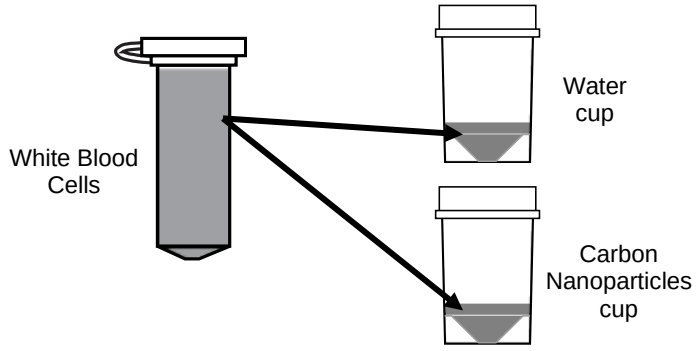
Part 4: *In Vitro* Studies

In vitro **("in glass") research** is done using cells that are cultured (grown) in test tubes, Petri dishes, or other containers. *In vitro* studies have two advantages. They are less expensive than *in vivo* studies. They are also better suited to studies in which scientists want to observe changes in cells or specific molecules in cells.

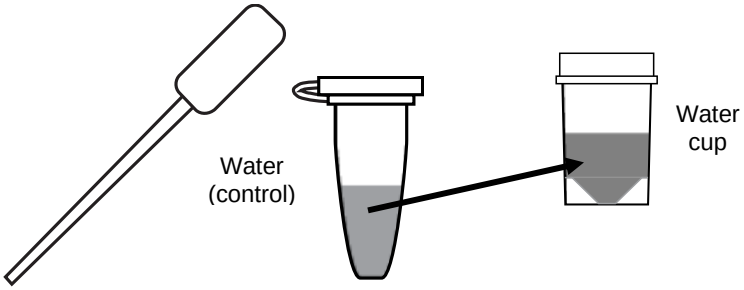
Researchers would like to use an *in vitro* study to determine the level of free radicals in white blood cells exposed to carbon nanoparticles. Free radicals are highly reactive molecules that can cause damage to the proteins, nucleic acids, and membranes found in cells.

You will conduct this experiment using human white blood cells cultured in blood plasma (the watery part of blood). You will treat some of the white blood cells with a low concentration of carbon nanoparticles and others with water (as a control). To determine the concentrations of free radicals, you will use a Free Radical Indicator.

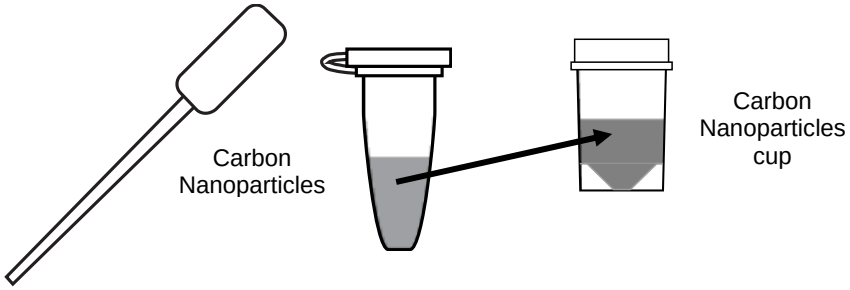
1. Place 5 drops of the white blood cell samples into the two small cups.



2. Add 5 drops of water to the cup labeled "Water."

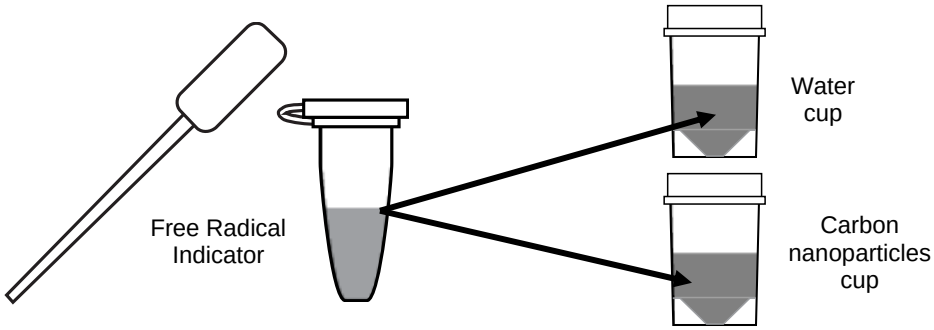


3. Add 5 drops of carbon nanoparticle solution to the cup labeled "Carbon Nanoparticles."

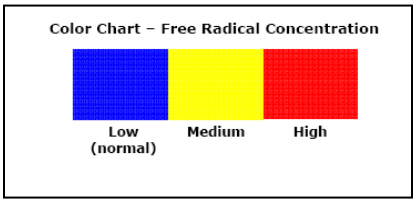


4. Use plastic stirrers to gently mix the contents of each of the cups for 2 minutes.

5. Add 2 drops of Free Radical Indicator solution into both of small cups. Stir to mix the indicator solution with the samples.



6. Use the *Color Chart – Free Radical Concentration* to determine the concentration of free radicals in each cup.



7. Record the concentration of harmful free radicals in the table below.

Cells that were mixed with:	Free Radical Concentration
Water (control)	***Low (normal)***
Carbon Nanoparticles	***Medium***

8. Does this simulated experiment suggest that exposure to carbon nanoparticles increases human risks for heart attacks? Support your answer with the data that you collected in this experiment.

The cells mixed with nanoparticles showed medium or above normal concentrations of free radicals. This may cause cell damage that increases risks for heart attacks.

Part 5: Conclusions

1. What did your **simulated** experiments suggest about the possible hazards of nanoparticles?

   The simulated experiments suggested that nanoparticles may increase the risks for cardiovascular diseases.

BUT…

It is important to remember that the experiments that you conducted were very simplified simulations that do not provide adequate evidence that inhaled nanoparticles are harmful to human health.

2. What is one problem with drawing conclusions about nanoparticle risks based on the experiments that you did?

   The experiments that we did were very simplified simulations that do not provide adequate evidence.

More Research Needed!

Currently, very little is known about the interaction of nanoparticles and biological systems. Initial toxicology experiments have shown that some types of nanoparticles can enter the body, affect organ function, and possibly lead to health problems. But these experiments must be repeated many times under precisely controlled conditions using specific types and concentrations of nanoparticles. Also, the changes to cells, tissues and whole organisms observed following nanoparticle exposure are often very slight. These small changes may or may not lead to health problems.

Scientific research is needed to identify short-term and long-term health effects of different kinds of nanoparticles on human health. This research should study the response of living organisms to the many different kinds of nanoparticles that have different chemical compositions, sizes, shapes, and surface areas. This research should also use realistic nanoparticle concentrations similar to those that people are likely to encounter at the work or in their daily lives. The results of this research should be communicated to the general public and to regulatory agencies in an unbiased manner so that they can effectively weigh the benefits and health risks of nanoparticles.

3. If one scientist conducts an experiment that shows that nanoparticles cause small changes in the circulatory system, is it safe to conclude that nanoparticles lead to health problems? Explain why or why not.

   No, these experiments need to be repeated by other scientists under precisely controlled conditions using specific types and concentrations of nanoparticles.

4. What does initial toxicology research indicate about the affect of nanoparticles on organisms?

   Initial toxicology experiments have shown that some types of nanoparticles can enter the body, affect organ function, and possibly lead to health problems.

5. What types of experiments need to be done to completely investigate the possible health risks associated with nanoparticles?

   This research should study the response of living organisms to the many different kinds of nanoparticles that have different chemical compositions, sizes, shapes, and surface areas. This research should also use realistic nanoparticle concentrations similar to those that people are likely to encounter at the work or in their daily lives.

6. After nanotoxicology experiments have been done, to whom should the results be communicated?

   The results should be shared with the general public and regulatory agencies.

7. In **your** opinion, are nanoparticles really hazardous to human health? Support your answer to this question with information from the "More Research Needed!" reading.

   Student answers will vary. But their answers should demonstrate an understanding that more research is needed to determine if nanoparticles are hazardous to human health.