Teacher's Preparatory Guide

Can We Absorb Nanoparticle Pollutants?

Overview: Many plastics are in our oceans due to pollution, including ocean dumping and land runoff. Sunlight and alkaline ocean water break these plastics down into small particles, many are nanoscale in size. These *nanoparticles* can easily enter our cells. How do these particles cross membranes into cells?

Purpose: This lab will help students understand how nanoparticles in our oceans can carry toxic pollutants into our cells, and how this may interfere with proper physiological function of ocean and human life

Time Required: Three 55-minute student lab periods, plus two pre-lab classes and one post-lab group presentations class

Level: High school environmental science, life science, chemistry, and physiology

Big Ideas of Nanoscale Science and Engineering: Forces and interactions, structure of matter, and models and simulations

Teacher Background Human-made pollutants, such as plastics, are in our soil, air, and ocean. Over time, sunlight, alkaline water, and heat break down plastic debris into smaller and smaller particles that can be one billionth of a meter in size (a nanometer)[1]. These ***nanoparticles*** have large surface areas that easily bind with persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). PCBs, have been banned since the 1970s, but they still persist in the environment due to their chemical stability[2]. They were originally used for electronic applications and coolants. PBDEs are used as flame-retardants in furniture, bedding, electronic equipment, and clothes. However, PBDEs can accumulate in the body and can be toxic in large quantities[3].

When plastic nano-size particles are released into the environment they can create problems due to binding with other oil-based pollutants and heavy metals[4,5]. Depending on their chemical properties the particles can even gain entryway into cells by crossing the cell membrane. The cell membrane is a phospholipid bilayer that is composed of a polar hydrophilic head (water-loving) and hydrophobic lipid tails (water-hating). The structure of the membrane regulates the transport of molecules in and out of the cell. Only small lipophilic molecules can easily cross the cell membrane. There are also proteins embedded into the membrane for transport of polar and water-soluble molecules that cannot cross the cell membrane. Lipid nanoparticles can easily enter the cell and can accumulate to dangerous levels.

Metal pollutants, like the inorganic mercury used in this lab, have a unique way of binding with phospholipids in the cell membrane. A special binding site exists on metals, so that the water-loving head of the phospholipid is attracted to this site and covalently bonds with the metal ion[6]. Other organic metals, such as methyl mercury, cross the bilayer using chloride as a

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complex mechanism. The chloride acts as a *facilitator*[7] for the mercury to cross the lipid bilayer. Plastics, being lipids, can attach to cell membranes via hydrogen bonding[4]. To some extent, this can cause the plastic to cross the membrane due to the attractiveness of this bonding. In this activity, students will replicate the process of how nanoparticle pollutants cross a phospholipid bilayer using mercury ions (Hg^{+2}). The cell used for this experiment will be a hen egg. The first part of the lab will involve removal of the egg shell using acetic acid (vinegar). The reaction for what is happening in removing the shell is: $CaCO_3 + 2H^+ \Rightarrow Ca^{+2} + H_2O + CO_2$. Students will then expose the egg to mercury, which simulates exposure of cells to environmental toxins including nanoparticles. Once the pathway of the pollutant is determined in the lab, students will predict the effects of toxic pollutants in the body, and research and present their results in a group oral presentation.

The lipophilic nature of plastics, metals, and polymers allow for cell membrane attraction due to the lipid tails within the bi-layer:

For some images and explanation of a lipid bilayer, please see:

- Bare Bones Biochemistry http://andersonlab.qb3.berkeley.edu/Tutorials/Bare_Bones_Biochemistry.htm
- Image of a Lipid Bilayer (plasma membrane) http://lamp.tu-graz.ac.at/~hadley/nanoscience/week4/membrane.jpg

Sources

1. M.A. Manzano, J.A. Perales, D. Sales and J.M. Quiroga. “Using Solar and Ultraviolet Light to Degrade PCBs in Sand and Transformer Oils” (accessed July, 2009) http://www.sciencedirect.com/science/article/pii/S0045653504006010
2. Roberta C. Barbalace. The Chemistry of Polychlorinated Biphenyls. EnvironmentalChemistry.com. Sept 2003. Accessed on-line: 6/23/2011 http://EnvironmentalChemistry.com/yogi/chemistry/pcb.html
3. Polybrominated diphenyl ethers (PBDEs). US Environmental Protection Agency. Accessed on-line: 6/23/2011 http://www.epa.gov/opptintr/pbde/
4. Simon J. Slater, Cojen Ho, Frank J. Taddeo, Mary Beth Kelly, Christopher D. Stubbs. **“**Contribution of Hydrogen Bonding to Lipid-Lipid Interactions in Membranes and the Role of Lipid Order: Effects of Cholesterol, Increased Phospholipid Unsaturation, and Ethanol” (accessed August, 2009) http://pubs.acs.org/doi/abs/10.1021/bi00065a025
5. Lorena M. Rios, Charles Moore and Patrick Jones. “Persistent Organic Pollutants Carried by Synthetic Polymers in the Ocean Environment” (accessed July, 2009) http://5gyres.org/media/Persistent_organic_pollutants.pdf
6. Cesar Santiago, Angela Ballesteros, Laura Martinez-Munoz, Mario Mellado, Gerardo G. Kaplan, Gordon J. Freeman, and José M. Casasnovas. “TIM-4 Structures Identify a Metal Ion-Dependent Ligand Binding Site Where Phosphatidylserine Binds” (accessed Aug., 2009) http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2330274
7. John Gutknecht. “Inorganic Mercury (Hg^{2+}) Transport Through Lipid Bilayer Membranes” (accessed August, 2009) http://www.springerlink.com/content/q321523h20121813/

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Materials per class

- a bottle of corn syrup
- a large container full of sample pollutant water (2 liters per class)
- 5 gal. distilled or filtered water
- receptacle for egg/sample water solution that has been tested with kits
- sharps container for disposal of used blades (cardboard box or glass jar will suffice)
- receptacle for sharp cutting instruments

Materials per group of 4 students

- gloves
- goggles
- lab aprons
- egg, raw and still in the shell
- tongs
- measuring tape
- triple beam balance scale or digital scale
- 2 Glad® 1/2 cup (118 ml) storage containers with lids
- labels or masking tape for labeling containers
- 1 Sharpie® marker
- distilled water (100 ml or enough for each group to cover their egg)
- plastic squirt bottle
- corn syrup (100 ml or enough for each group to cover their egg)
- sample pollutant water (100 ml or enough for each group to cover their egg)
- 2 HMT mercury test kits
- Exacto® knife, razor, scalpel, or dissecting probe
- strainer
- 3 small "Dixie®" cups, 5 oz (142 ml)

Source/Website	Material
Osumex (http://heavymetalstest.com/_hgkit.php) (Richard Long is the contact person for these kits and is very educator-friendly. Request the NNIN curriculum discount.)	 • HMT Mercury Test Kits
Harbor Freight (http://www.harborfrieght.com) or any educational supply company	· calipers · scales
Grocery store	· eggs · tape · distilled vinegar · corn syrup

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	· Glad® ½ cup storage containers with lids · distilled water

Advance Preparation Purchase materials, which may be found here:

1. ***Collect water with pollutants.***
   The best time to collect is after a rain; the runoff will contain more pollutants. Streams and storm drains are the best place to collect samples. A quick test of the water on site will determine if mercury is present in the sample. Although the scale seems to be light at the 0.05 ppm, this is still relatively high. The big uproar of mercury in fish was at 0.8 ppm. A sample of 0.05 ppm should provide good results.

2. ***Soak the eggs in vinegar at least 2 days beforehand.***
   Soak the demonstration egg <u>and several extra backup eggs</u> in case students accidentally pop their egg in vinegar. After 24 hours, replace the vinegar with fresh vinegar. The eggs do not need to be refrigerated and can be stored outside for up to 2 weeks and still work for this lab. The egg is ready when the shell has been removed. The eggs should look yellowish, like the image in the *Procedure Checkpoints* section. Students will put their own eggs in vinegar on Day 1 of the unit after you do the osmosis demonstration.

Safety Information Students must wear goggles, gloves, and aprons; no open-toed shoes, no shorts, and long hair should be tied back. Acetic acid is an eye irritant; students should wear goggles. Ask students before the lab begins whether they have a latex allergy. Have a box of nitrile gloves on hand, just in case. Eggs spurt when cut; students should wear lab aprons to protect clothing. Students should wash hands thoroughly before and after this lab. Pollutants in stream or ocean water are of an undetermined nature. Razors, scalpels, and dissecting probes are a cutting hazard. Dispose of all sharps in an appropriate container. Instruct students to use with caution. Keep a first aid kit handy.

Instructional Procedure

Time	Activity	Goal
Day 1	**Introduction to Osmosis**	
5 min	**Note:** The day before, put an egg in vinegar. **Today:** *Demonstration: Osmosis Eggsperiment*. Remove the egg from the vinegar, and rinse with distilled water. Ask students to describe its appearance. Place the egg in corn syrup. Set it aside for the next day.	Reinforce the concept of osmosis by showing cell membrane permeability. Discuss interactions of lipids with other lipids (attract) and water (repel).
40 min	Review or introduce cell transport by using the *Teacher Background* section as a guide. Check for understanding.	Students must understand cell membrane physiology and how it interacts with water.
5 min	Review safety procedures for laboratory practices.	Students practice lab safety.

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5 min	Distribute *Student Worksheets* to students. Students follow *Demonstration Day* procedures: they measure and record initial data and observations of store-bought eggs. They put eggs in vinegar in labeled containers, leaving lids loose since CO_2 is given off during reaction. Eggs soak in vinegar for about 2 days prior to actual lab to allow enough time to remove the shell.	To get the experiment started.
Day 2	**The day before the lab**	
5 min	Remove the egg from the corn syrup, rinse with distilled water. Ask students how the egg has changed. Put the egg in iodine. Soak it for 45 min.	Reinforce the concept of osmosis showing cell membrane permeability.
25 min	Lead a class discussion using the *Guided Dialog* section. Discuss calculations to be used.	Students consider how the nanoparticles can enter cells.
20 min	Show video segment from *Voyage to Kure.* (Refer to the *Resources* section to get this video.)	Show students how debris can impact the environment.
5 min	Remove the egg from the iodine, rinse with distilled water. Ask students: How is this different than before we soaked it in the iodine? *The inside of the egg turns a bright reddish-orange.* What happened? *Osmosis*. Students should explain their answer using terms in the *Guided Dialog* section.	Demonstrate how osmosis works via cell membrane permeability.

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Day 3	**Day 1 of the student lab**	
10 min	Students wash hands, and get materials.	Focus students for class.
25 min	Students follow *Day 1 of Lab* procedures on the *Student Worksheet*.	Students make measurements and observations.
10 min	Clean up. Students assign/delegate group duties for their oral presentation.	Students begin to prepare for oral presentation.
Day 4	**Day 2 of Student Lab**	
10 min	Group students and do safety/materials checks.	To focus students for class.
25 min	Students follow *Day 2 of Lab* procedures on *Student Worksheet*.	Students to observe how the egg shrinks due to osmosis, measure and observe. Students compare/contrast.
20 min	Groups clean up and store eggs. Introduce group project to students with few details at this point.	
Day 5	**Day 3 of Student Lab**	
40 min	Have students follow pre-lab checks and safety; ask students to follow *Day 3 of Lab* procedures on *Student Worksheet*.	To have students begin to analyze results and form conclusions.
15 min	Review results and possible explanations as a class. Go over post lab questions.	To have students get ideas as to how to answer the post lab questions.
Day 6		
50 min	Present group activities to students complete with rubric; group presentation will be on the impact of pollutants. Activities are in the *Going Further* section of this lab, and include the *Student Challenge.*	Students provide: □poster showing normal and affected gland of the endocrine system □letter to or from EPA discussing plastic debris and effects on the environment □PowerPoint presentation OR poster discussing what PCBs and PBDEs are □PowerPoint presentation tying each role to the big picture

Teaching Strategies The lab should be done in cooperative lab groups of 4 students each. A lecture of covalent, ionic, and dipole-dipole bonding should provide a background for the students. A quick overview of physiology and anatomy may also be beneficial for this lab since this lab demonstrates pollutants crossing the membrane. Before the lab begins, discuss the information in *Teacher Background* section.

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Guided Dialog *Before* beginning the lab, review the meaning of these terms:

Phospholipid bilayer *Cell membrane surrounding the cell. It is a two layer (bi) structure composed of hydrophilic (water loving) heads and hydrophobic (water hating) fatty tails.*

Permeability *The ability of substances to cross a barrier.*

Covalent Bond *The sharing of electron(s) between two or more elements.*

Nanoscale *Anything that is 1–100 nanometers (a billionth of a meter) in size.*

Homeostasis *The state at which all metabolic pathways work most efficiently.*

Tissue *Groups of cells performing a similar function.*

Organ *Groups of tissues performing a similar function.*

Organ system *Groups of organs that cooperate to perform a function inside the body.*

Endocrine system *The system that regulates hormones in glands throughout the body.*

Lipophilic *Attracted to lipids.*

Ionic Bond *Formed between two ions, one negative and one positive. The positive ion takes the electron from the negative ion.*

Ion *A charged particle that is either positive due to loss of an electron (cation), or negative due to gaining an extra electron (anion).*

Ask students these questions to review what they already know:

1. What is the cell composed of? *a bilayer of phospholipids, organelles, membranes, etc.*
2. Why is this fact important when talking about nanoscale particles? *The nanoparticles are lipophilic. They are attracted to the membrane and tend to easily get into the cell, carrying along toxic persistent organic pollutants. (Based on lecture given previously.)*
3. How are our cells exposed to nanoscale particles? *They are found in our environment, bioaccumulation throughout food web, and in the air we breathe.*
4. What factors affect how many pollutants we are exposed to? *Reduce, Recycle, Reuse.*

List any last-minute details that the students must remember, including reiterating all safety precautions. Now, begin the lab.

Procedure Checkpoints

1. *Soaking eggs in vinegar:*

Soak the eggs in vinegar for three days before lab begins.

This is how the egg should look after soaking in vinegar.

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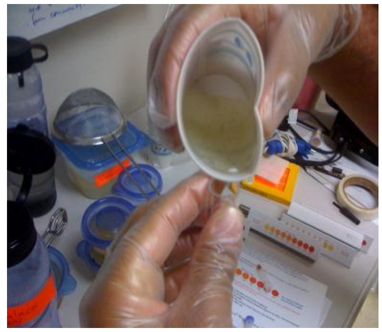
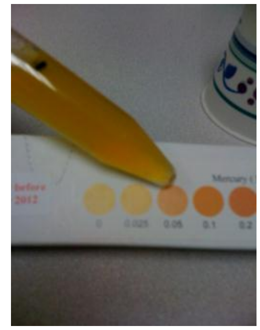
2. *HMT mercury test*:

HMT mercury test



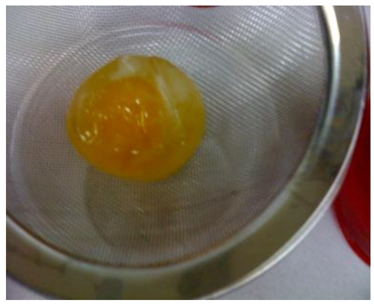
Use the HMT mercury test to test the polluted water



Positive test for mercury in water.

3. *Soaking eggs in corn syrup*:

Eggs should soak overnight in corn syrup after having been soaked in vinegar.



Egg shrivels in corn syrup.

4. *Soaking eggs in polluted water*:

Soak eggs in polluted water overnight.

Notice appearance of the egg after soaking in polluted water.

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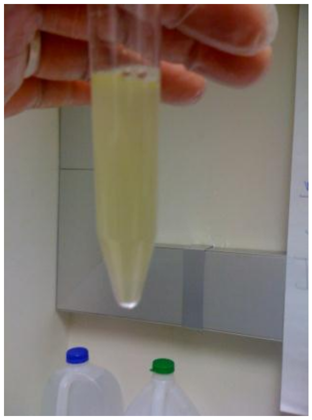
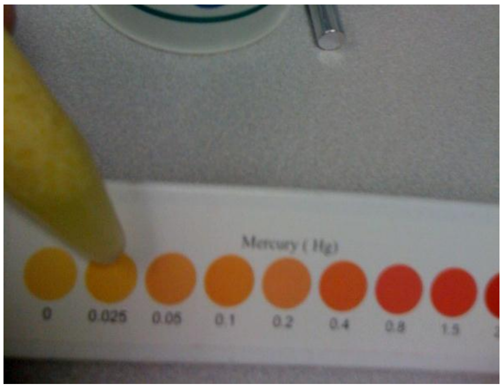
5. *Testing water inside egg:*

Cut open the egg so the water inside can be tested; careful—it does spurt.



Eggs whites in testing tube after soaking in polluted water.



Checking the egg for mercury. This is a positive test for mercury—notice color of egg is reddish yellow.

Control test—egg *not* soaked in polluted water. There is no color, so there is no presence of mercury.

Cleanup Collect the tested solutions and sharp tools in separate receptacles. Students should wash and dry all surfaces, including their hands, with soap and water. Pour the ocean water down the sink. Flush the tested water/egg solution down the toilet.

Enhancing Understanding Cover this section *after* the activity. Students who have a good grasp of the content of the lab can be further challenged with these questions:

1. Where are PCBs and PBDEs located initially? *Mandated by Federal Government, they are synthesized and put into all foam and upholstery in our furniture.*
2. Since mercury and other pollutants like PCBs and PBDEs are attracted to lipids (fats and oils), does BMI (Basic Mass Index) affect the body's ability to absorb pollutants? If so, how? *This answer will vary* 180^0*. Since there have been few studies if any on this topic, the correctness of this answer will depend on student rationale. In other words, does someone who is more on the heavier side have more or less of a chance of pollutant-related sickness than someone who is physically fit?*

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3. Flame retardants (PCBs and PBDEs) are mandatory in all furniture upholstery and foam products. What is the best way, other than reduce, reuse, and recycle, to lower the amount of PCBs and PBDEs in the environment? How should one go about making this change? *The laws need to be changed and the cost-to-benefit factor must be analyzed. Lobbying to have the laws changed is a good start. Education and public awareness will also be a powerful tool in changing minds of those elected officials in Washington D.C.*

Going Further Have students work on group projects asking them to inquire and learn about the impact of pollution on physiology. Computer labs are needed for Internet reference research. Float around class and provide feedback and/or check on progress of groups. Ask students to present their findings to the class. A rubric is available in the *Assessment* section.

Student Challenge This activity is designed for the students after the presentation on the physiological impact of nanoscale pollution. A rubric is available in the *Assessment* section.

- Groups must come up with public awareness/education ideas to help reduce the amount of pollution and exposure.
  - Public service announcements (ex., beach cleanup day)
  - K–8 class visitations
  - Contact existing public awareness/outreach programs and volunteer with the programs. Organize groups to help volunteer with these programs.
    - Ocean Futures Society (http://www.oceanfutures.org)
    - Ambassadors of The Environment (http://www.aote.org)
    - Coastal Keepers
    - Channel Keepers
  - Any other creative ways approved by instructor
- Find positive ways to get the attention of the legislature to make law amendments.

Assessment

1. Students should be able to measure the amount of water the egg up took or lost through the process of osmosis. (5 points)
2. Students should be able to determine if the carrier mechanism for toxins was or was not achieved. (15 points)
3. Students should have completed lab worksheets. (20 points)

Student presentations:

4. Students should be able to give a rational argument on how the pollutants can be absorbed and what effect they have on the body during the group presentation. (20 points)
5. Students are conducting scientific inquiry during their group presentation so these following criteria must be present:
   a. research cited (20 points)
   b. facts stated on any argument they propose (20 points)

Role	5 Points	10 Points	15 Points	20 Points
Leader: PowerPoint to tie all aspects together. Oral presentation unifying all parts of the group duties.	☐ Very few visuals ☐ Incomplete ☐ Words are copied and pasted; student does not know meaning ☐ Student has incorrect posture ☐ Mumbling and long pauses	☐ Visuals complete ☐ Captions explaining pictures ☐ Student understands more of the content ☐ Student enunciates somewhat clearly ☐ Intermediate pauses	☐ Visuals are clearly labeled ☐ PowerPoint flows in an orderly fashion ☐ Student has 75% terminology in own words ☐ Student enunciates clearly ☐ Short pauses to think	☐ Visuals are clearly defined ☐ Student has an orderly flowing PowerPoint ☐ All the terminology is in student's own words ☐ Speaks clearly
EPA Official	☐ Letter is incomplete ☐ Many grammatical mistakes (>75%) ☐ Many misspellings ☐ Handwritten ☐ Informal vocabulary ☐ Arguments are not clearly stated and no evidence to support ☐ Mumbles/reads presentation	☐ Letter is complete ☐ Many misspellings ☐ Grammatical errors (<50%) ☐ Letter is typed ☐ Somewhat formal vocabulary ☐ Arguments are stated and some evidence is provided ☐ Student reads letter	☐ Letter is complete ☐ Grammatical errors /misspellings (<25%) ☐ Arguments are stated and moderate evidence supports the arguments ☐ Formal vocabulary and format are used ☐ Note cards heavily used	☐ Letter is typed and complete ☐ Formal format and academic vocabulary used ☐ Arguments are stated and significant evidence supports the arguments ☐ No grammatical errors or misspellings ☐ Student can recite letter without reading it

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Role	5 Points	10 Points	15 Points	20 Points
Systems Specialist	☐ Poster is incomplete ☐ Very few organs/tissues of the system are shown and labeled ☐ Functions of the organs/tissues not present ☐ Sloppy hand-drawn labeling ☐ No comparisons of normal function to toxic systems ☐ Mumbling	☐ 50% of the organs are drawn ☐ Labels are sloppily hand labeled; copied and pasted ☐ Not all organs/tissues identified ☐ Some normal functions listed ☐ Some toxic effects listed ☐ Enunciation needs improvement	☐ All of the organs are drawn and clearly labeled ☐ Labels are typed and in student's own words ☐ >75% of comparative toxic effects are stated and drawn ☐ Student speaks fairly clearly	☐ The system is clearly drawn ☐ Labels are typed in student's own words ☐ Organs/tissues are compared to normal system
Analytical Specialist	☐ PowerPoint is not complete ☐ Visual of PCBs and PBDEs are not present ☐ No definitions of PCBs and PBDEs ☐ Student does not know carrier mechanism	☐ Definitions are copied and pasted ☐ Visual of either PCBs or PBDEs (but not both) are present ☐ No mention of how PCBs or PBDEs interact with membrane	☐ PowerPoint flows smoothly for the most part ☐ Definitions are written in student's own words ☐ Student has some knowledge of how these polymers interact with membrane ☐ Student is familiar with carrier mechanism	☐ Student has an orderly flowing Powerpoint ☐ Student has both PCBs and PBDEs with complete definitions in student's own words ☐ Student can explain carrier mechanism when asked ☐ Student knows terminology ☐ Student speaks clearly

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Resources You may wish to use these resources either as background or as a resource for students to use in their inquiry-based design.

- The Osmosis "Eggsperiment" that inspired this lab (downloadable pdf) http://userpages.monmouth.com/~skifast/worksheets/EggLab.pdf
- Many films from "Ocean Futures Society" (http://www.oceanfutures.org) are great. We recommend: *Call of the Killer Whales, Gray Whale Obstacle Course,* and *Voyage to Kure*
- Plastics in the ocean
  - http://www.whoi.edu/science/B/people/kamaral/plasticsarticle.html
  - http://en.wikipedia.org/wiki/Great_Pacific_Garbage_Patch
  - http://news.nationalgeographic.com/news/2009/08/090820-plastic-decomposes-oceans-seas.html

- Public awareness/outreach programs
  - Ocean Futures Society (http://www.oceanfutures.org)
  - Ambassadors of The Environment (http://www.aote.org)
  - Donsnetcafe (http://www.donsnetcafe.net)

National Science Education Standards (Grades 9–12)

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B: Physical Science

- Structure and properties of matter
- Chemical reactions

Content Standard C: Life Science

- The cell

California Science Education Standards (Grades 9–12)

Chemistry, Content Standard 2: Chemical Bonds

a. Students know atoms combine to form molecules by sharing electrons to form covalent or metallic bonds or by exchanging electrons to form ionic bonds.
b. Students know chemical bonds between atoms in molecules such as H_2, CH_4, NH_3, $HCCH_2$, N_2, Cl_2, and many large biological molecules are covalent.

Investigation and Experimentation, Content Standard 1

a. Select and use appropriate tools and technology to perform tests, collect data, analyze relationships, and display data.
b. Identify and communicate sources of unavoidable experimental error.
c. Identify possible reasons for inconsistent results, such as sources of error or uncontrolled conditions.
d. Formulate explanations by using logic and evidence.

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