ocean explorer www.oceanexplorer.noaa.gov

The NOAA Ship *Okeanos Explorer*

NOAA Ship *Okeanos Explorer:* America's Ship for Ocean Exploration. Image credit: NOAA. For more information, see the following Web site: *http://oceanexplorer.noaa.gov/okeanos/welcome.html*

What Killed the Seeds

An essential component of the NOAA Office of Ocean Exploration and Research mission is to enhance understanding of science, technology, engineering, and mathematics used in exploring the ocean, and build interest in careers that support ocean-related work. To help fulfill this mission, the Okeanos Explorer *Education Materials Collection is being developed to encourage educators and students to become personally involved with the voyages and discoveries of the* Okeanos Explorer*—America's first Federal ship dedicated to Ocean Exploration. Leader's Guides for Classroom Explorers focus on three themes: "Why Do We Explore?" (reasons for ocean exploration), "How Do We Explore?" (exploration methods), and "What Do We Expect to Find?" (recent discoveries that give us clues about what we may find in Earth's largely unknown ocean). Each Leader's Guide provides background information, links to resources, and an overview of recommended lesson plans on the Ocean Explorer Web site (*http://oceanexplorer.noaa.gov*). An Initial Inquiry Lesson for each of the three themes leads student inquiries that provide an overview of key topics. A series of lessons for each theme guides student investigations that explore these topics in greater depth. In the future additional guides will be added to the Education Materials Collection to support the involvement of citizen scientists.*

This lesson guides student inquiry into the key topic of Human Health within the "Why Do We Explore?" theme.

Focus
Bioassays

Grade Level
7-8 (Life Science)

Focus Question
How can the biological effects of chemicals be studied?

Learning Objectives
• Students will be able to explain and carry out a simple process

for studying the biological effects of chemicals.
- Students will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Materials

- Radish seeds; at least 60 for each student group (ten seeds for each replicate)
- 10% household bleach solution, about 50 ml for each student group
- Kitchen strainer; may be shared among several student groups
- Zip-top plastic freezer bags, 1-quart size, or disposable plastic petri dishes, 100 mm x 10 mm (Carolina Biological Supply No. WW-74-1248); at least six for each student group
- Felt tip markers
- Paper towels
- Disposable plastic pipettes with rubber bulb or aspirator, one for each student group
- Ruler graduated in millimeters
- Distilled water
- Clean glass containers with stoppers or caps for collecting water samples; minimum capacity about 100 ml
- Copies of *Bioassay Inquiry Guide*, one for each student group

Audiovisual Materials

- Marker board, blackboard, or overhead projector with transparencies for group discussions

Teaching Time

One or two 45-minute class periods, plus time for student observations over several class periods

Seating Arrangement

Groups of 2-3 students

Maximum Number of Students

32

Key Words and Concepts

Natural products
Drugs from the sea
Bioassay

Background Information

People who are not familiar with ocean exploration often believe that the primary reason for investigating deep-sea ecosystems

Some drugs derived from marine invertebrates:

Ecteinascidin – Extracted from tunicates; being tested in humans for treatment of breast and ovarian cancers and other solid tumors

Topsentin – Extracted from the sponges *Topsentia genitrix*, *Hexadella* sp., and *Spongosorites* sp.; anti-inflammatory agent

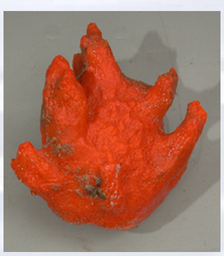
Lasonolide – Extracted from the sponge *Forcepia* sp.; anti-tumor agent

Discodermalide – Extracted from deep-sea sponges belonging to the genus Discodermia; anti-tumor agent

Bryostatin – Extracted from the bryozoan *Bugula neritina*; potential treatment for leukemia and melanoma

Pseudopterosins – Extracted from the octocoral (sea whip) *Pseudopterogorgia elisabethae*; anti-inflammatory and analgesic agents that reduce swelling and skin irritation and accelerate wound healing

w-conotoxin MVIIA – Extracted from the cone snail *Conus magnus*; potent pain-killer




Though they may be visually unimpressive, *Forcepia* sponges (left) are the source of the lasonolides and tunicates (right) are the source of ecteinascidin, potential new drugs for treating cancer. Image credit: NOAA.
http://oceanexplorer.noaa.gov/explorations/03bio/logs/hirez/lasonolide1_hirez.jpg
http://oceanexplorer.noaa.gov/explorations/03bio/logs/hirez/figure4_hirez.jpg

is little more than scientific curiosity. This perspective quickly changes, however, when they learn that these ecosystems are the source of promising new drugs for treating some of the most deadly human diseases.

Despite the many advances of modern medicine, disease is still the leading cause of death in the United States. Cardiovascular disease and cancer together account for more than 1.5 million deaths annually (40% and 25% of all deaths, respectively). In addition, one in six Americans have some form of arthritis, and hospitalized patients are increasingly threatened by infections that are resistant to conventional antibiotics. The cost of these diseases is staggering: $285 billion per year for cardiovascular disease; $107 billion per year for cancer; $65 billion per year for arthritis. Death rates, costs of treatment and lost productivity, and emergence of drug-resistant diseases all point to the need for new and more effective treatments.

Most drugs in use today come from nature, and almost all of these are derived from terrestrial organisms. Aspirin, for example, was first isolated from the willow tree. Morphine is extracted from the opium poppy. Penicillin was discovered from common bread mold. But recently, systematic searches for new drugs have shown that marine invertebrates produce more antibiotic, anti-cancer, and anti-inflammatory substances than any group of terrestrial organisms. Particularly promising invertebrate groups include sponges, tunicates, ascidians, bryozoans, octocorals, and some molluscs, annelids, and echinoderms.

Most of these animals do not appear particularly impressive. Many are sessile, and live all or most of their lives attached to some sort of surface. Several reasons have been suggested to explain why these animals are particularly productive of potent chemicals. One possibility is that they use these chemicals to repel predators, since they are basically “sitting ducks.” Since many of these species are filter feeders, and consequently are exposed to all sorts of parasites and pathogens in the water, they may use powerful chemicals to repel parasites or as antibiotics against disease-causing organisms. Competition for space may explain why some of these invertebrates produce anti-cancer agents: If two species are competing for the same piece of bottom space, it would be helpful to produce a substance that would attack rapidly dividing cells of the competing organism. Since cancer cells often divide more rapidly than normal cells, the same substance might have anti-cancer properties.

Harbor Branch Oceanographic Institution researcher Dr. Shirley Pomponi removes a bright yellow sponge from a rock collected by an underwater robot during the 2003 Medicines from the Deep Sea expedition. Extracts from the sponge were tested for anti-cancer properties. Image credit: Laura Rear, NOAA.
http://oceanexplorer.noaa.gov/explorations/03bio/logs/summary/media/10249_bio_600.jpg

The potential for discovering important new drugs from deep-ocean organisms is high, because most of Earth's seafloor is still unexplored, and deep-sea explorations routinely find species that have never been seen before. In 2003, the Ocean Explorer Deep Sea Medicines Expedition visited the Gulf of Mexico to search for new resources with pharmaceutical potential. The expedition collected selected benthic invertebrates from deep-water bottom communities in the Gulf of Mexico (sponges, octocorals, molluscs, annelids, echinoderms, tunicates), and tested extracts of these organisms to identify those that may be useful in treatment of cancer, cardiovascular disease, infections, inflammation, and disorders of the central nervous system. This lesson guides student inquiries into bioassays, which are tests that use biological organisms to study the action of chemicals or physical changes in the environment.

Learning Procedure

1. To prepare for this lesson:
   - If you have not previously done so, review introductory information on the NOAA Ship *Okeanos Explorer* at http://oceanexplorer.noaa.gov/okeanos/welcome.html. You may also want to consider having students complete some or all of the Initial Inquiry Lesson, *To Boldly Go…* (http://oceanexplorer.noaa.gov/okeanos/edu/leadersguide/media/09toboldlygo.pdf).
   - Review background essays on Deep Sea Medicines, Microbiology, Natural Products and Molecular Biology linked from the Deep Sea Medicines 2003 Expedition welcome page (http://oceanexplorer.noaa.gov/explorations/03bio/welcome.html).
   - Review procedures on the *Bioassay Inquiry Guide*, and assemble materials listed on Page 2 of this lesson plan. To prepare a 10% bleach solution, mix 50 ml household bleach with 450 ml tap water. Keep the solution away from sunlight. The Guide instructs students to prepare at least three replicates for each solution being tested and for each control solution. This is the minimum number of replicates needed for statistical analysis; more is better, if time and materials permit.

2. If you have not previously done so, briefly introduce the NOAA Ship *Okeanos Explorer*, emphasizing that this is the first Federal vessel specifically dedicated to exploring Earth's largely unknown ocean. Lead a discussion of reasons that ocean exploration is important, which should include human health.

3. Discuss the importance of finding new drugs for the treatment of cardiovascular disease, cancer, inflammatory diseases, and infections. Describe the potential of marine communities as sources for these drugs, and briefly discuss some potentially useful drugs that have been discovered from these communities. Ask students to list some reasons that these kinds of drugs might be found primarily among sessile invertebrates. Tell students that they will be learning to use a technique for studying the effects of chemicals on living organisms. Explain that a bioassay uses a biological organism to study the effects of chemicals or physical environmental change (such as radiation or heat). When toxicity is being studied, bioassays provide an integrated measure of all changes to which a test organism is suggested, and provide a different type of understanding than would be obtained from direct measurements of specific chemical or physical factors.

   Tell students that they will be using radish seeds as a bioassay organism. Two responses will be investigated: germination and growth rate. Lead a discussion to identify one or more substances (liquids are easiest) whose toxicity is to be tested. Runoff water from a street (usually contaminated with vehicle emissions) or a nearby water body suspected of being polluted are common test subjects. Have students collect the substances to be tested. A sample of 100 ml is adequate for the test. Remind students to wash their hands thoroughly after handling water that is suspected of being contaminated. (***Washing hands is a good idea after ANY laboratory procedure!***)

4. Have students perform bioassays using the procedure described on the *Bioassay Inquiry Guide*.

5. Lead a discussion of students' results. Students should realize that different organisms are not equally sensitive to chemical agents. For example, the concentration of copper in water that would kill algae or a snail is harmless to most fish. When choosing a bioassay organism, investigators need to consider which compounds or organism responses are of most concern. Seed bioassays are very sensitive to herbicides and fairly sensitive to metals. They are less sensitive than fish or invertebrate assays to industrial chemicals like polychlorinated biphenyls (PCBs) or solvents. A full evaluation of a sample's biological activity requires performing several different bioassays. Bioassays for drug screening, for example, often include bacteria (to screen for potential antibacterial activity) and specific tissue cultures (to screen for anti-cancer activity).

The BRIDGE Connection

www.vims.edu/bridge/ – Enter "pharmaceutical" in the Search box for resources on drugs from the sea.

Click on "Ocean Science Topics" then "Habitats," then "Deep Sea" for resources on deep-sea communities.

Click on "Ocean Science Topics," then "Human Activities" then "Technology" for resources on biotechnology.

The "Me" Connection

Have students write a short essay on how bioassays might be of personal benefit.

Connections to Other Subjects

English/Language Arts, Mathematics (Statistics)

Assessment

Written reports prepared in Step 2 offer opportunity for assessment.

Extensions

1. Visit http://oceanexplorer.noaa.gov/explorations/03bio/welcome.html to find out more about the Deep Sea Medicines 2003 Expedition.

2. Visit http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon09no1.pdf for more examples and ideas for using bioassays.

Multimedia Discovery Missions

http://www.oceanexplorer.noaa.gov/edu/learning/welcome.html Click on the links to Lesson 12 for an interactive multimedia presentation and learning activities on Medicine from the Sea.

Other Relevant Lessons from NOAA's Ocean Explorer Program

While each lesson is targeted toward a specific grade level, most can be adapted for use in other grades as well.

Chemists with no Backbones (Grades 5-6; 4 pages, 356k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_ChemNoBackbones.pdf

Focus: Benthic invertebrates that produce pharmacologically-active substances (Life Science)

In this activity, students will be able to identify at least three groups of benthic invertebrates that are known to produce pharmacologically-active compounds and will describe why pharmacologically-active compounds derived from benthic invertebrates may be important in treating human diseases. Students will also be able to infer why sessile marine invertebrates appear to be promising sources of new drugs.

Microfriends (Grades 5-6; 6 pages, 420k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_Microfriends.pdf

Focus: Beneficial microorganisms (Life Science)

Students will be able to describe at least three ways in which microorganisms benefit people, describe aseptic procedures, and obtain and culture a bacterial sample on a nutrient medium.

Living by the Code (Grades 7-8; 5 pages, 400k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_LivingCode.pdf

Focus: Functions of cell organelles and the genetic code in chemical synthesis (Life Science)

In this activity, students will be able to explain why new drugs are needed to treat cardiovascular disease, cancer, inflammation, and infections; infer why sessile marine invertebrates appear to be promising sources of new drugs; and explain the overall process through which cells manufacture chemicals. Students will also be able to explain why it may be important to sythesize new drugs, rather than relying on the natural production of drugs.

Cell Mates
(Grades 9-12; 6 pages, 444k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_CellMates.pdf

Focus: Bacterial endosymbionts and organelles of eukaryotic cells (Life Science)

In this activity, students will be able to compare and contrast prokaryotic and eukaryotic cells, explain the endosymbiont theory for the origin of eukaryotic cell organelles, and explain evidence that suggests an endosymbiotic origin for at least two common eukaryotic cell organelles.

The Benthic Drugstore (Grades 9-12; 4 pages, 360k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_Drugstore.pdf

Focus: Pharmacologically active chemicals derived from marine invertebrates (Life Sscience)

In this activity, students will be able to identify at least three pharmacologically active chemicals derived from marine invertebrates, describe the disease-fighting action of at least three pharmacologically active chemicals derived from marine invertebrates, and infer why sessile marine invertebrates appear to be promising sources of new drugs.

The Electric Sieve (Grades 9-12; 5 pages, 400k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_ElecSieve.pdf

Focus: Separation of complex mixtures (Chemistry)

In this activity, students will be able to explain and carry out a simple process for separating complex mixtures, and will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Watch the Screen! (Grades 9-12; 5 pages, 428k)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/Meds_WatchScreen.pdf

Focus: Screening natural products for biological activity (Life Science)

In this activity, students will be able to explain and carry out a simple process for screening natural products for biological activity, and will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Other Resources

The Web links below are provided for informational purposes only. Links outside of Ocean Explorer have been checked at the time of this page's publication, but the linking sites may become outdated or non-operational over time.

Rathbun, J. 1996. A Simple Bioassay Using Lettuce Seeds. The *Volunteer Monitor*. Spring, 1996. Available online at http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon09no1.pdf

Mayer, A. M. S. and K. R. Gustafson. 2003. Marine pharmacology in 2000: Antitumor and cytotoxic compounds. Int. J. Cancer 105:291-299. Available online at http://marinepharmacology.midwestern.edu/docs/MP2000_Anticancer_Mayer_Gustafson.pdf

Tim Batchelder, T. 2001. Natural products from the sea: Ethnopharmacology, nutrition and conservation. Townsend Letter for Doctors and Patients, Feb, 2001. Available online at http://www.findarticles.com/p/articles/mi_m0ISW/is_2001_Feb/ai_70777319/pg_1.

Faulkner, D. J. 2000. Marine pharmacology. Antonie van Leeuwenhoek 77: 135-145.

www.nci.nih.gov – Web site of the National Cancer Institute

http://www.woodrow.org/teachers/bi/1993/ – Background and activities from the 1993 Woodrow Wilson Biology Institute on biotechnology

National Science Education Standards

Content Standard A: Science As Inquiry

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

Content Standard C: Life Science

- Behavior of Organisms

Content Standard D: Earth and Space Science

- Structure of the Earth system

Content Standard F: Science in Personal and Social Perspectives

- Personal and community health
- Natural resources
- Enviromnental quality

Ocean Literacy Essential Principles and Fundamental Concepts

Essential Principle 5.

The ocean supports a great diversity of life and ecosystems.

Fundamental Concept c. Some major groups are found exclusively in the ocean. The diversity of major groups of organisms is much greater in the ocean than on land.

Fundamental Concept d. Ocean biology provides many unique examples of life cycles, adaptations and important relationships among organisms (such as symbiosis, predator-prey dynamics and energy transfer) that do not occur on land.

Essential Principle 6.
The ocean and humans are inextricably interconnected.

Fundamental Concept b. From the ocean we get foods, medicines, and mineral and energy resources. In addition, it provides jobs, supports our nation's economy, serves as a highway for transportation of goods and people, and plays a role in national security.

Essential Principle 7.
The ocean is largely unexplored.

Fundamental Concept a. The ocean is the last and largest unexplored place on Earth—less than 5% of it has been explored. This is the great frontier for the next generation's explorers and researchers, where they will find great opportunities for inquiry and investigation.

Send Us Your Feedback

We value your feedback on this lesson, including how you use it in your formal/informal education setting.
Please send your comments to: oceanexeducation@noaa.gov

For More Information

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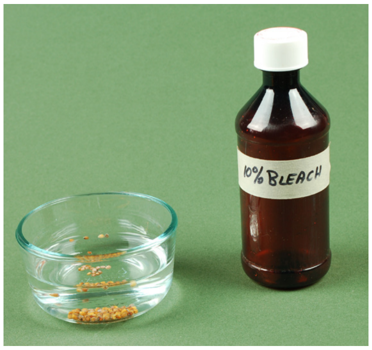
Bioassay Inquiry Guide

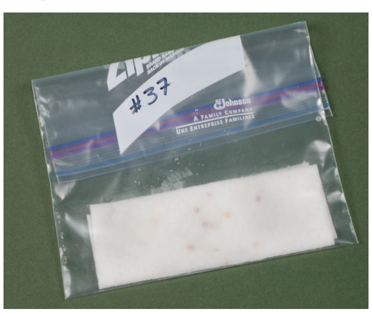
Step 1a:



Step 1b:

Step 3:

Step 4:



Step 6:

(Adapted from an article by Joe Rathbun in the spring 1996 issue of the Volunteer Monitor)

1. Soak seeds for 20 minutes in a 10% solution of household bleach in distilled water, then rinse thoroughly under running tap water. The solution kills fungi, which could interfere with seed germination.

2. Cut paper towels into pieces approximately 11” x 6”. You will need at least three pieces for each solution being tested, as well as at least three pieces for each control solution.

3. Place 10 seeds in the middle of each paper towel, leaving about 1/2” space between the seeds. Fold the edges of the paper towel over to cover the seeds.

4. Place each paper towel with the seeds into a zip-top plastic freezer bag or disposable plastic petri dish. Pipette enough undiluted sample solution into the bag or dish to saturate the paper towel. Prepare at least three replicates for each sample being tested, as well as at least three controls using distilled water instead of sample water. Use the same volume in each bag or dish.

5. Incubate bags or dishes at room temperature, in the dark, for five days. (It is OK to briefly check the dishes during incubation. If the paper seems dry, pipette a few ml of distilled water onto the paper.)

6. When incubation is complete, record the number of seeds that germinated in each bag or dish, and measure (to the nearest mm) the length of the root that has emerged from each germinated seed (the image shows a seed after 24 hours’ incubation). If fewer than 80% of the seeds in the control sample germinate, this indicates a problem with the assay (e.g., bad seeds, poor incubation conditions). If this happens, the test should be re-run.

7. For each sample (including the controls), calculate the mean and standard deviation of root lengths. Comparisons can be made by using the Student’s t-test. A more approximate method is to compare the mean ±1 standard deviation of each sample to the control. If a sample’s mean plus 1 standard deviation is less that the mean of the control minus 1 standard deviation, there is a strong likelihood that the sample is significantly more toxic than the control. Prepare a written report of your results, including a discussion of the outcome.