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*

Section 5: Key Topic – Human Health

What Killed the Seeds?

Focus
Bioassays

Grade Level
7-8 (Life Science)

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

Learning Objectives
- Students will explain and carry out a simple process for studying the biological effects of chemicals.
- Students will 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. 741248); 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 Investigation 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

Ocean Exploration and Research

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

NOTE: Explanations and procedures in this lesson are written at a level appropriate to professional educators. In presenting and discussing this material with students, educators may need to adapt the language and instructional approach to styles that are best suited to specific student groups.

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

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

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

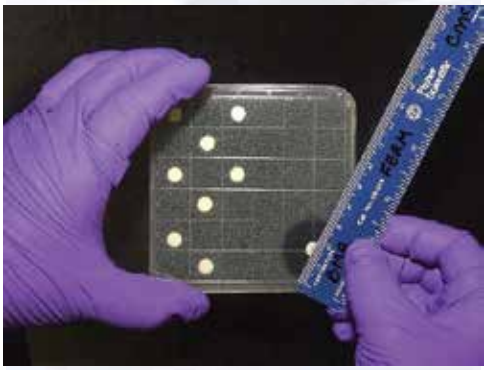
Lasonolide – Extracted from the sponge *Forcepia* sp.; anti-tumor agent

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

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



This bioassay plate detects antimicrobial activity. Small disks of blotter paper are dipped in marine-organism extracts and placed on an agar plate with various bacteria. The clear zone indicates that the extracts are inhibiting microbial growth. Image credit: NOAA. *http://oceanexplorer.noaa.gov/explorations/03bio/background/microbiology/media/figure_04.html*

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*

rapidly than normal cells, the same substance might have anti-cancer properties. 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 deepwater bottom communities (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 investigations 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:
   - 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 lesson, *To Boldly Go*….
   - 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 Investigation 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 a test organism's response to chemicals or environmental change, and give a more complete 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 considered standard practice after ANY laboratory procedure!)**

4. Have students perform bioassays using the procedure described on the *Bioassay Investigation 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 and class discussions offer opportunities 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 Lesson Plans 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)
(from 2003 Medicines from the Deep Sea Expedition)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_chemnobackbones.pdf

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

Students will 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 infer why sessile marine invertebrates appear to be promising sources of new drugs.

Living by the Code (Grades 7-8)
(from 2003 Medicines from the Deep Sea Expedition)
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)

Students will 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 explain why it may be important to synthesize new drugs, rather than relying on the natural production of drugs.

Cell Mates (Grades 9-12) (from 2003 Medicines from the Deep Sea Expedition)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_cellmates.pdf

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

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)
(from 2003 Medicines from the Deep Sea Expedition)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_drugstore.pdf

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

Students will 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)
(from 2003 Medicines from the Deep Sea Expedition)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_elecsieve.pdf
Focus: Separation of complex mixtures (Chemistry)
Students will explain and carry out a simple process for separating complex mixtures, and will infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Watch the Screen! (Grades 9-12)
(from 2003 Medicines from the Deep Sea Expedition)
http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_watchscreen.pdf
Focus: Screening natural products for biological activity (Life Science)
Students will explain and carry out a simple process for screening natural products for biological activity, and will infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Other Resources

See page 217 for Other Resources.

Next Generation Science Standards

Lesson plans developed for Volume 1 are correlated with *Ocean Literacy Essential Principles and Fundamental Concepts* as indicated in the back of this book. Additionally, a separate online document illustrates individual lesson support for the Performance Expectations and three dimensions of the Next Generation Science Standards and associated Common Core State Standards for Mathematics and for English Language Arts & Literacy. This information is provided to educators as a context or point of departure for addressing particular standards and does not necessarily mean that any lesson fully develops a particular standard, principle or concept. Please see: *http://oceanexplorer.noaa.gov/okeanos/edu/collection/wdwe_ngss.pdf.*

Send Us Your Feedback

We value your feedback on this lesson, including how you use it in your formal/informal education settings. Please send your comments to:
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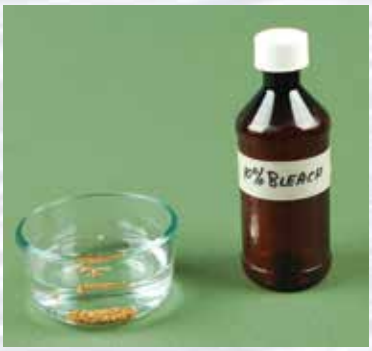
Bioassay Investigation Guide

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 than 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.

Notes: