



## The Good, the Bad and the Nasty Tasting

### Focus

Chemical ecology: Chemically-mediated interactions

### Grade Level

9-12 (Biology, Chemistry)

### Focus Questions

How do seaweeds and soft-bodied sessile invertebrates living in habitats where predatory fishes and invertebrates are abundant avoid being eaten?

How do scientists determine the ecological roles of natural products produced by marine organisms?

Are compounds that consumers "taste" and reject harmful (i.e., bad in the sense that they cause damage to the taster), or do they just taste nasty?

### Learning Objectives

Students will be able to describe and explain chemical defense strategies used by many marine seaweeds and sessile invertebrates to protect them from predation, ward off disease, help defend living space, and reduce the impact of environmental stresses.

Students will be able to describe and explain at least three ways in which chemicals produced by marine organisms can benefit humans.

Students will be able to describe a first-hand experience that demonstrates how chemical defenses can be effective at deterring predators.

### Materials

- ▶ Palatable Control Cookie (1 per student) – see recipe below
- ▶ Distasteful Treatment Cookie (1 per student) – see recipe below
- ▶ Satiation Control Cookie (1 per student) – use Oreo cookies
- ▶ Paper plates

### Audio/Visual Equipment

- ▶ (Optional) Internet-connected computer to view slide presentation showing examples of chemically-defended marine organisms, their secondary metabolites, and pertinent information

### Teaching Time

One 45-minute class period; allow for more time with in-depth data analysis and interpretation

### Seating Arrangement

Classroom style

### Key Words

Allelopathy  
Antifouling  
Chemical ecology  
Chemical defense  
Ecology  
Natural products  
Secondary metabolite  
Control  
Hypothesis  
Treatment  
Replication

### Background Information

*Aquarius* is an undersea laboratory owned by the National Oceanic and Atmospheric Administration (NOAA). Its purpose is to support research on oceans and coastal resources by allowing scientists to live and work on the seafloor for extended periods of time. *Aquarius* is presently deployed three and a half miles offshore in the Florida Keys National Marine Sanctuary. It operates 62 feet beneath the surface at Conch Reef. Missions typically last 10 days and aquanaut candidates undergo five days of specialized training before each mission starts. Visit <http://www.uncw.edu/aquarius/> for more information, including a virtual tour of the *Aquarius* laboratory.

*Aquarius* missions are focused on understanding our changing ocean and the condition of coral reefs. Ecologists strive to understand factors that influence the diverse and complicated interactions that occur among species, such as:

- predator-prey interactions;
- competitive interactions;
- microbial infections; and
- intra-specific interactions (i.e., between

individuals of the same species), such as mate location.

Terrestrial ecologists have long known that toxins and other compounds commonly produced by diverse plant and insect species play a large role in the dynamics of terrestrial communities; but until about 20 years ago, little was known about chemically-mediated interactions in the marine environment. As chemists began making discoveries of novel chemistry in marine plants and animals, they found that the most chemically-prolific marine species tended to be seaweeds and soft-bodied invertebrates that inhabit coral reefs. Given the great abundance of grazers and predators on coral reefs, as well as the intense level

of competition for limited bottom space, the chemists frequently speculated that the compounds they were isolating acted to deter predators and competitors. Several of these chemists began collaborating with marine ecologists to test these hypotheses through rigorous field and lab experimentation. These collaborations played a critical role in establishing the foundations of marine chemical ecology. You can view with your class the MS PowerPoint presentation on marine chemical ecology (Nutritional Boosts in the Marine Environment) and the related MS PowerPoint presentation on practical uses for marine natural products (Chemical Ecology). Both of these presentations can be found on the *Aquarius* web site at <http://uncw.edu/aquarius/education/lessons.html>.

We now know that some compounds produced by marine plants and animals help them survive the ecological battles that often exist among organisms. These chemicals can work in many different ways. They can deter potential predators, ward off pathogens, keep living space free from competing organisms, and even reduce the impact of exposure to environmental stresses, such as high levels of solar UV radiation. These chemicals can have an enormous impact on the outcome of interactions between species and, thus, also on the structure and function of communities and ecosystems. The study of these chemicals is known as natural products chemistry because plants and animals produce the chemicals.

In addition to their ecological importance, chemicals that affect the ecology of various organisms are the source of most modern medicines. Humans have lived with terrestrial plants and used them medicinally for thousands of years. Today, interesting results from ecological studies sometimes identify new chemicals with pharmaceutical potential

too. Extensive exploration of the oceans, their inhabitants, and marine natural products began only about four decades ago with the development of SCUBA and deep-diving submersibles. In this short time, marine chemists and pharmacologists have learned much about the compounds produced by marine organisms. Many are unlike any chemicals produced by terrestrial plants, both structurally and in their pharmacological profiles. The structural novelty of the marine compounds is what first compelled scientists to dive into the oceans to find chemically-rich marine species.

Collaborations between chemists and pharmacologists soon began to show that, in addition to their structural novelty, the marine chemicals affected crucial cellular processes in unique ways that provided new pathways to fight diseases like cancer, AIDS, and arthritis. Marine natural products have proven to be a rich source of new leads for the development of new drugs. Several marine compounds are now being given to small groups of patients to test the effectiveness and safety of these emerging treatments. Even though it often takes a decade or more and millions of dollars to complete the drug development process, new marine-based drugs will soon be added to your doctor's arsenal for fighting human ailments (see also the presentation on practical applications of marine natural products).

Chemical ecology research often uses advanced technology to purify bioactive natural compounds and to determine their molecular structures. To purify chemical compounds, scientists use a piece of equipment known as a High Pressure Liquid Chromatograph (HPLC). The HPLC processes complex mixtures of compounds dissolved in a carrier solvent by pushing them through a long, thin tube (or column) that contains another material that attaches to the chemicals of interest.

This attachment is not permanent, and different compounds stay attached for different amounts of time. As a result, individual compounds come out of the column at different times. This is the property of chromatography that allows chemicals to be separated and isolated for further analysis.

Once a compound has been purified and isolated, its molecular structure needs to be determined to evaluate its relationship to other compounds present in the source organism and to bioactive compounds present in other marine organisms. Structure "elucidation" typically involves measuring the 'spectral' characteristics of the molecules, including the wavelengths of ultra-violet (UV) and infrared (IR) radiation they absorb. Each compound has a unique pattern of absorbance that provides clues about its structure, much like a fingerprint helps identify individual people.

It is also important to determine the molecular weight of the isolated compound. This is typically done using a technique called mass spectrometry (MS). From the MS data, the molecular formula of the compound can be established.

Two powerful techniques used to further determine the structure of compounds are called Nuclear Magnetic Resonance (NMR) and x-ray crystallography. NMR is similar in principle to Magnetic Resonance Imaging (MRI), which is used to diagnose various human ailments in hospitals. NMR provides information on the micromagnetic environment of each atom in a molecule, which varies predictably among different functional groups that make up organic molecules and how these functional groups are bonded together. X-ray crystallography, which measures the dispersion of x-rays passing through the crys-

tal of a compound, can pinpoint the position of every atom in a molecule and thereby its complete structure. However, to use this technique, the chemist must be able to “grow” a crystal of the compound, which is not always possible. Chemical ecology thus requires partnerships between field biologists who are interested in how these chemicals influence the biology and ecology of organisms and chemists who use sophisticated laboratory equipment to isolate, identify, and sometimes even synthesize the chemicals brought to them by the field biologists.

Chemical ecologists have used *Aquarius* to carefully monitor feeding experiments under natural conditions and to conduct complex experiments that provide more accurate information about predator-prey preferences. The extended bottom time available by working from *Aquarius* makes it possible to rigorously test detailed hypotheses.

This activity is intended to introduce students to the field of marine chemical ecology and marine natural products.

### Learning Procedure

1. Prepare palatable “Control” and unpalatable “Treatment” cookies using the following recipes:

a. Palatable “Control” Cookie Recipe (alternatively, use your own favorite cookie recipe):

- 1 cup all-purpose flour
- ¼ teaspoon baking soda
- 1/8 teaspoon salt
- 5 tablespoons butter
- 7 tablespoons unsweetened cocoa
- 2/3 cup granulated sugar
- 1/3 cup packed brown sugar
- 1/3 cup plain low-fat yogurt
- 1 teaspoon vanilla extract

Cooking spray oil

Combine flour, baking soda and salt. Melt butter in a saucepan, remove from heat, and then stir in cocoa powder and sugars. Next, stir in yogurt and vanilla. Add flour mixture and stir until moistened. Form 1-tablespoon size scoops into a small circle and then place them on a baking sheet coated with cooking spray. Bake at 350° for 8 to 10 minutes.

Allow to cool on the baking sheet until firm (at least 2-3 minutes). Yields about 24 cookies.

b. Unpalatable “Treatment” Cookie Recipe

Same recipe as above, except add 1 teaspoon of salt. Each “treatment” cookie, if made according to this recipe, contains less salt than a hamburger from a fast food restaurant. Form each bit of dough for the individual cookies into a square before baking so that the cookies have a shape that will allow you to distinguish between the remains of control and treatment cookies. To use an ingredient other than salt to make cookies nasty tasting, try onion powder! A tablespoon of onion powder added to the above recipe should do the trick.

2. The data from your feeding assay are easily analyzed statistically by Fisher’s exact test. If you want to do this analysis, check with the mathematics/statistics department at your school to obtain a statistical analysis program to quickly run a Fisher’s exact test with your data. Find someone at the school or elsewhere who can also show you how to input your data into the analysis program. Fisher’s exact test can be calculated by hand, but it is time consuming to do so. You can find several web pages that are set up to calculate Fisher’s

exact test, quickly and easily. Search using the keywords, “Fisher’s exact test.” Make sure you run the sample data through the web site table to make sure you get the same answers.

### Sample Data for 15 Students

(All of Whom Ate the Oreo Cookie):

	Eaten	Rejected	% Consumed
Control	15	0	100%
Treatment	3	12	20%

The P value calculated from Fisher’s exact test for the sample data set is  $<0.0001$ , meaning there virtually no chance the observed frequencies for rejection of the treatment and control cookies would have occurred simply by chance. At P values of 0.05 and 0.01, there is a 5% and 1% chance, respectively, that the observed data sets would have been obtained in the absence of a treatment effect. So,  $<0.0001$  means the treatment effect is very strong.

- Review the basic concepts of scientific experimentation. A scientific experiment is one that addresses a hypothesis, and uses appropriate experimental methodologies to test the hypothesis. These experiments typically have “controls” to distinguish “treatment” effects, and sufficient levels of replication.

The “mouths-on” feeding activity mirrors the experimental methods used by marine ecologists to examine the effect of a chemical isolated from marine plants or animals on the feeding preferences of predators. In this activity, students are the test subjects and are offered equal opportunities to eat each of two cookies: a “control” cookie that tastes good and a “treatment” cookie

that is identical to the first one except that extra salt is added to the dough. The excess salt should make the treatment cookie distasteful to most students and thereby act as an analogue for a toxic or noxious-tasting compound produced by a marine organism.

- Conduct the mouths-on feeding assay as follows:  
Give each student in the class one control and one treatment cookie and then ask them to taste each one. Then tell the students to eat the ones they like.

When conducting feeding assays, scientists usually are not “grossed out” when a predator spits out a test food. However, teachers, and especially the students, probably would not enjoy seeing their neighbor spit out a bad tasting cookie.

Suppressing the desire to spit out a bad tasting cookie could skew the results of the feeding assay and needs to be avoided. To reduce this risk, pass out napkins with the cookies in case a student needs to discretely empty their mouth.

For each student, record whether or not he or she ate each type of cookie. If more than 25% of a cookie is left uneaten, score this as a rejection because some predators require a few bites to decide that a “prey” is inedible. Collect and discard any uneaten cookies. Then give students an Oreo cookie and tell them it is, in fact, an Oreo cookie, fresh out of the bag, and to eat it if they care to do so—but they do not have to eat it or even taste it. After giving the students a few minutes to eat the Oreo cookie, record for each student whether or not they ate it. The Oreo cookie serves as a “satiating control” to ensure that the

students were hungry and thus had motivation to sample and eat the control and treatment cookies during the actual test. Now it is time to examine the outcome of your feeding assay. Begin with the Oreo cookie data. Here you are making an untested assumption that all students like Oreo cookies. You could test this assumption the day before the assay by offering each of your students an Oreo cookie to see if they eat it—if most do not, find another cookie that most of the students will eat. This is important because you will disregard data from the control/treatment feeding experiment for each student that did not completely eat the Oreo cookie because you have to assume they were full during the experiment and thus not sufficiently motivated to eat either the treatment or the control cookie.

Summarize data on a chalk board, marker board, or overhead transparency. Tell students to score a cookie type as rejected if more than 25% of it was not eaten, and to disregard data from students that rejected the Oreo cookie. Have each student prepare a brief report analyzing the results of the experiment. You may want to have students use Fisher's exact test as described in Step #2.

5. Lead a group discussion of the results. Often in feeding assays with fishes, the fishes will attack and then spit out a food item several times before deciding to reject it. If a significant part of a food pellet is not eaten, although plainly visible to the fish, this is scored as a rejection. It is possible that students who ate a bad-tasting cookie are just not interested in eating anything else. This could also affect the use of the Oreo as a control. During the discussion of results, you should ask the

students who did not eat the Oreo cookie whether their choice not to eat it was the result of them not being hungry or whether it was the result of them being disgusted by the bad-tasting cookie and thus, not being interested in eating any more. Did they trust you that this was a real Oreo? Could one bad-tasting cookie affect interest in all other cookies? What might happen if you used a piece of candy as the control instead of a cookie? Discuss how this might affect feeding behavior of a fish or sea urchin.

### The BRIDGE Connection

[www.vims.edu/bridge/](http://www.vims.edu/bridge/) – Click on “Ocean Science” in the navigation menu to the left, then “Ecology,” then “Coral”

### The “Me” Connection

Have students write an essay on the importance of natural products to their own lives, and why preservation of complex natural systems, such as coral reefs, could be personally important.

### Connections to Other Subjects

English/Language Arts, Mathematics

### Evaluation

Reports prepared in Step #4 and participation in group discussions provide opportunities for assessment.

### Extensions

One interesting finding from studies of both terrestrial and marine chemical ecology is that it often takes higher doses of a chemical to deter feeding as the nutritional quality of the food increases, for example with higher protein content, because nitrogen is often a limiting nutrient for most consumers. With this in mind, the results of this lesson's activity can be discussed in the context of food type

vs. salt content. Above it was stated that each “treatment” cookie, if made according to the recipe, contains less salt than a hamburger from a fast food restaurant (this information expressed as mg sodium is currently available at [www.olen.com/food](http://www.olen.com/food)). Obviously, the amount of salt needed to make a cookie distasteful is far less than what most people crave in their hamburger. Determine the weight of 1½ teaspoon of salt and divide by the number of treatment cookies made. Then use your chemical knowledge to determine the mg of sodium per cookie. How do the cookie and hamburger compare? Although there is an obvious mismatch in the protein content of a cookie and a hamburger, there are probably also subtle, or not so subtle, interactions between flavors and taste perception that make a little salt in sweet cookies distasteful, while the same amount of salt might stimulate feeding on a hamburger. This comparison demonstrates that there is still much we do not know about the complex interactions between predators and their prey in the marine environment.

### Resources

<http://chemistry.org/portal/Chemistry?PID=acsdisplay.html&DOC=%5Ceducation%5Ccurriculum%5Cchematters%5Cdrugssea.html> – American Chemical Society web site

<http://www.science.fau.edu/drugs.htm> – An overview article on drugs from the sea

Faulkner, D. J. 2000. Marine pharmacology. *Antonie van Leeuwenhoek* 77: 135-145. Available online at [http://www.reefcheck.org/headlines/june/pdf/marine\\_pharmacology.pdf](http://www.reefcheck.org/headlines/june/pdf/marine_pharmacology.pdf).

[http://www.uncw.edu/aquarius/virtual\\_tour/ipix.html](http://www.uncw.edu/aquarius/virtual_tour/ipix.html) – Virtual tour of the *Aquarius* undersea laboratory

<http://www.marinebiology.org/science.htm> – Odyssey Expeditions web site, with lots of information about coral reef fishes and reef ecology

### 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**

- Interdependence of organisms
- Diversity and adaptations of organisms
- Behavior of organisms
- Structure and function of living things

#### **Content Standard E: Science and Technology**

- Understandings about science and technology

#### **Content Standard F: Science in Personal and Social Perspectives**

- Populations, resources, and environments

*Activity developed by Dr. N. Lindquist,  
University of North Carolina*