Teacher Version: Toxicity of Copper Ions to Yeast

This activity was developed by Dr. Susan Hershberger for the Fighting with Food project, a partnership between Miami University, the University of Cincinnati, and the University of Kentucky. The project is funded through the National Center for Research Resources and the Division of Program Coordination, Planning, and Strategic Initiatives of the National Institutes of Health through Grant Number R25 ODO01190-02. These materials have been developed and reviewed upon the advice of for the following research scientists at partnering institutions: University of Kentucky—Dr. Bernhard Hennig, Dr. Lisa Gaetke, and Dr. Lindell Ormsbee; University of Cincinnati: Dr. Kim Dietrich and Dr. Mary Beth Genter. Pedagogical review has been provided by Dr. Aeran Choi, Kent State University.

Background

One aspect of studying the effects of toxicants on human health is using test organisms that may model potential human responses. At the University of Kentucky, Superfund Research Program, scientists are using whole cells or spore responses to detect environmental contaminants, especially organic contaminants such as PCBs. A search for similar research using an organism that could easily translate to the classroom led to the work of a Superfund research group at UC Berkeley. This Superfund Research Program called *Functional Profiling of Susceptibility Genes* uses yeast (*Saccharomyces cerevisiae*) to investigate how genetic differences affect susceptibility to toxicant exposure in eukaryotic cells. The UC Berkeley research project focuses on toxic metals (e.g. copper, cadmium, iron), metalloids (e.g. arsenical compounds) and aromatic hydrocarbons (benzene and its metabolites), as well as pesticides and emergent contaminants (including flame retardants). Yeast are used as the test organism in this project because humans and yeast share fundamental genes and cellular pathways, hundreds of human disease genes also exist in yeast, yeast are susceptible to toxicants, and yeast are easy to use in the laboratory. (http://www.vulpelab.net/toxicant-susceptibility.html) This student laboratory, *Toxicity of Copper Ions to Yeast,* provides a hands-on opportunity to investigate one toxicant (copper) with yeast as the test organism. Copper is a useful metal: copper wires conduct electrical current, and copper surfaces store information in electronic devices. In our bodies and other biological systems, copper ions are found associated with some proteins and other biological molecules where the copper-containing protein is involved in signaling or other energy transformations. Thus, copper is an essential metal. A small (microgram) amount of copper is necessary in the diet. The wide distribution of small amounts of copper ions in soils and plants means that our need for copper is usually met through varied food consumption. Despite being an essential nutrient, copper ions in higher concentration can be toxic. One of the earliest food safety laws was passed in England to outlaw the addition of copper to canned peas or other products to improve their green color.

Copper sulfate is the form of copper used in the activity. This salt exists as a series of compounds that differ in their degree of hydration. Copper sulfate is the active ingredient in some products to control sewer line blockage, where the copper sulfate is a toxic deterrent to plant roots that might grow into the small space between pipes. Copper sulfate is also known as an algaecide and fungicide and can be added to ponds to inhibit the growth of fungi and algae. Copper sulfate is also the active ingredient in

Bordeaux spray used to control fungi in grapes and other fruit crops. While copper sulfate can be found for these plumbing and agricultural uses in some states, in other states it is outlawed, presumably to prevent copper ion accumulations in natural waters. In the following activity, students will explore at what level copper ions are clearly toxic to yeast.

Concept checklist

To help you plan for using this lesson in your classroom and potentially modify it to meet your needs, consider the following list of targeted concepts and note whether your students will be introduced to them for the first time or will be revisiting concepts they have been exposed to previously.

Targeted concept	Introducing	Revisiting
states of matter		
gas collection and measurement		
observations vs. inferences		
chemical reactions		
catalysts (enzymes)		

Materials

- active dry yeast, preferably rapid rise
- granulated sugar or corn syrup
- snack size zipper bags (to minimize leakage use Ziploc or other major brand)
- graduated cylinders or measuring spoons and cups
- copper sulfate (available as Copper (II) Sulfate 5-Hydrate from science supply companies such as http://sciencekit.com/ or can be purchased at a hardware or aquatic supply store as drain cleaner or as pond algae treatment)
- calcium chloride (as an alternative or in addition to copper sulfate, can be purchased as ice melter or as a brewing aid)
- warm water bath
- quart jar (for measuring water displacement)
- empty small soft drink bottles
- balloons

Safety

Copper sulfate is harmful if swallowed and irritating to eyes and skin. Students must wear goggles while handling the copper sulfate solution and observe all standard laboratory safety practices. Gloves are also recommended.

Getting Ready:

By preparing a stock copper sulfate solution that can be diluted in the class study, potential exposure is limited, as students will not be required to handle the solid material. Distribution of sugar into test bags or bottles is easier with a stock sugar solution. A 50% corn syrup and water solution was used during the development and testing of this activity.

- Prepare a stock 0.4M solution of copper sulfate by dissolving 1 gram $CuSO_4 \bullet 5 H_2O$ per 10 mL water. 30-40 mL of stock solution will cover 4-5 groups of students doing all 5-6 recommended experiments.
- Prepare a stock sugar solution for each group by diluting 60 mL corn syrup with 60 mL water to make a stock solution that is approximately 25% sugar in water. (120 mL of solution will cover 5–6 experiments requiring 20mL sugar solution each.)

Opening Activity and Demonstration:

To introduce the basic concept of yeast growth in a sugar solution, we suggest two things: giving the students an opportunity to "proof" yeast and making the demonstration bottle described below.

The idea of proofing yeast originally came about as a way of checking that the yeast was still active before adding it to an entire recipe and potentially wasting a lot of ingredients. Give each group of students 1 gram (1/4 teaspoon) or more of dry active yeast in a small bowl or cup. Have them carefully observe the dry yeast and then add about 15 mL warm water and about 1 teaspoon of sugar. Have students observe carefully as the water dissolves the dry coating around the granules of yeast, releasing the active yeast inside. Have students check the yeast mixture again in about ten minutes. They will see bubbly foam start to form on the surface from the carbon dioxide being released. This foam is proof that the yeast is active. Prompt the students to share what they know about the reaction of sugar and yeast, what the products are, and how might they attempt to quantify the reaction (measure how much the yeast grow under different treatments). The reaction equation is as follows:

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$

Making a demonstration bottle provides students with another way to look at the gas produced by the yeast. In a small empty soft drink bottle, mix 30 mL corn syrup and 30 mL water (or 15 grams granulated sugar with 45 grams water) with 1 gram (1/4 teaspoon) or more of dry active yeast. Stretch a balloon over the mouth of the bottle. Place in a warm water bath and leave where students can observe the slow inflation of the balloon. (Balloon inflation begins after about 20 minutes and is clearly obvious after 40–60 minutes.) We suggest assembling the bottle of yeast and sugar solution earlier in the day so that a visible amount of gas has been produced by class time.

Note: You may want to offer your students the chance to explore the properties of CO_2 either before continuing this lesson or at a later time. One option is using limewater to test for the presence of carbon dioxide gas. (See "Capturing Carbon Dioxide" from Terrific Science Press at http://www.terrificscience.org/downloads/NCW/NCW2007.pdf.) Another option is pouring CO_2 into a container in which a lit candle is positioned. You can find instructions in "The Burning Candle" from Terrific Science Press at http://www.terrificscience.org/lessonpdfs/BurningCandle.pdf.

Prompt students to compare the demonstration bottle with their proofed yeast and discuss the why the balloon has expanded. Ask students to consider how they could measure the amount of gas produced by the yeast. Prompt them to discuss factors that may make quantification difficult. For example, if using balloons to trap gas produced, what measurements of the balloons can be made? Can one assume that all balloons of the same basic size and shape will all inflate equally even if the amount of gas is exactly the same? How else could the gas be collected and measured?

Pre-Activity Discussion

The pre-activity brainstorming discussion outlined below is designed for instructors to engage students in understanding the goals of the investigation and possible approaches to doing the investigation. In cases where teachers use inquiry and facilitate student proposed and designed investigations, this discussion provides a starting point for that process. In cases where teachers supply students with a procedure for the investigation, this discussion incorporates some elements of student inquiry into the activity.

Introduce the idea of using yeast as a test organism to measure the impact of a toxicant. Engage students in a discussion of the variables in such an experiment, such as the amount of yeast, the amount of nutrients for the yeast, the type of toxicant used, and the concentration of the toxicant. Listing some of the variables and leaving space for adding others may help to emphasize the importance of this step. A good strategy here is to have each student write his/her ideas prior to small group or class discussion. This “brain drain” approach encourages all students to think, contribute to and be empowered by the discussion. Also discuss the expected outcome (variations in yeast growth) and how it could be measured. (Depending on your time constraints and the experience of your students, you may use this discussion to lead students to developing their own test procedures or use the provided procedure.)

Based on the opening demonstration, students will probably recognize that one way to measure the growth of yeast is to measure the volume of gas produced. They may suggest running the reaction in bottles and measuring the volume of the gas produced in a balloon by measuring the circumference of the balloon. Gas volume pipets, which some high schools may have, are another option for measuring the gas. Alternatively, the reaction might be run in snack size Ziploc baggies and the volume of gas measured by water displacement. This is the version we tested and developed.

An ideal concentration and reaction size for the snack size Ziploc baggies is one that produces observable results in a reasonable time but does not risk bursting open the bag. If students are developing their own procedures, you could ask them to find this ideal concentration and reaction size through trial and error as part of designing the experiment. However, in the service of time efficiency, we suggest that students developing their own procedures be informed that 25 mL of total test solution (20 mL sugar solution and water + copper sulfate solution totaling 5 additional mL) will visibly react with 1 gram of yeast (1/4 teaspoon of yeast) in a warm water bath in approximately 15 minutes.

Possible Procedure

The procedure outlined below is written to the teacher. In cases where teachers have students propose and design their own investigations, this procedure serves as an example for the teacher of a tested procedure that produces observable outcomes. While finding no observable outcome is a viable result in itself, and certainly may happen in student-directed investigations, having a tested procedure as *a reference point can help teachers facilitate discussions with students as they propose their own procedures. In cases where teachers supply students with a procedure for the investigation, the written procedure can be adapted for students to read directly.*

1. Measure ¼ teaspoon yeast or 1 gram of yeast into each of six snack size Ziploc bags.
2. Add the appropriate amount of copper sulfate solution to each bag. (5 mL, 1 mL, 0.5 mL, 0.10 mL, .05 mL, and none)
   Note: With the exception of 5 mL, these amounts are too small to measure with a graduated cylinder. Students will add these amounts in drops, as listed on the sample data table. Note using a standardized pipette or dropper is very important.
3. Add the corresponding amount of water to total 5 mL of liquid to each bag. (0, 4 mL, 4.5 mL, 4.9 mL, 4.95 mL, and 5 mL).
   Note: For practical purposes, when using a graduated cylinder, 4.9 and 4.95 mL will measure as 5 mL. This is fine.)
4. Add 20 mL of stock sugar solution to each bag.
5. Push as much air as possible out of each bag and seal completely.
   Note: The air is easily pushed out by laying the bag flat on the table with just the zipper portion slightly lifted to prevent the liquid from leaking out and carefully closing the zipper.
6. Place all bags into a warm water bath to hasten the reaction. (Depending upon classroom time, the reaction may have to be assessed before the completion of the reaction is approached.)
7. Qualitatively assess the volumes of gas by observing the bags and ranking them from most to least full.
8. Option: Quantitatively assess the volumes of gas produced by using a ruler to measure the thickness of each bag when laid flat on a table.
9. Option: Quantitatively assess the volumes of gas produced through the displacement of water.
   a. Begin with a quart jar completely filled with room temperature water that is placed in a larger diameter dish or container. Carefully lower the partially inflated snack bag into the jar of water allowing the displaced water to flow into the outside dish. Push the bag completely below the surface with a flat hand, taking care not to immerse the fingers.
   b. Remove the snack bag from the jar and carefully measure the volume of water displaced into the dish by pouring the water into an appropriately sized graduated cylinder or other graduated volume measuring device. Be sure to record the concentration and results of each experiment in an appropriate data table. To calculate the amount of gas produced, subtract the "base" volume (bag + solution) from the final volume. (A bag where copper ion has completely inhibited the reaction may be used for the base volume.)

Sample data table

Bag	Drops of 0.4 M Cu^{2+}	Volume of 0.4 M Cu^{2+}	Volume of H_2O	Volume of stock	Resulting concentration	Qualitative Result	Initial Quantitative	Final Quantitative

				sugar solution	of Cu^{2+} ions in each reaction mixture**		Result Volume of snack size bag	Result Volume of bag minus bag plus solution
1	0	0	5 mL	20 mL	0	Bag inflated nearly completely	285 mL	285-25 mL= 260 mL
2	1	0.05 mL	4.95* mL	20 mL	0.00080 M			
3	2	0.10 mL	4.9* mL	20 mL	0.0016 M			
4	10	0.5 mL	4.5 mL	20 mL	0.080 M			
5	20	1.0 mL	4.0 mL	20 mL	0.016 M			
6	100 (5 mL)	5.0 mL	0 mL	20 mL	0.08 M			

M = molar = moles/liter   *an actual measurement of 5.0 mL is fine   **High school level students should calculate these values

Post-Activity Discussion

The post-activity discussion is designed to help the teacher facilitate student learning as students summarize their observations and make claims about the outcome of the investigation using their data as evidence. Whether students use a provided procedure or have designed one of their own, this discussion incorporates key components of inquiry-based learning into the lesson.

What claims can you make about the toxicity of copper ion to yeast? How strong is the evidence for copper ion being toxic to yeast? How reasonable is the data from your investigation? Do your results fit with the results found by other investigators in your class? How can the results of other students help your interpret any unexplained results in your data? What conclusions can you draw about the level at which copper ion is toxic to yeast? Very small amounts of copper are required in the human diet. Do you think copper a small amount of copper may be required for optimal yeast growth? Why or Why not? How might you or other researchers determine if copper is an essential nutrient for yeast?

Molar Calculations

For high school students, we suggest you use this activity to give them practice calculating the resulting concentration of Cu^{2+} ions in each reaction mixture.

Resulting concentrations can be calculated from $M_1V_1 = M_2V_2$ where M_1 and M_2 are molar concentrations and V_1 and V_2 are volumes of solution in liters. M_1 = 0.4 M (0.4 moles/liter), the concentration of the stock copper sulfate solution. V_1 = the amount of 0.4 M solution in a given test. M_2 is the unknown molar concentration of Cu^{2+} ions in the reaction mixture. V_2 is the final volume of the reaction mixture, which is 25 mL (.025 L) for each test.

For example, for bag #6:

$M_1 \quad V_1 \qquad\qquad = \qquad M_2 \quad V_2$

(0.4 M)(.005 L)	=	(x M) (.025 L)
$\frac{(0.4\ \text{M})(.005\ \text{L})}{(.025\ \text{L})}$	=	$(x\ \text{M})\ \frac{(.025\ \text{L})}{(.025\ \text{L})}$
(0.4 M)(.2)	=	x M
0.08M	=	x

Or, another way to write the same thing:

(0.4 moles/liter) x (.005 liter)	=	(x moles/liter) (.025 liter)
.0020 moles	=	(x moles/liter) (.025 liter)
$\frac{.0020\ \text{moles}}{.025\ \text{liter}}$	=	$\frac{(x\ \text{moles/liter})\ (.025\ \text{liter})}{.025\ \text{liter}}$
0.08 moles/liter	=	x moles/liter

Calcium Chloride (Ice-Melt) as an Alternate Toxicant to Copper Sulfate

You may want your students to investigate calcium chloride is an alternate to copper sulfate or as an additional model toxicant. Like copper, calcium is essential in the human diet in appropriate quantities. Calcium chloride, $CaCl_2$ is widely known for its ability to de-ice and prevent ice formation on road surface. Calcium chloride may contain zero, one, two, four, or six water molecules in its hydration sphere. It is considered an irritant and can also burn skin when its less hydrated forms become more hydrated and draw water from the skin surface. Calcium chloride is currently listed in the Generally Regarded as Safe (GRAS) list of food additives where it is an added electrolyte in sports drinks and water, and has been used in pickling, preserving, brewing, and cheese production.

Compared to copper sulfate, larger amounts of calcium chloride are required to observe potential toxic effects on yeast. The greater concentration of calcium and chloride ions may inhibit yeast growth through dehydration (where the solution is hypertonic with respect to the cell solution) in addition to specific calcium or chloride ion toxicity. In our tests, the source of calcium chloride tested was ice melt or the brewing aid, so the level of hydration was not known. We tested a stock calcium chloride solution of 8 grams of calcium chloride dissolved in 10 mL of water. Since the hydration of the calcium chloride is unknown, but the material was not stored to prevent absorption of water, we assumed that it was approximately a mixture of 50% tetrahydrate and 50% hexahydrate, which would provide a solution concentration of 4 M (moles/liter) — ten times more concentrated than the previously described copper sulfate solution. This calcium chloride stock solution was added to each reaction bag using the same scheme as described previously for copper sulfate.

Sample data table for Calcium Chloride

Bag	Drops of 4 M Ca^{2+}	Volume of 4. M Ca^{2+}	Volume of H_2O	Volume of stock sugar solution	Resulting concentration of Ca^{2+} ions in each reaction mixture**	Qualitative Result	Initial Quantitative Result Volume of snack size bag	Final Quantitative Result Volume of bag minus bag plus solution
1	0	0	5 mL	20 mL	0	Bag inflated nearly completely	285 mL	285-25 mL= 260 mL
2	1	0.05 mL	4.95* mL	20 mL	0.0080 M			
3	2	0.10 mL	4.9* mL	20 mL	0.016 M			
4	10	0.5 mL	4.5 mL	20 mL	0.08 M			
5	20	1.0 mL	4.0 mL	20 mL	0.16 M			
6	100 (5 mL)	5.0 mL	0 mL	20 mL	0.8 M			

M = molar = moles/liter *an actual measurement of 5.0 mL is fine **High school level students should calculate these values