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LETTERS

I recently came across an article called "Why is that dog paralyzed?" in the January 2013 issue of the *ABT* (Milanick et al., 2013). This is a problem-based learning activity that is recommended for AP Biology teachers. As a neuroscientist, a former college professor, and the Biology Staff Scientist at Vernier Software & Technology, I have some serious reservations about recommending this exercise to high school teachers.

The concept behind the activity is excellent, but this exercise uses the compound acetylthiocholine, which is toxic, and the article provides no safety precautions for the teacher or the students. I read through the appendix at the website provided by the authors and did not find any safety precautions there, either. Although most colleges have a chemical safety officer who oversees safety and disposal of toxic compounds, many high schools do not have a person on staff who knows that the compounds used in this assay are toxic. The teacher is going to be handling acetylthiocholine powder, as well as DNTB powder when mixing up stock solutions. The students are going to be handling these solutions. Acetylthiocholine is a substance that high school students should not use without taking proper safety precautions (goggles and gloves).

The enzyme assay used in this exercise (for the enzyme acetylcholinesterase, AChE) is commonly performed in many upper-division neuroscience courses. At Vernier Software & Technology, we have published an activity that uses the reagents presented in the article (this experiment can be found in "Introduction to Neurotransmitters using AChE" in the *Advanced Biology with Vernier* lab book). We provide the safety information for the chemicals in the teacher information pages. The Safety and Hazard Information for the two compounds used for the AChE activity are listed below.

> **Acetylthiocholine iodide:** Hazard code T, Toxic. Respiratory, skin and eye irritant; May be harmful if inhaled, toxic by ingestion, harmful if absorbed through skin. Wear gloves and eye protection and appropriate full face particle respirator (N99 or equivalent).
>
> **5,5′-Dithiobis(2-nitrobenzoic acid), (DTNB), (Ellman's Reagent):** Hazard code Xi, Irritant. Respiratory, skin and eye irritant; May be toxic by ingestion, inhalation, absorption through skin. Wear gloves and eye protection and appropriate dust mask (N95 or equivalent).
>
> Hazard information reference: Sigma-Aldrich, MSDS, 800-325-5832, http://www.sigmaaldrich.com

Standard safety precautions should be taken when performing this activity and when mixing up solutions for this lab. The person (teacher) mixing up the stock solutions should wear eye protection, gloves, and a respirator, in addition to a lab coat, since they will be working with the compounds as solids. The students performing the activity should wear eye protection and gloves, in addition to a lab coat.

Given the safety requirements for using acetylthiocholine, the instructor may want to consider mimicking the AChE reaction when performing the "Why is that dog paralyzed?" activity from the January issue of the *ABT* (Milanick et al., 2013). This is an excellent problem-based learning activity, but it does not appear to be designed to teach students how to perform quantitative enzyme assays or how to perform antibody assays. For example, in the first part of the exercise, fish muscle samples are added to test tubes to make the results of the AChE reaction fit the story line. In the second activity, turmeric is used to mimic the results of a fluorescent antibody reaction. Mimicking the data for both parts of the activity may be a safer and simpler alternative to performing the AChE reaction.

It would be very simple to mimic the AChE reaction. You just need a solution that will turn yellow in color after adding another solution to it. For example, Bio-Rad Laboratories has a very nice BioFuel Enzyme kit that uses p-nitrophenol as a standard. This compound turns yellow when it is mixed with the stop solution found in the kit. The teacher can easily make solutions that "mimic" the AChE reaction by following the steps below.

1. Label two 50-mL bottles Positive and Negative.
2. Put 10 mL of deionized water into each bottle.
3. Add 2 mL of standard + 8 mL of deionized or distilled water to the bottle labeled Positive and mix.
4. Add 10 mL of deionized water or distilled water to the bottle labeled Negative.
5. Mix up the stop solution as directed in the instructions for the BioFuel Enzyme kit.

The teacher can easily make up a series of solutions that can stand in as negative or positive AChE reactions. Simply substitute the solution from the bottle labeled Positive for the fish homogenate in the "Why is that dog paralyzed?" activity. To watch the reaction turn yellow, have the students put 2 mL of the solution from the bottle labeled Positive in a test tube. Then have them add 2 mL of stop solution to the test tube. For negative reactions, have the students put 2 mL of the solution from the bottle labeled Negative in a test tube. Then add 2 mL of stop solution. The instructor can always split the samples into test tubes that are labeled accordingly

so that the students have samples for Normal Dog (Positive), Coon Hound Disease (Positive), Pesticide Exposure (Negative), and Coon Hound Disease with Pesticide Exposure (Negative). The stop solution is basic. Make sure that students take proper safety precautions as described in the manual for the BioFuel Enzyme kit.

In any case, as a former educator and current curriculum provider, I was rather concerned that this activity was recommended for AP Biology without providing safety precautions. I would recommend that high school teachers "mimic" the AChE reaction for this exercise.

Reference

Milanick, M., Graham, K. & Wessel, M. (2013). Why is that dog paralyzed? A problem-based case & laboratory exercise about neuromuscular transmission. *American Biology Teacher*, *75*, 36–39.

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Correction

Please note that the caption for the Wood Frog in the January 2013 *ABT* should have read: "At the time of capture it was 2.8 cm (and not mm) from snout to vent and weighed 1.55 g."

Roy Rea
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I wanted to call your attention to a pretty significant mistake in the article "A Socratic Method for Surveying Students' Readiness to Study Evolution" in the February 2013 issue of the *ABT* (Stansfield, 2013).

On page 103, right column, List A, number 1, the author states, "Mitosis in humans normally produces haploid gametes, of variable genetic composition, by at least three processes." And then on page 104, left column, List B, number 1, the author states, "This statement is true." I believe he has mistaken mitosis for meiosis.

As David Kirk, a colleague of mine, mentioned to me when noticing this mistake, "Students do not need any help from the instructor to get confused about mitosis versus meiosis. They manage this quite well on their own, thank you!"

Reference

Stansfield, W.D. (2013). A Socratic method for surveying students' readiness to study evolution. *American Biology Teacher*, *75*, 102–105.

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