

Laboratory Safety and Scientific Method

1. Pre-Lab Reading

Be sure to complete the following reading assignment before lab:

Chapter 1 Section 1 (1.1) “Scientific Method” [Biology2e](#), OpenStax College

Chapter 2 "The Chemical Foundation of Life" [Biology2e](#), OpenStax College

Chapter 3 Section 3 (3.3) “Lipids” [Biology2e](#), OpenStax College

2. Purpose and Objectives

The purpose of this lab is to:

1. Introduce laboratory safety and general lab procedures.
2. Become familiar with the steps of the scientific method.
3. Become familiar with the general structural features and chemical properties of lipids.
4. Introduce carbon compound structure and notation.

Upon completion of this investigation, you should be able to:

- follow proper laboratory safety and general laboratory procedures.
- explain the role of positive and negative controls in experimentation.
- write a hypothesis and prediction for an experiment.
- construct a table that portrays the results of an experiment.
- perform a qualitative test that can be used to detect lipids.

3. Laboratory Safety

General Lab Safety Guidelines

- Follow your instructor's safety guidelines as described for each lab.
- **NO FOOD OR DRINK IN LAB!!**
- Store book bags and other items in the storage cubes provided.
- Only wear shoes that cover your **entire** foot (NO SANDALS OR SLIDES).
- Wear long pants and a shirt that is long enough to reach the pants and/or a safety apron.
- Wear goggles and use care when handling glassware and chemicals. Report all accidents, including minor ones, to your instructor immediately.
- **WASH YOUR HANDS AS SOON AS YOU LEAVE CLASS!**

4. General Laboratory Procedures

Stock containers: It is not proper laboratory procedure to return stock solutions or solids to their containers because this is likely to contaminate the entire stock. Check with your instructor regarding proper disposal if you accidentally pour out too much of a reagent.

Cross contamination: Pipettes, graduated cylinders, spatulas, and weigh boats should be used for only one stock. If you cross contaminate materials, you put not only your own experiment at risk but also the work of your classmates.

Water: There are two types of faucets that will provide the water needed for labs. One contains tap water and the other deionized water. Be sure you know which is which and check your lab instructions before filling containers.

Clean Up: At the end of a lab, dispose of materials and clean glassware and as directed by the instructor. Make sure all equipment is put back where it belongs. Clean your bench using wipes or paper towels.

5. Scientific Method Background Information

The scientific method will serve as a general guideline for conducting experiments in Biology lab. The scientific method consists of the following steps (Hillis).

Making observations: The first step is simply to examine the situation. For example, if you wanted to study pond life you might catalog the organisms present and count how many of each you find. You might also use instruments such as a thermometer to measure water temperature. Some of the labs that we do will simply consist of observations.

Asking questions: Observations often raise questions. For example, you might ask why a particular species of fish is present in one pond but not in a nearby one.

Forming hypotheses: A hypothesis is a tentative answer to a question (Hillis). You might hypothesize that a fish species is present in pond A but not in pond B because the temperature differs between the two ponds.

Making predictions: The next step is to predict the outcome that you expect when you perform an experiment or make additional observations. Based on the example above you might predict that the fish species observed will be present in ponds with a temperature that matches pond A but not in ponds that have a temperature like that of pond B.

Testing the predictions: Finally, design an experiment to test your hypothesis. The experiment should be constructed so that it will determine if your predictions are correct. The best hypotheses are falsifiable, that is, they can be proven to be incorrect if your results do not match your prediction. For the pond example you might observe fish presence and temperature in a number of ponds. If the fish is present in ponds that have a variety of temperatures, then this disproves your hypothesis. If the fish is only present in ponds with a temperature that matches pond A, then the results of your study support your hypothesis. **Note: Scientists generally will not say that a result proves a hypothesis.** The hypothesis must always stand up to further experiments and observations.

Variables: A number of factors can affect the outcome of an experiment. These factors are called variables. In an ideal experiment all variables except one are held constant. One variable, called the **independent variable**, changes from sample to sample. For the example above the independent variable is the water temperature of the ponds sampled. The **dependent variable**

changes depending on the value of the independent variable. For this example, the dependent variable is the number of fish of a particular species that is present in each pond. If the hypothesis is supported, then this number will vary depending on the temperature of the ponds.

6. Background Information for Lipids

Lipids are a diverse group of molecules composed primarily of carbon and hydrogen with a small amount of oxygen. Lipids are soluble in non-polar solvents such as acetone or ether but are insoluble in polar solvents such as water. Due to their limited ability to interact with water, lipids are described as hydrophobic molecules. The most important types of lipids are waxes, fats, phospholipids and steroids.

Fats (triglycerides) are constructed from two different molecules: glycerol and fatty acids. Fats have different properties depending on what types of fatty acids are bonded to the glycerol molecule. Fatty acids can differ both in the length of the hydrocarbon tail (normally between 16 and 22 carbon atoms in length) and in their degree of **saturation**. Saturation refers to how many hydrogen atoms are bound to the carbons in the hydrocarbon tail of a fatty acid. A **saturated fatty acid** has the maximum number of hydrogen atoms bound to the carbon atoms of the tail. In **unsaturated fatty acids** some of the hydrogen atoms are removed, allowing double bonds to form between carbon atoms. Fats containing mostly saturated fatty acids are generally solids at room temperature while those containing unsaturated fatty acids are usually liquids at room temperature and are often referred to as oils.

Phospholipids are important constituents of cell membranes. They are similar to fats but have a phosphate-containing group attached to glycerol in place of a third fatty acid. Phospholipids are **amphipathic** molecules, meaning they have both **hydrophobic** and **hydrophilic** regions. The tail, composed of fatty acids, is hydrophobic while the head region where the phosphate group is bound to glycerol is hydrophilic. Diversity in phospholipid molecules arises not only from variation in the fatty acids used in their construction, but also in the chemical groups that can be bound to the phosphate group.

7. Experiment 1: Lipids

Safety Guidelines for Lipid Lab

- **Wear safety goggles.**
- Do not taste any of the reagents or foods used in the lab.
- Work in the fume hood. Keep the dye tank covered.
- Wear **gloves** when handling the dye solution.

Sudan IV is a dye that is soluble in lipids but not in water. When lipids are present, Sudan IV will form a pink/orange/ red color.

You will work in groups of two during the investigation. In the first part of the exercise, you will create **positive and negative controls** for the qualitative tests you will conduct. A positive control gives a positive result for the presence of the substance being tested. For the lipid test you will create a positive control by using vegetable oil, which contains lipids. When the proper indicator is added, a color change will occur indicating the presence of lipid (a positive result). A negative control provides a negative result. When the indicator is added, no color change should be observed (a negative result). Deionized water is used for the negative control.

Record all results and observations in your lab book. Model your data tables after the one provided by your instructor. If the containers for the foods being sampled are available, check the labels to see if lipid is likely to be present.

Procedure

1. Prepare your lab notebook by recording the **lab title, date, lab section,** and your **lab partner's name.** Write a **purpose** and a **procedure** statement. Remember that the procedure statement refers to the appropriate pages in the lab manual. See the "Lab Assignments and Expectations" pages for more information.
2. Choose 2 foods to test. These should be two different versions of the same food. For example, two kinds of milk or two kinds of potato.

3. Write a **hypothesis** and **prediction** for your experiment. Record these in your lab notebook. For a simple experiment like this, the hypothesis is a guess as to whether each food will contain lipids. The prediction tells what results you expect if your hypothesis is true, i.e. what color you expect the samples to be at the end of the experiment. You may combine your hypotheses for both foods into one sentence. Combine your predictions for both foods into another sentence. **Label these as “Hypothesis” and “Prediction”.**
4. Read the instructions for constructing and labeling graphs and tables in the syllabus. Construct a table in your lab book for recording your results. The table should have 5 rows (one column label, two controls and 2 samples) and should include the following columns: Food Tested (list food type, not just number), Initial Color, Final Color (record the color of the samples before and after the experiment), and Lipid Presence (record a + if lipid is present and a – if it is not). **Don’t forget to include a title.**
5. Place one piece of filter paper on clean paper towels.
6. Using a **pencil** (pen ink will dissolve), draw a line dividing the paper into quarters. Label one section “water” (negative control) and another section “oil” (positive control). Label the others to identify the food samples you are testing.
7. Place one drop of water in the “water” section and one drop of vegetable oil on the “oil” section. Add one drop of each food sample to the other sections. For thick or solid foods rub a sample of the food on the paper and let it sit for at least one minute. **Be sure to scrape off as much solid material as you can before proceeding with the next step.** Set the paper aside to dry.
8. Wearing gloves and goggles, place the filter paper in a dish filled with Sudan IV solution and stain for 3 minutes. Work in the fume hood and keep the dish covered.
9. After the 3 minutes are up, use the large tweezers to remove the filter paper and place it into a bowl filled with deionized water. Allow it to sit for 1 minute.

10. Remove the filter paper from the water and place on paper towels. Observe the color of the spots. Compare your results to the positive and negative control samples. A dark red/orange spot indicates a positive test for lipids. Record your observations in your lab notebook.

8. Chemical Composition of Biological Molecules

Living things are composed of cells which are, in turn, composed of chemical entities such as atoms and molecules. Cells contain a variety of atoms but some of the most common are carbon, hydrogen, oxygen, nitrogen and phosphorus.

Many molecules found in cells are carbon compounds. These are described as "**organic**" compounds. Carbon is key to the chemistry of life largely because it can form four bonds with other atoms. Thus, carbon atoms can form various chains, branched structures and even ring structures as carbon atoms form bonds with each other and with other types of atoms.

Some molecules are described as **hydrocarbons**. These are composed of only carbon and hydrogen. An example is octane, which is found in gasoline. Octane is a chain of eight carbon atoms plus enough hydrogen atoms so that each carbon atom in the chain is bonded to four other atoms.

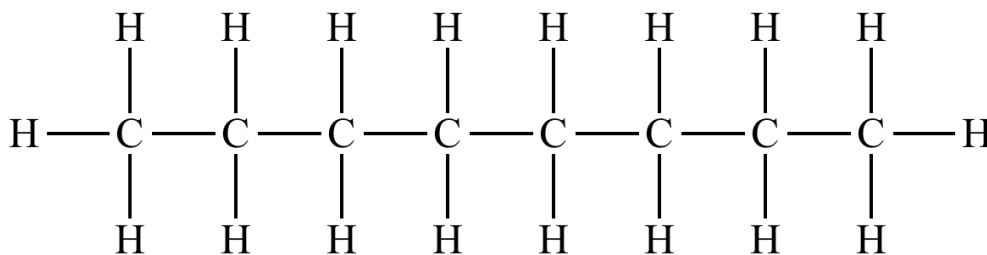


Figure 1: Octane Structure; <https://www.chem.ucla.edu/~harding/IGOC/O/octane.html>

Many organic molecules also have other atoms attached to the carbon/hydrogen backbone structure. These are referred to as **functional groups**. Common functional groups include

hydroxyl groups (OH) and amino groups (NH₂). An example is ethanol, which contains a hydroxyl group:

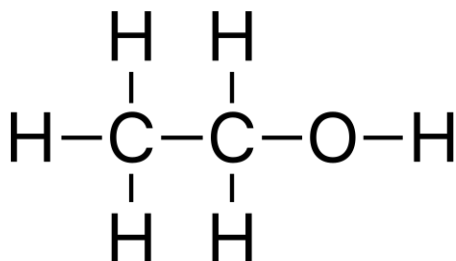


Figure 2: Ethanol, Wikimedia Commons

Structures of carbon molecules can be written as shown in figures 1 and 2 for octane or ethanol where each line represents a bond between two atoms. It is common, however, for these structures to be written in shorthand or line form as shown below for ethanol.

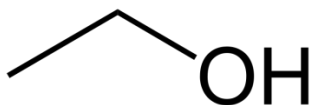


Figure 3: Ethanol, [Bryan Derksen](#), Wikimedia Commons

Here carbon and most hydrogen atoms are not shown. Each line represents a bond between atoms and the open ends and intersections of these lines represent carbon atoms. Each carbon is assumed to be bonded to the proper number of hydrogen atoms.

In text these structures are often described by their **molecular formula**. Octane is written C₈H₁₈; ethanol is C₂H₆O.

While there are a variety of carbon compounds in cells, some of the most abundant fall into four categories described as **macromolecules**. These relatively large molecules are classified into the following groups: lipids, carbohydrates, proteins and nucleic acids.

Lipids, as described above, contain primarily carbon and hydrogen along with a small amount of oxygen (CHO). Carbohydrates are also composed primarily of these atoms but generally contain more oxygen (CHO). All proteins contain carbon, hydrogen and oxygen but also nitrogen (CHON). The last type of macromolecule is nucleic acids. These contain carbon, hydrogen, oxygen and nitrogen plus phosphorus (CHONP)

Next week we will continue our study of organic molecules with special attention to carbohydrates and proteins.

Procedure

1. Write the names of the atoms represented by the atomic symbols listed below in your lab notebook:

C

H

O

N

P

2. Write the structure of propane, C_3H_8 , in complete form (all atoms shown) **and** shorthand/line form in your lab notebook. Remember that each carbon will be bonded to four other atoms.
3. Match (in your lab notebook) the four types of macromolecules with their chemical composition as listed below:

CHO

CHO

CHON

CHONP

9. Conclusion and Summary

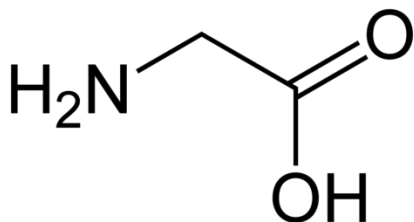
Write a conclusion and summary for your investigation that addresses the following questions.

Please number your answers.

1. Write a conclusion regarding your hypotheses for the lipid experiment.

Example: Suppose that your hypothesis states that potato flakes contain lipid. If you saw the predicted red color change in the potato sample for the lipid test, then this result **supports** your hypothesis. If you did not see the predicted color change then this result **does not support** your hypothesis.

2. If your positive control (oil) fails to change color in the lipid experiment then what can you conclude about your results?
3. Were any results from the lipid experiment unexpected? Explain. Are you confident in the validity of your results? Note: this should not be just a "yes" or "no" answer.
4. What are at least two possible sources of error that could have affected your results in the lipid experiment? Please don't just say "human error"; be specific in describing sources of error.
5. Both lipids and carbohydrates are composed primarily of carbon, hydrogen and oxygen (CHO). How do their compositions differ?
6. Below is the structure of glycine, an amino acid found in proteins. What atoms are found in the structure of glycine?



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<https://commons.wikimedia.org/w/index.php?curid=1738773>

Sources:

Principles of Life, 3rd edition (2019), Hillis et al. ISBN 978-1-3190-1771-2.

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