

## Course Work - Toxins

### Part A – Movies

The environment we live in is made up of many thousands of different chemicals. These are required for the cellular functions of plants, animals, and microorganisms. Without chemicals, life would not exist.

#### Watch 'Modern Chemistry: An Introduction' and 'The Perception of Cancer: Do Pesticides Play a Role?'

1. Give two reasons why our society has developed the notion that all chemicals are "bad" and that "natural" things do not contain chemicals.
2. Ames's cancer research began about 25 years ago over a bag of potato chips. Ames was reading the ingredients on the bag. It struck him that no one knew what each chemical did to human genes, and there was no easy way to find out. Give the name of the chemical found in potato chips that shows potential for causing cancer.
3. According to Ames why would it be extremely difficult to totally reduce our exposure to carcinogens by banning certain carcinogenic man-made chemicals?
4. What are some concerns that Ames has with animal cancer testing?
5. What is the significance of the numbers 60 % and 99 %? Have most chemical tests for carcinogenicity been carried out on natural or synthetic chemicals?

### Part B - The Ames Test

#### Read 'Part B' (on next page) and then answer the following questions:

6. Use the 'Virtual Ames Test' to test various chemicals for carcinogenicity, do not forget to use an experimental control. Record your results.

Hint: you will need to remove the effects of spontaneous mutants by choosing the correct starting number of bacteria ( $10^5$ ). Use this number for all experiments. The only variable in your experiments should be the chemical that is being tested.

7. Are there flaws in using the Ames test to predict carcinogenicity?
8. Should we base our choices about which foods to eat on the Ames test?
9. If a major pesticide was very mutagenic in the Ames test, would you advocate that farmers not use it?
10. If your favorite food was very mutagenic in the Ames test, would you stop eating it?

## Part B – The Ames Test

The Ames test is based on the following theories and assumptions:

- Normal development of animals and other multicellular organisms depends on the control of cell growth. Cancer is the uncontrolled proliferation of cells in animals.
- Cancer is often caused by a genetic change in the cell.
- Genetic changes in cells can be induced by chemicals that alter DNA, causing mutations.
- DNA is the universal genetic material in plants, animals, and microbes. Therefore, the ability of a chemical to cause mutations in the DNA of a bacterium may be used to predict its ability to cause mutations in human DNA, which, in turn, may be correlated with its potential to induce cancer.

### Some definitions that may be useful for this experiment

**Streptomycin:** the antibiotic that provides the selection; it differentiates strains of bacteria that are sensitive (do not grow on streptomycin) from those that are resistant (grow on streptomycin).

**Mutagen:** a chemical (or a physical phenomenon, such as X-rays) that causes changes in the sequences of bases in DNA; exposure to a mutagen will increase the frequency of mutants in a population.

**Mutagenicity:** the potential of a chemical for causing mutations or changes in DNA.

**Mutant:** an organism that differs genetically from its parent; in this case, bacteria that are resistant to streptomycin.

**Spontaneous mutant:** a mutant that arises from random changes in the DNA; changes can be caused by mistakes during DNA replication, damage from X-rays or ultraviolet light.

**Frequency of mutants:** the proportion of the population accounted for by mutants; for spontaneous bacterial mutants, the frequency is typically one mutant for a given trait in every million to 10 million cells.

**Carcinogenicity:** the ability to cause cancer in animals.

### Key Concept

The Ames test is based on the assumption that mutagenicity is associated with carcinogenicity and that mutagenic activity in bacteria is predictive of mutagenic activity in humans.

### Your Challenge

**Mutations in DNA appear randomly in a population of bacteria.** A chemical mutagen *increases the frequency* at which mutations appear. The Ames Test measures the ability of a chemical to increase the rate of mutation in bacteria. The mutations are detected by measuring a characteristic that results from changes at the gene level, such as the ability to grow under certain conditions.

**In this lab simulation, you will conduct a modified Ames Test.**

**You will be provided with:**

- **A virtual strain of *Escherichia coli* that is sensitive to (killed by) the antibiotic streptomycin.** Natural cultures contain rare mutants that grow in the presence of the antibiotic because of a small change in their DNA that makes them resistant to the action of the drug. You can choose the number of bacterial cells (from 0 – 10) that you wish to incubate.
- **Several virtual chemicals ("Test Substances") to test for mutagenicity.** Chemicals that cause mutations should increase the frequency of streptomycin-resistant mutants in a population of cells.
- **Two types of media.** Nutrient Agar and Nutrient Agar + Streptomycin. Nutrient Agar is a general medium that allows many culturable bacteria, including *E. coli*, to grow well.
- **70 hours of incubation time at 28 °C.**
- **Endless amounts of new media – just click on the "Reset" button.**

**To run the experiments:**

**Install JAVA:** <http://www.java.com/en/download/index.jsp>

**Experiment:**

<http://profhorn.meteor.wisc.edu/wxwise/kti/bacteria/experiment/spudswin.html>

Adapted from Handelsman, J. (2002) *Biology Brought to Life Laboratory Guidebook*. McGraw Hill, New York.