

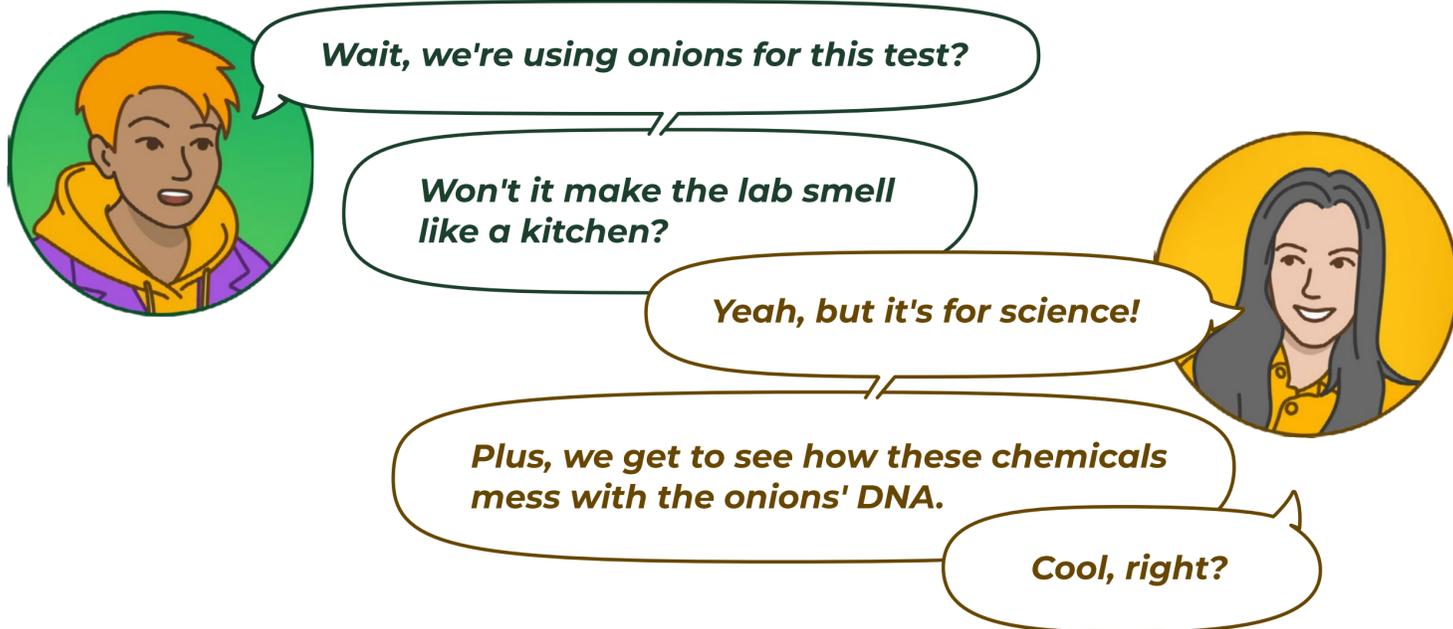
# ALLIUM TEST

## Investigating the Impact of Cosmetic Chemicals on Onion Growth and its DNA

### BACKGROUND

The Allium test is a biological method used to assess the potential toxicity of chemicals in the environment. While chemical analyses provide quantitative information about pollutants, biological tests reveal their effects on organisms, offering insights into cytotoxicity and genotoxicity. **Cytotoxicity** refers to a substance's ability to damage or destroy cells, while **genotoxicity** affects genetic material (DNA) and may lead to cytotoxic effects.

Onions, scientifically called *Allium cepa* L., can serve as a bioindicator - an organism that helps assess environmental conditions, such as pollution or toxicity, by showing measurable biological responses. The test itself is therefore called the Allium test.



The Allium test is quick, cost-effective, sensitive to low chemical concentrations, versatile in assessing both cytotoxicity and genotoxicity. It is non-invasive, widely used for environmental monitoring, and provides clear observations of plant chromosomes.

The test works by exposing onion bulbs (*Allium cepa*) to different concentrations of a chemical solution. The roots of the onions are then monitored for growth over a set period, typically between 72–96 hours (3–4 days). Cytotoxicity is determined by measuring the root length — shorter roots indicate higher toxicity. In addition, genotoxicity is assessed under a microscope by examining chromosomal abnormalities during cell division (mitosis) in stained root tip cells.

In this experiment, students will detect harmful effects of environmental chemicals on both cellular growth and reproduction of *Allium cepa* L., generating valuable data for environmental risk assessment.



So, we're basically onion detectives, looking for clues in their cells?



Exactly! And the shorter the roots, the more toxic the chemical.

It's like a mystery we need to solve.

## AIM OF THE EXPERIMENT

To assess the **cytotoxic** and **genotoxic** effects of a chemical (e.g. cosmetic ingredient) on the onion plant (*Allium cepa*) by observing root growth and chromosomal changes in root tip cells.



## LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

### General Learning Objectives

- ✓ Understand the importance of environmental toxicology.
- ✓ Learn the difference between cytotoxicity and genotoxicity.
- ✓ Enhance laboratory skills and techniques.
- ✓ Develop scientific inquiry and critical thinking skills.

### Specific Learning Objectives

- ✓ Comprehend the Allium test procedure and its significance in assessing cytotoxicity and genotoxicity.
- ✓ Measure root length to determine cytotoxicity and identify chromosomal abnormalities to assess genotoxicity.
- ✓ Evaluate the impact of tested chemicals on plant growth and genetic material.
- ✓ Understand how tested environmental toxins can affect plant growth and chromosomal integrity.



- ✔ Apply findings to environmental risk assessment.

## TIME

### Cytotoxicity test:

Part I: 90 min

Part II (after 96 h (4 days)): 90 min

### Genotoxicity test:

Part III (after extra 24 h (1 day)): 90 min

It is possible to conduct only the **Cytotoxicity test** – the first and second parts of the experiment; especially in the case of less equipped laboratories or if the students are not directly from a science- or technology-oriented field.

Otherwise, we recommend also carrying out the third part – **Genotoxicity test**.

## MATERIALS NEEDED



### Equipment:

- 15 glass test tubes without the lid (adopt the final number to the number of your samples and tested concentrations)
- 6 test tube racks (suitable for at least 5 tubes)
- Plastic Pasteur pipettes (droppers)
- Automatic pipettes with tips
- Beakers (100, 250 and 500 mL)
- Volumetric flasks (100 mL)
- Tweezers
- 15 onion bulbs (*Allium cepa* L.) (or more)
- Millimetre ruler
- Microscope slides
- Coverslips
- Hot plate
- Light microscope with a magnification of 400× (or at least 100×)

### Chemicals:

- Tested chemical/cosmetic product (EXAMPLE: 30% hydrogen peroxide or Shampoo)
- Tap water (negative control)
- 1% solution of 0.1 M HCl or simply just vinegar (positive control)
- Orcein-acetic stain
- 1% solution of HCl
- Fixative (ethanol and acetic acid 3:1)

## SAFETY PRECAUTIONS



Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.

Additionally, be aware of the following specific safety precautions:

- ⚠️ **Be Cautious with Heating:** When heating substances, be careful to avoid burns. Use appropriate tools and heat sources.
- ⚠️ **Be Careful with Stain:** Work with dyes in a fume hood as they have a strong odour. Dyes are strong, so always use nitrile gloves and a protective lab coat.



*Yeah, I'd rather cry from the onions, not from an accident.*

*Safety first! We don't want any onion-related injuries.*



## EXPERIMENT SETUP



### Step 1 → Prepare the Work Area

Ensure your workspace is clean and free from distractions. Set out all necessary materials. Wear your safety gear.

### Step 2 → Prepare the Experiment

**Concentrations:** Prepare 3 different concentrations ( $c_1$ – $c_3$ ) or dilutions ( $R_1$ – $R_3$ ) of tested chemical. You can also choose the cosmetic product. Test each concentration in 3 replicates (A, B and C).

TESTED CHEMICAL/COSMETIC PRODUCT: \_\_\_\_\_

PICTOGRAMS: \_\_\_\_\_

**Volume:** Each test solution should have a final volume of 100 mL.

**If using a specific chemical:**

Calculate the amount of stock solution ( $R_0$ ) and tap water ( $H_2O$ ) needed for each test solution ( $R_1$ – $R_2$ ).

<i>DILUTION</i>		<i>Chemical Concentration</i>		<i>Volume of Chemical (mL)</i>	<i>Volume of H<sub>2</sub>O (mL)</i>
<b>R<sub>0</sub></b>	Undiluted (stock)	$c_0$		/	/
<b>R<sub>1</sub></b>	10×	$c_1$			
<b>R<sub>2</sub></b>	100×	$c_2$			
<b>R<sub>3</sub></b>	1000×	$c_3$			

**If using a cosmetic product:**

Estimate rough dilutions based on volume percentages.

<i>DILUTION</i>		<i>Product Concentration</i>		<i>Volume of product (mL)</i>	<i>Volume of H<sub>2</sub>O (mL)</i>
<b>R<sub>1</sub></b>	25%		$c_1$		
<b>R<sub>2</sub></b>	10%		$c_2$		
<b>R<sub>3</sub></b>	1%		$c_3$		

**Control Samples:** Use tap water ( $C_{neg}$ ) as negative control and 1% solution of 0.1 M HCl or vinegar as a positive control ( $C_{pos}$ ).

Positive and negative controls are essential for validating the results. A positive control shows the expected effect to confirm the experiment works, while a negative control shows no effect to ensure any observed changes are due to the treatment being tested.

**Test tubes:** Prepare glass test tubes without threads and caps.

Appropriately label the test tubes (e.g., c1 A) and place them in plastic tube racks. All three replicates of each test concentration should be placed in its own tube rack. Label also tube racks to avoid mixing samples.



*And we don't want any party crashers, so label carefully!*

*Labelling everything? This feels like organizing a science party!*



**Onion *Allium cepa* L.:** Choose onions (3 tested concentrations in 3 replicates each + 2 controls – all together 15 onion bulbs) of similar size to fit well on top of the test tubes, minimizing liquid evaporation.

Carefully cut off a minimal layer (up to 2 mm) of the dried root base to expose the root tissue to the test solution. Ensure the onion base remains intact.

### Step 3 → Conduct the Experiment

#### Part I:

1. Use a dropper to carefully fill the test tubes with the prepared solutions to the top.
2. Gently place the onions on top of the test tubes, ensuring they are submerged in the test solution.
3. Add the evaporated or used solution daily with a dropper, or water in the case of controls.
4. Keep the racks with test tubes in a well-lit area, such as a bench facing a window.
5. After 4 days, measure the root lengths (Part II) and \*prepare microscopic slides for cytogenetic analysis (Part III), respectively.

**Part II: CYTOTOXICITY:**

6. Remove the onions from the test tubes and measure the lengths of the five longest roots with a millimeter ruler.

**\*Part III: GENOTOXICITY:**

7. Cut 0.5 cm tips from the three longest roots for each concentration.

**8. FIXATION:**

- Immerse the root tips in a fixative (ethanol-acetic acid, 3:1).
- Store them in a freezer for approx. 24 h.

Fixation preserves cells and maintains similarity to living organisms.

**9. STAINING:**

- Prepare orcein stain (one solution per group).
- Prepare a water bath at 60°C.
- Pour 1% HCl into a beaker and heat it in the water bath.
- Take the root tip from the fixative and immerse it in heated HCl for 5 minutes.
- After 5 minutes, rinse the root tip with distilled water.
- Place the root tip on a slide, add orcein stain.
- Cover the sample with a cover slip and squash the root tip to prepare a smear (microscope slide).

**Step 4 → Monitor and Record Data****CYTOTOXICITY:**

After 96 h (4 days) record the measurements in a table and calculate the average root lengths.



*Measuring roots? I didn't know onions had beauty contests.*



*Well, the longest roots win the prize for least toxic!*

### Root Length Measurements

Concentration	$C_{neg}$		$C_{pos}$		$C_1$		$C_2$		$C_3$	
Replicate	A	B	A	B	A	B	A	B	A	B
Root 1 (mm)										
Root 2 (mm)										
Root 3 (mm)										
Root 4 (mm)										
Root 5 (mm)										
<b>Average Root Length (mm)</b>										

### \*GENOTOXICITY:

MICROSCOPY: Observe cell division and chromosomes under a light microscope. Identify at least 100 metaphase cells and assess chromosome condition. Report the percentage of all metaphase cells and cells with chromosomal damage.

1. Count and record the total number of cells observed in each sample (at least 100, the optimum 1000). Students can pool their results to reach as high number counted cell as possible.
2. Count and record the number of cells undergoing mitosis.
3. Count and record the number of cells in metaphase.



*Counting cells? This is like a microscopic treasure hunt!*

*And the treasure is understanding how chemicals affect our environment!*



### Indices Calculation:

**Mitotic Index:** Number of cells in mitosis per 1000 cells examined.

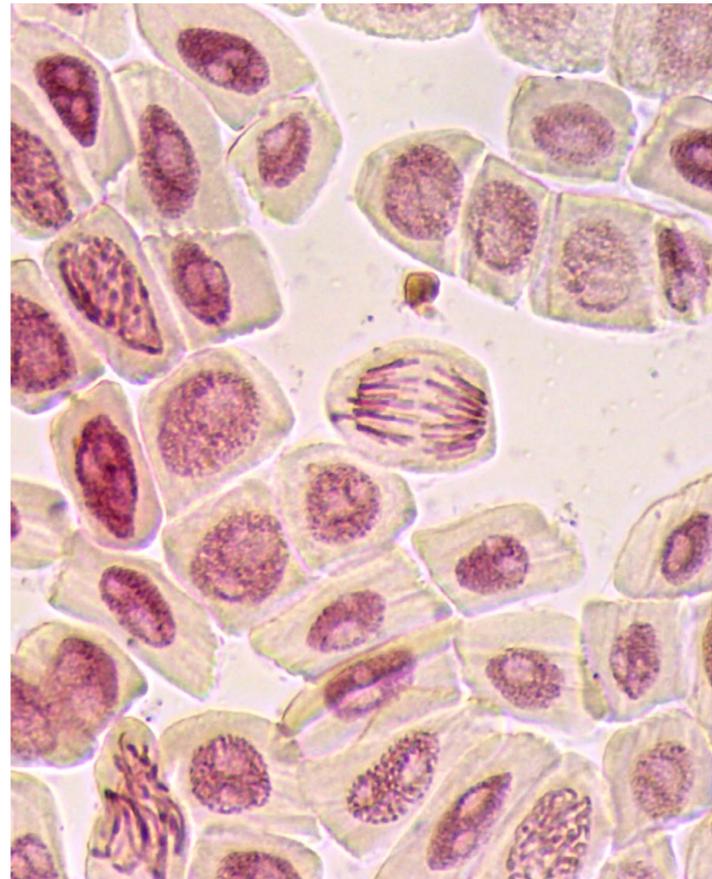
$$\frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \times 100$$

**Metaphase Index:** Number of metaphase cells per 1000 cells in mitosis.

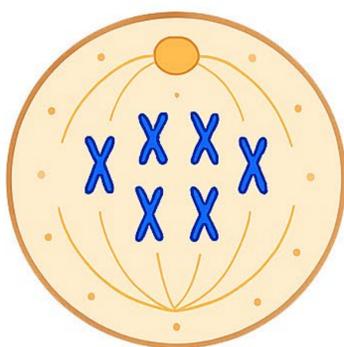
$$\frac{\text{Number of cells in metaphase}}{\text{Number of cells in mitosis}} \times 100$$



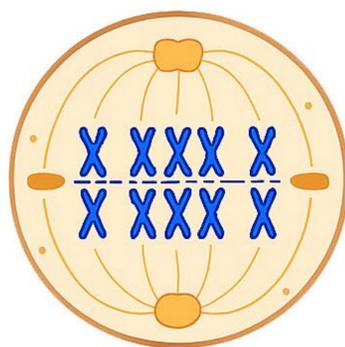
Cytotoxicity test



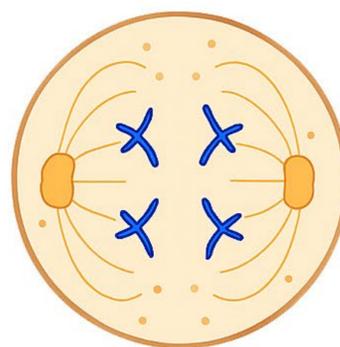
Genotoxicity test



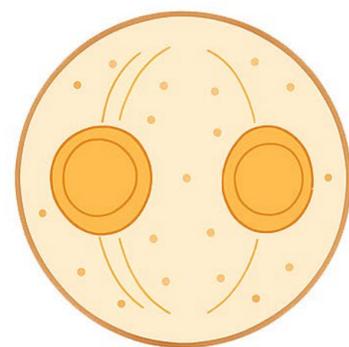
1. Prophase



2. Metaphase



3. Anaphase



4. Telophase

**Cell division phases** (Interphase – The cell grows and copies its DNA; **1. Prophase** – Chromosomes condense and become visible; **2. Metaphase** – Chromosomes line up in the middle; **3. Anaphase** – Chromatids are pulled apart; **4. Telophase** – Two new nuclei form; Cytokinesis – The cell splits into two)



**\*GENOTOXICITY****Data Presentation Table for Mitotic and Metaphase Indices**

<i>Sample</i>	<i>Total Number of Cells</i>	<i>Number of Cells in Mitosis</i>	<i>Number of Cells in Metaphase</i>	<i>Mitotic Index (%)</i>	<i>Metaphase Index (%)</i>
$C_{neg}$					
$C_{pos}$					
$C_1$					
$C_2$					
$C_3$					



## ANSWER KEY QUESTIONS



Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!

1. What is ecotoxicology and why is it important in relation to our everyday used cosmetics?
2. How can chemicals in the environment affect plant and animal life?
3. What are some common sources of environmental pollutants?
4. How do cosmetics and their chemical ingredients influence the environment?
5. Can the chemicals in cosmetics also affect human health? If so, how?
6. What differences did you observe in root lengths between the control group and the treated groups?
7. Would the exposure time affect the root growth in the Allium test in your opinion?
8. Why is it important to use a control group when conducting the Allium test?
9. Can you define all stages of mitosis?
10. What types of cell division abnormalities did you notice under the microscope in the treated samples?
11. How can observing cell division help determine the genotoxic effects of a chemical?
12. What conclusions can you draw about the genotoxicity of the tested chemical based on your observations?



***Do we really need to know this?***

***Can't we just ask the plants and animals?***

***If only they could talk! But since they can't, it's up to us.***



**Let's think critically:**

13. What do you think it means when we refer to a "chemical cocktail" in cosmetics?
14. Why is it important to consider the combined effects of multiple chemicals in a single product?
15. In your opinion, what are the potential risks of using products that contain a mixture of various chemicals?



*Chemical cocktail? Sounds like a party I don't want to attend.*



*Yeah, mixing chemicals can be a recipe for disaster.*

## FOR EDUCATORS



### Additional Activities/Extensions (Optional):

Propose a new experiment by modifying one variable – for example, add a different chemical or chemical mixture. You can also monitor cell division over different time scales (after 24, 48, 72, and/or 96 hours) of exposure.

Optionally, photograph the Petri dishes daily and create a photographic collage of germination.

### Adapting the Experiment for **Secondary School Students:**

Simplify the experiment:

- ➔ **Focus on Root Length Measurement:** Instead of conducting cytogenetic analysis, have students measure and compare the root lengths of onions exposed to different concentrations of a household or cosmetic product.
- ➔ **Use Fewer Concentrations:** Reduce the number of samples to three (e.g., control, low, and high concentration) to simplify data collection and analysis.
- ➔ **Visual Observation:** Encourage students to make detailed visual observations of root growth and any visible changes in root morphology.

#### Example:

- ✓ **Objective:** Measure the effect of a household chemical (e.g., vinegar) or a cosmetic product (e.g. shampoo) on onion root growth.
- ✓ **Procedure:** Use three concentrations (0%, 10%, and 50% vinegar/shampoo solution) and measure root lengths after 96 hours.
- ✓ **Data Collection:** Record root lengths and compare the average lengths across different concentrations.

### Adapting the Experiment for **Primary School Students:**

Simplify the experiment:

- ➔ **Basic Root Growth Observation:** Focus on observing and measuring root growth without involving chemical treatments.
- ➔ **Use Safe Materials:** Use safe, non-toxic substances like water, saltwater, and sugar water diluted hand soap, lotion, or conditioner to observe their effects on root growth.
- ➔ **Hands-On Activities:** Include hands-on activities like drawing and labelling parts of the onion and roots.

**Example:**

- ✔ **Objective:** Observe how different types of water affect onion root growth.
- ✔ **Procedure:** Use three types of water (tap water, diluted hand soap, and diluted conditioner) and measure root lengths after 96 hours.
- ✔ **Data Collection:** Record root lengths and make simple comparisons. Have students draw pictures of their observations.

# General safety precautions



The following general safety precautions apply to all experiments in this handbook.

Please review them carefully before conducting any lab work. Some experiments may also have additional specific precautions listed within their respective tutorials.

-  **Follow Instructions:** Always listen to your teacher/educator/assistant and follow the lab instructions carefully. If you're unsure about any step, ask for clarification before proceeding.
-  **Know Safety Equipment:** Familiarize yourself with the location and proper use of safety equipment like eyewash stations and fire extinguishers.
-  **Be Careful with Glassware:** Exercise caution when handling and washing glassware to avoid breakage and injury.
-  **Safety Gear:** Always wear a lab coat, safety goggles, and gloves. Ensure you have closed-toe shoes and tie back long hair.
-  **Handle Chemicals Safely:** Handle chemicals and equipment with care. Never taste or sniff chemicals. Always label containers or tubes.
-  **Check Pictograms:** Before using any chemical, review the safety pictograms on the label to understand the hazards associated with it.
-  **Handle Solvents Carefully:** Use solvents in a fume hood to avoid inhaling fumes and ensure proper ventilation.
-  **Dispose of Waste Properly:** Follow proper procedures for disposing of chemical and biological waste. Do not pour chemicals down the drain unless instructed.
-  **Report Accidents:** Immediately inform your teacher/educator/assistant of any accidents, spills, or injuries, no matter how minor they seem.