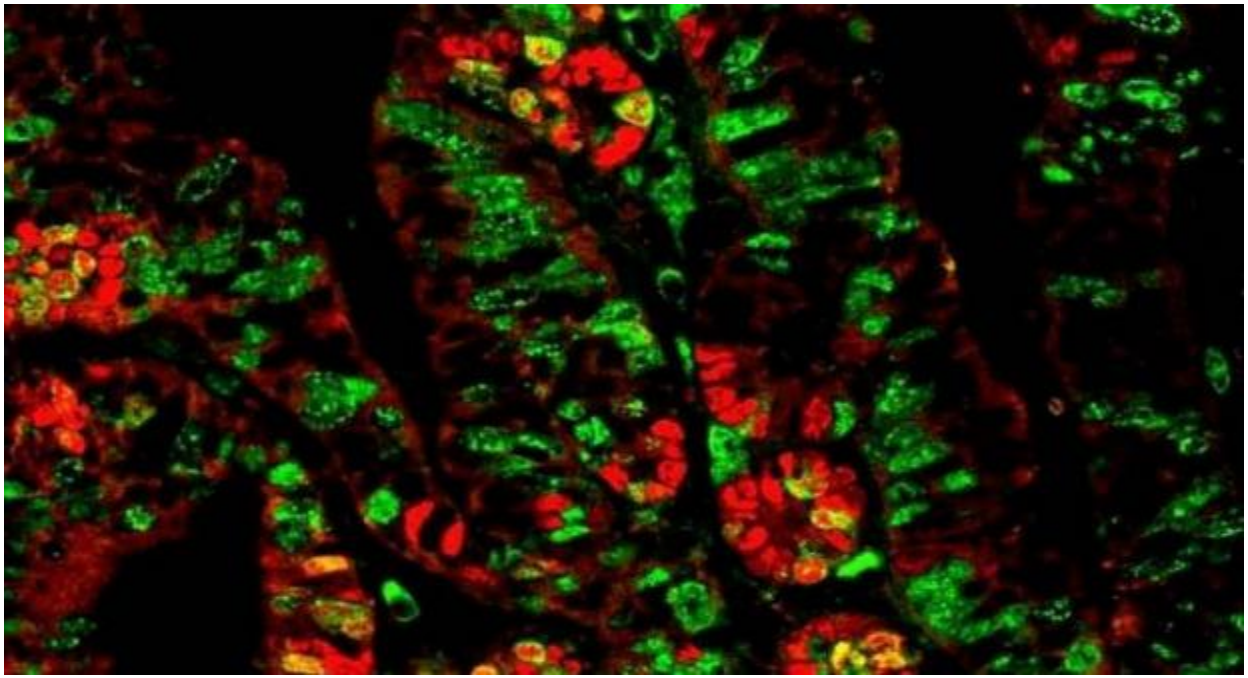
# The Effects of Xenoestrogens on MG-63 Cancer Cells



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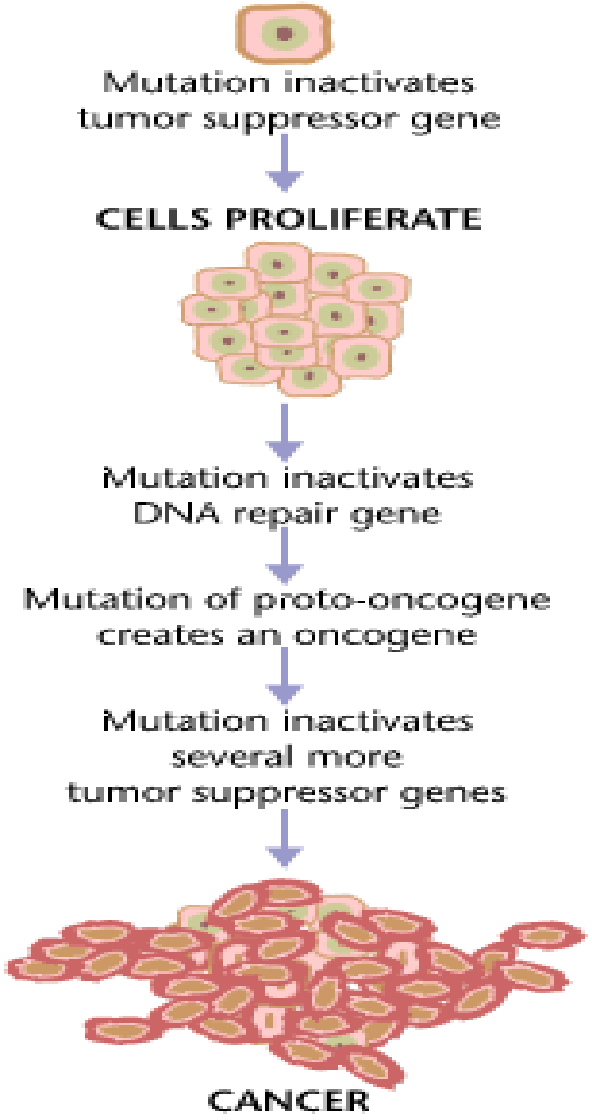
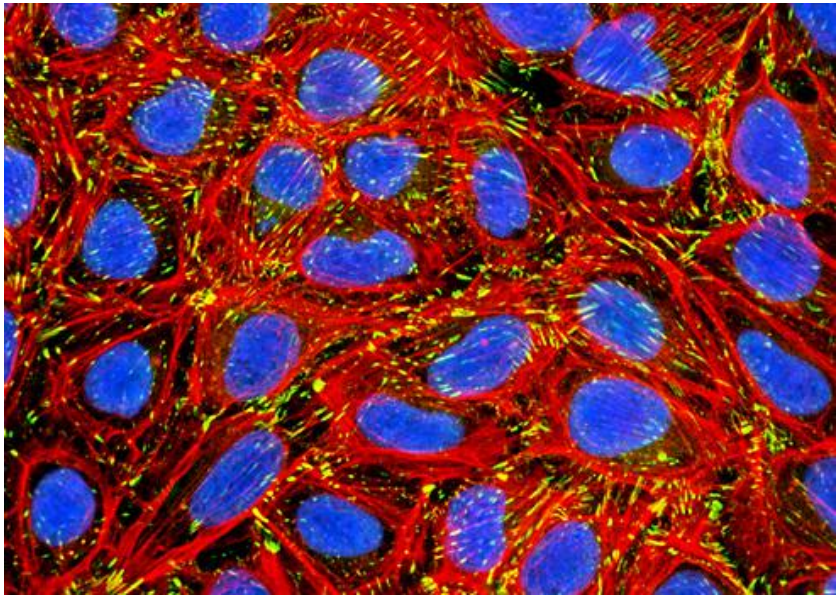
# An Overview of Cancer Cells

- Cancer cells are cells that grow and divide at an irregular, unregulated pace.
- Apoptosis does not occur in cancerous cells; their mutations are passed on to the second generation, eventually clustering and forming tumors.
- Tumors can be malignant (aggressive) or benign.



# MG63 Cancer Cell Line

- Human cancer cell line
- *Osteosarcoma* cells, an aggressive form of bone cancer
- Useful model to test the effects of variables on cancer cell proliferation



# Endocrine Disruptors and Cancer

- Scientists are constantly researching chemical factors that either cause cancer, promote cancerous growth, inhibit cancer growth, or alter the progression or phenotype of cancer

- Some cancers are promoted by the work of xenohormones

    -mimic the actions of a particular hormone

    -might promote cancer growth

# Overview of Xenoestrogens

- Type of xenohormone that imitates estrogen
- Can be either natural or synthetic  chemical compounds
- Synthetic xenoestrogens  are widely used in industrial compounds which have estrogenic effects on a living organism
- Clinically significant because they can mimic the effects of endogenous estrogen and thus have been implicated in the precocious puberty and other disorders of the reproductive system

# Atrazine

- An organic compound widely used as a herbicide
- Prepared from cyanuric chloride, which is treated sequentially with ethylamine and isopropyl amine
- Some studies still show controversial effects of atrazine
  - -Banned in the European Union in 2004 because of its persistent groundwater contamination
  - -In the United States it is one of the most widely used herbicides, with 76 million pounds of it applied each year, in spite of the restriction that used to be imposed

# Purpose

- To determine the effect of atrazine exposure on cancer cell proliferation.

# Hypothesis

**Null Hypothesis**

- Atrazine **WILL NOT** have an effect on cancer proliferation.

**Alternative Hypothesis**

- Atrazine **WILL** significantly effect the proliferation and survivorship of cancer.

# Materials

- Cryotank
- 75mm$^2$ tissue culture treated flasks
- 25 mm$^2$ tissue culture treated flasks
- Fetal bovine serum (FBS)
- MG63 Osteosarcoma Cancer Cell Line
- Trypsin-EDTA
- Pen/strep
- Macropipette + sterile macropipette tips (1 mL, 5 mL, 10, mL, 20 mL)
- Micropipettes + sterile tips
- DMEM Serum - 1% and Complete Media (4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate + [ 10% fetal bovine serum for complete])

- 75 mL culture flask
- Atrazine
- Incubator
- Nikon Inverted Microscope with imaging technology
- Laminar Flow Hood
- Laminar Flow Hood UV Sterilizing Lamp
- Sharpie pen
- Hemacytometer
- Sterile PBS
- Ethanol (70%)
- Sterile Water
- Purple Nitrile gloves

# Procedure: Cell Culturing

- A 1 mL aliquot of MG63 cells from a Cryotank was used to inoculate 30 mL of 10% serum DMEM media in two 75mm$^2$ culture flask yielding a cell density of approximately $10^6$ to $2x10^6$ cells.

- The media was replaced with 15 mL of fresh media to remove cryo-freezing fluid and incubated (37° C, 5% $CO_2$) for 2 days until a cell density of approximately $4x10^6$ to $5x10^6$ cells/mL was reached.

- The culture was passed into two sets of 3 flasks in preparation for experiment and incubated for 2 days at 37° C, 5% $CO_2$.

# Procedure: Proliferation Experiment- Day 0 (Addition of Variable)

- After trypsinization, cells from all of the flasks were pooled into 1 common 75mm$^2$ flask (cell density of approximately 1 million cells/mL).
- 0.1 mL of the cell suspension was added to six 25 mm$^2$ tissue culture treated flasks containing 5 mL of DMEM (com) media, creating a cell density of approximately $10^5$ cells per flask.
- Two stock solutions of atrazine were created using sterile water: 1/100x and 1/10,000x. X = the concentration of the undiluted Atrazine product.
- The following concentrations of variable (next page) were added to the flasks.
- The cells were incubated at 37°C, 5% $CO_2$ for the remainder of the study.

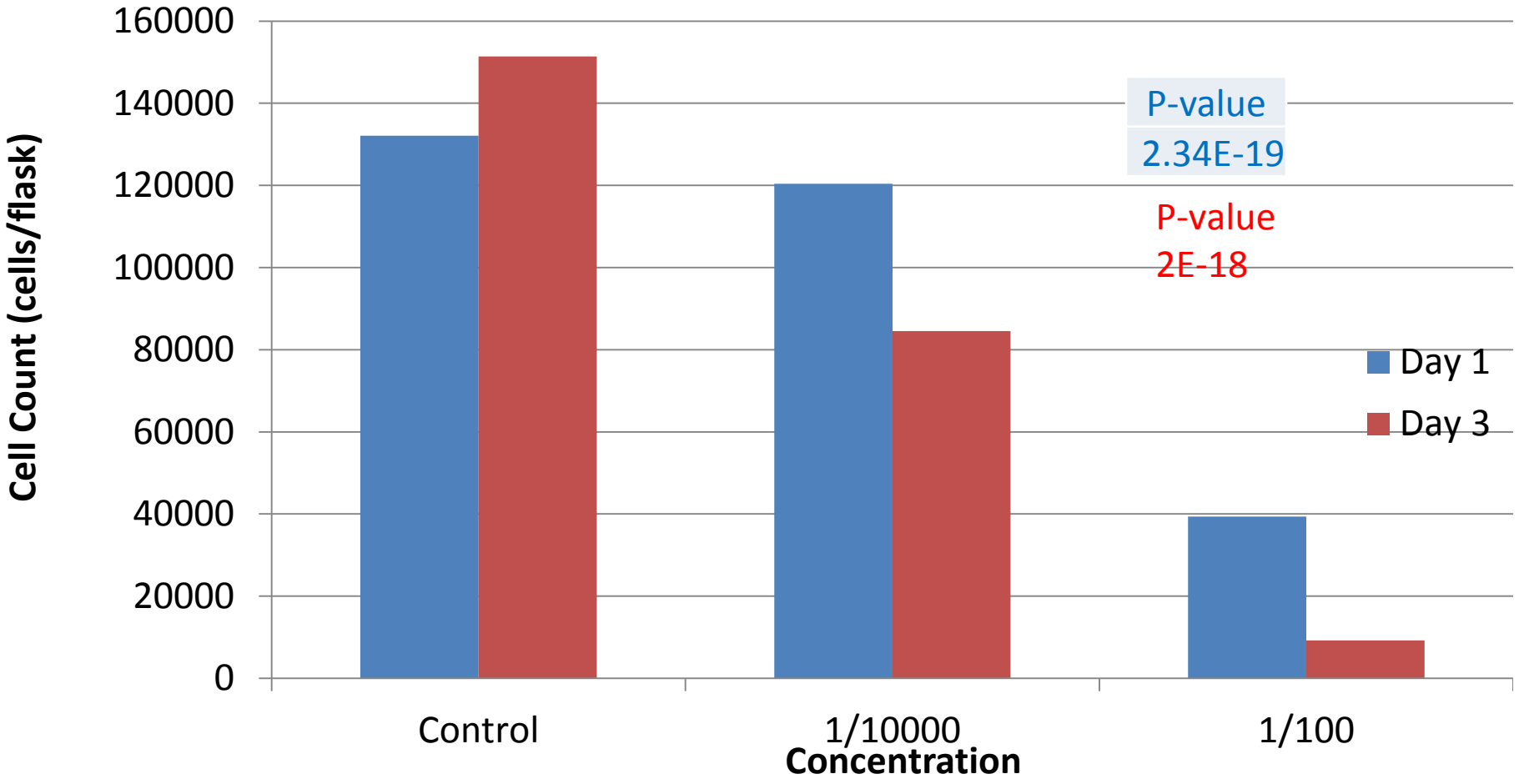
# Concentrations of Variable

| | 0 | $10^{-6}$X | $10^{-4}$X |
|---|---|---|---|
| Stock | 0 mL | 0.05 µL of 1/10,000 stock | 0.05 µL of 1/100 stock |
| Media | 5 mL | 4.950 mL | 4.950 mL |
| Total | 5 mL | 5 mL | 5 mL |

# Procedure- Days 1 and 3

- Day 1 and Day 3
  - Cell densities were determined as follows:
    - The cells were trypsinized and collected into cell suspension.
    - 25 µl aliquots were transferred to a Hemacytometer for quantification (four counts per flask).
- Day 1 and Day 3
  - Nikon Inverted Microscope was used to take images of representative areas of each flask.

# Results of Proliferation Analysis

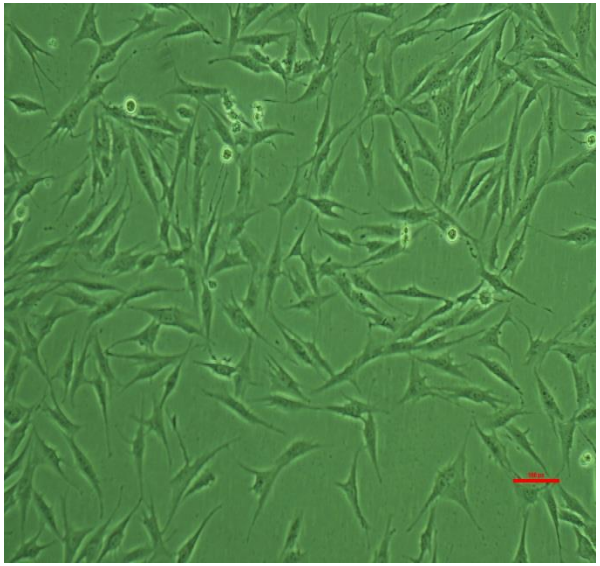


# Dunnett's Test Results

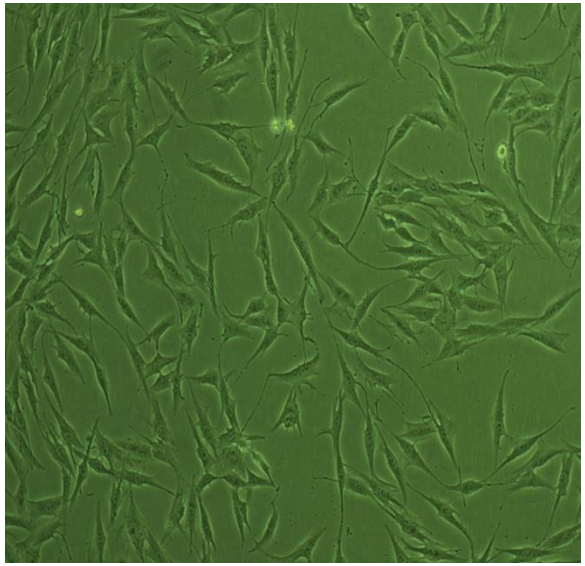
$$t_d = \frac{M_i - M_c}{\sqrt{\frac{2MSE}{n_h}}}$$

| Concentration | T-Value | T-Critical (0.05) | Variation |
|---|---|---|---|
| ***Day 1*** | - | - | - |
| 1/100X | 8.18 | 2.67 | **Significant** |
| 1/10000X | 1.02 | 2.67 | Insignificant |
| ***Day 3*** | - | - | - |
| 1/100X | 11.55 | 2.67 | **Significant** |
| 1/10000X | 5.44 | 2.67 | **Significant** |

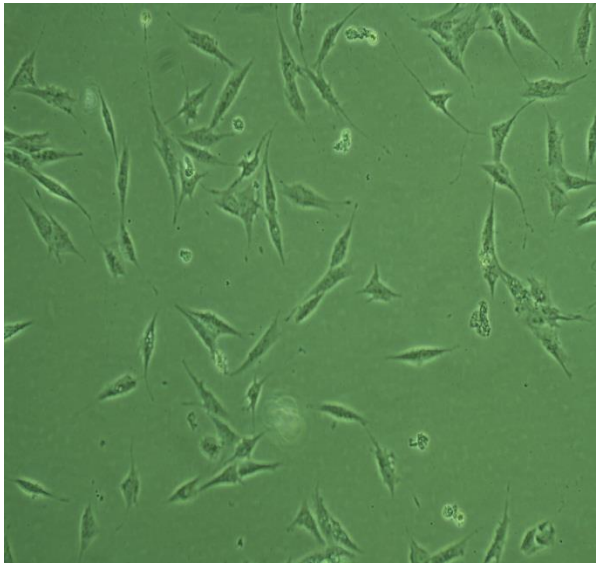
# Proliferation Results: Day 1



Control

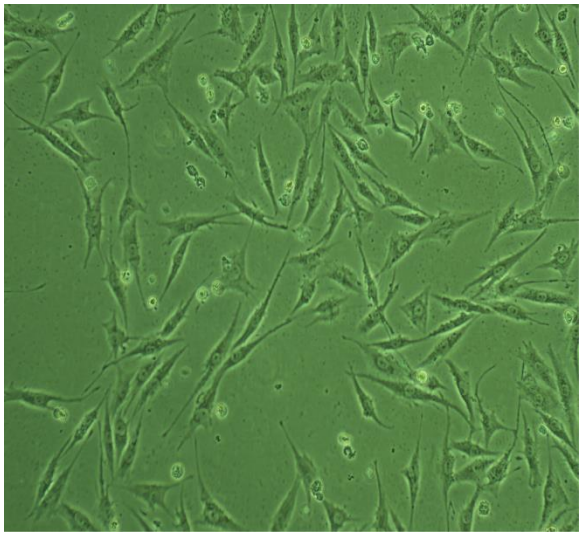


Low

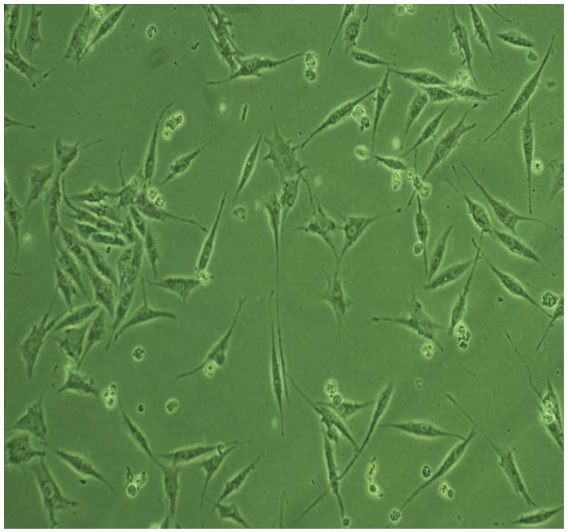


High

# Proliferation Results: Day 3



Control



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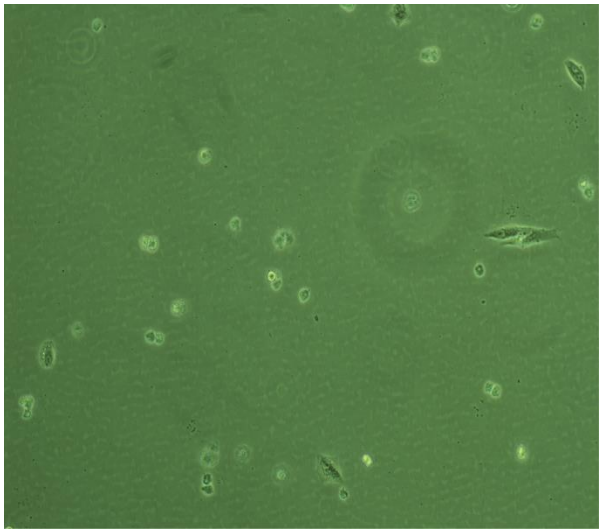

High

# Conclusions

- Proliferation
  - MG63
    - Based upon the results gathered from the ANOVA and Dunnett's statistical analyses, it appears that the addition of atrazine in high concentration significantly affected cancer cell proliferation. Alternatively, time of exposure may significantly affect the survivorship rates.

# Future Changes

## Limitations

- Hemacytometer counts in proliferation experiment can vary
- Cell culture health can vary
- Low number of replicates
- Lag time

## Extensions

- Obtain an LD-50 for atrazine
- Use a wider range of concentrations
- Test the effects of atrazine on other cell lines
- Test synergistic effects of atrazine
- MTT Assay
- Tritiated Thymidine Incorporation Assay

# Statistical Analyses of the Proliferation Results

- ANOVA
  - Statistical analysis that allows a comparison of means of different groups.

- Dunnett's test
  - Determines significant variance between the control group and experimental group.

# Works Cited


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