

The Effects of Pesticide on MG63 Cancer Cell Lines

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Pesticides

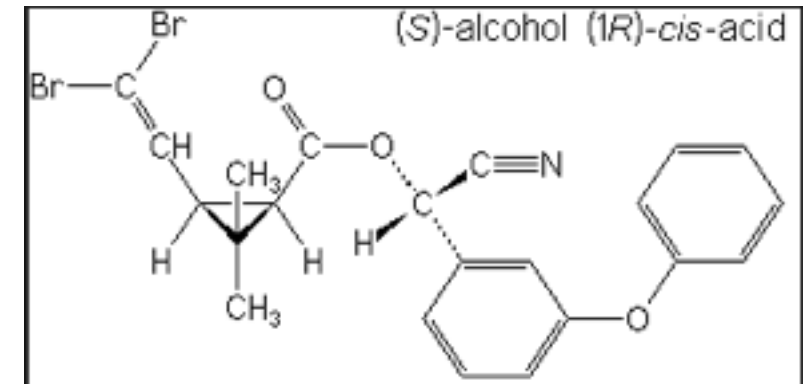
- Include any chemical, antibacterial, biological agent, used to kill or repel unwanted species.
- Grouped in other categories: bactericides, fungicides, herbicides, rodenticides, and insecticides.
- May harm non-target species.
- Long-term effects still unclear.

Raid Max Bug Barrier

- Active Ingredient: 0.03% Deltamethrin
- Insecticide
- Used in houses to create a barrier that kills insects on contact.
- **Kills:** Ants, Crickets, Gnats, Ground Beetles, Mosquitoes, Roaches, Spiders, Stink Bugs, etc.



Deltamethrin



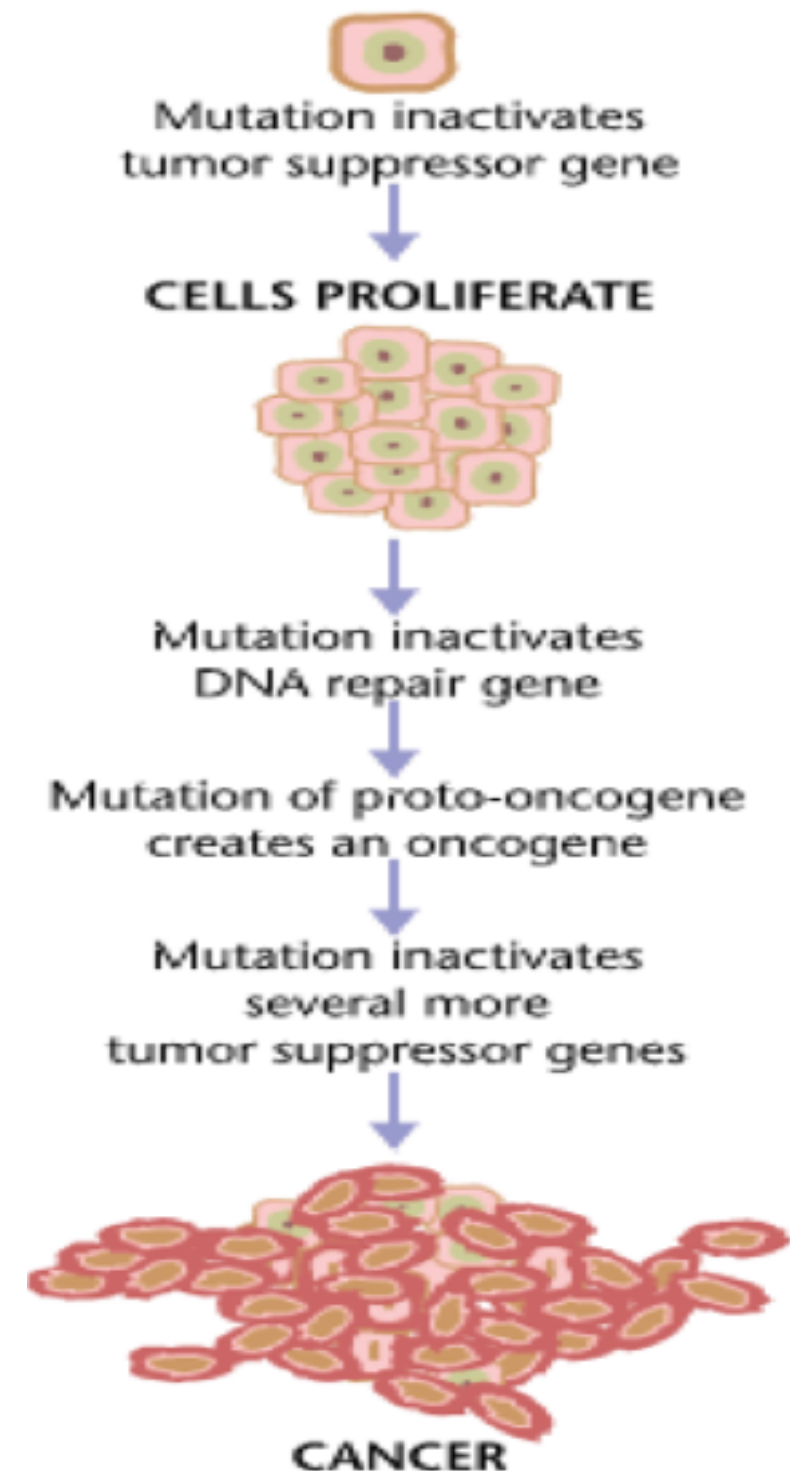
- Commonly found in Insecticides
- common uses are agricultural and home pest control
- pyrethroid ester insecticide with the chemical formula $C_{22}H_{19}Br_2NO_3$
- Targets the nervous system
- extremely effective in killing pests

Studies on Deltamethrin

- Chronic studies conducted on rats, mice, and dogs
- Slight toxicity in mammals, causing temporary paralysis
- Highly toxic to aquatic life
- Side effects: unsteadiness, convulsions, hypersensitivity, and decreased motor activity
- There is little to no side effects observed in past dermal, ingestion, and inhalation studies. (In cases of prolonged exposure, Toxicity and other side effects have been observed)
- Considered unlikely to be carcinogenic to humans (EPA)

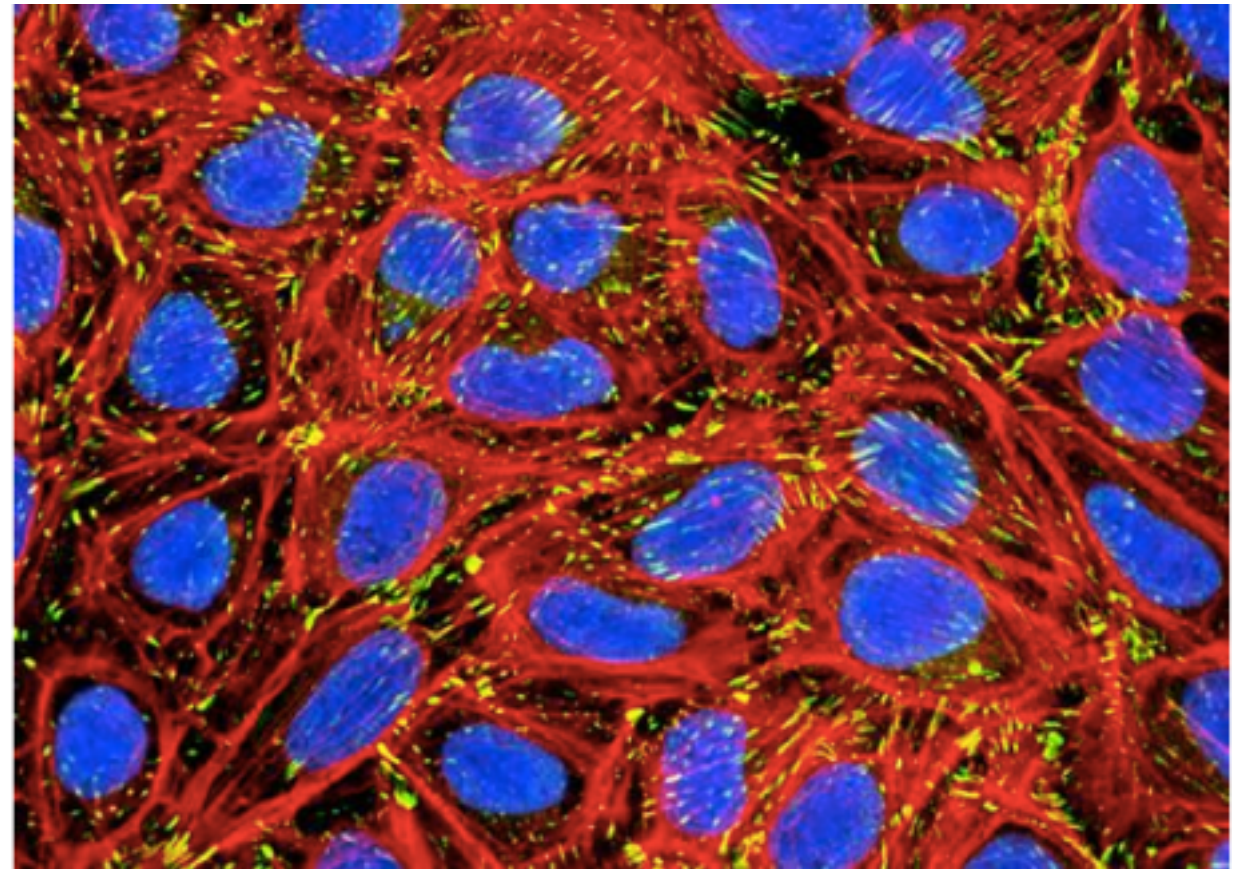
An Overview of Cancer Cells

- 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



Purpose

- To examine the effect of pesticide on MG63 cell proliferation and survivorship

Hypotheses

- Null: The different concentrations of pesticide will not have a significant effect on the proliferation and survivorship of the Mg63 cells.
- Alternative: The different concentrations of pesticide will have a significant effect on the proliferation and survivorship of the Mg63 cells.

Procedure (Cell Line Culture)

- A 1 mL aliquot of MG63 cells from a Cryotank was used to inoculate 30 mL of 10% serum DMEM media in a 75mm² culture flask yielding a cell density of approximately 10^6 to 2×10^6 cells
- The media was replaced with 15 mL of fresh media to remove cryo-freezing fluid and incubated (37° C, 5% CO₂) for 2 days until a cell density of approximately 4×10^6 to 5×10^6 cells/mL was reached
- The culture was passed into 12 flasks in preparation for experiment and incubated for 2 days at 37° C, 5% CO₂

Procedure (Addition of Variable on Day 0)

Procedure (Addition of Variable on Day 0)

- Cells from a T75 flask were resuspended after trypsinization to a density of approximately 300-500K/mL.
- 4 mL of 10% DMEM media was added to each T25 flask
- 0.5 mL of cell suspension was transferred to 12 T25 flasks. Flasks were placed back into incubator and cells were allowed to attach for several hours
- 0.1 mL of pesticide (not the active ingredient) was added to 9.9 mL of media to create a 1% stock (stock A). Serial dilution to create a 0.1% (stock B) and a .01% (stock C). All were sterile filtered using a 0.22 micron filter.
- T25 flasks were removed from incubator and variable was added to reach desired concentrations

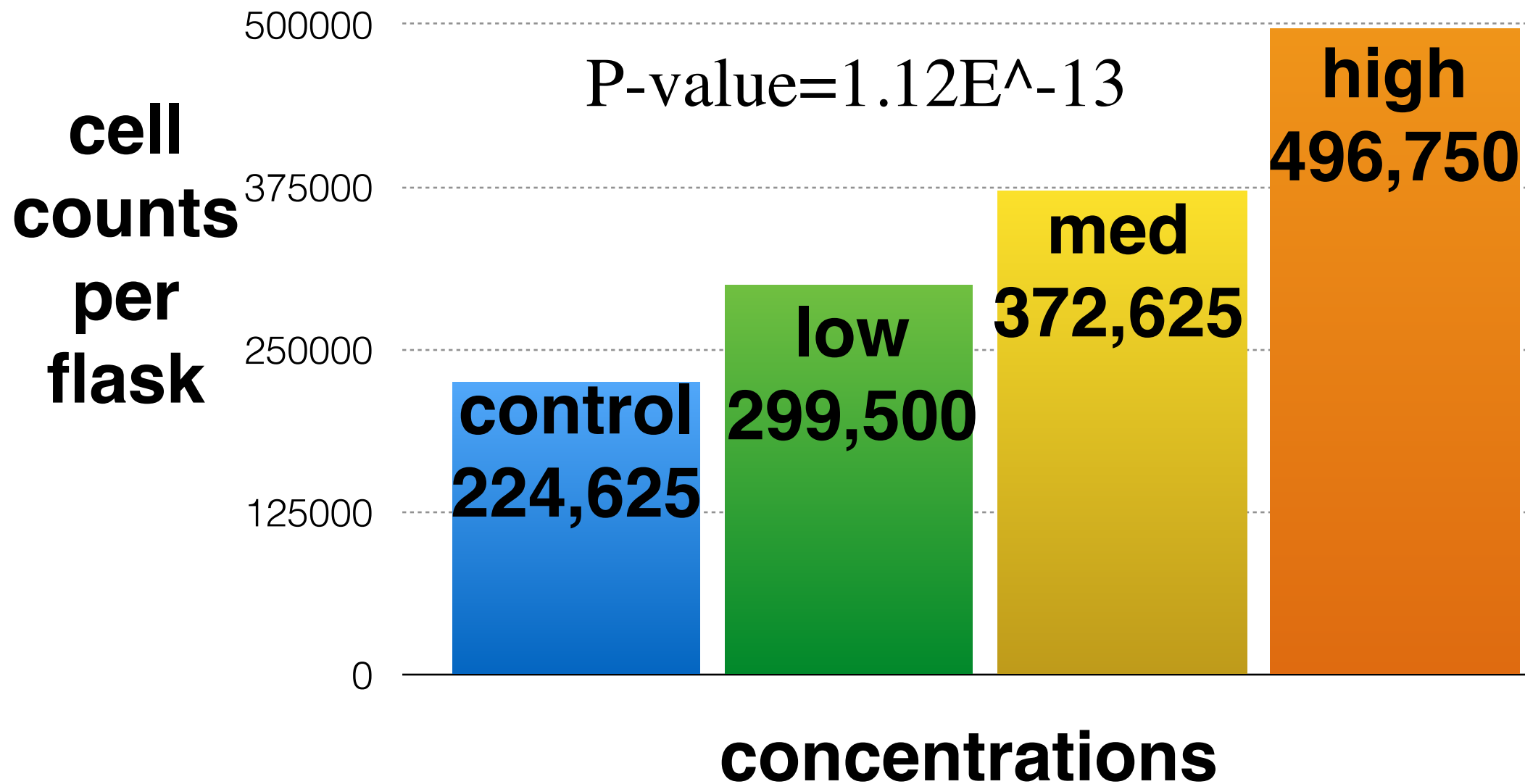
Procedure (Cell Counts)

- Day 1 and Day 3
- Cell densities were determined as follows:
- The cells were trypsinized and collected into cell suspension.
- 20 μ l aliquots were transferred to a Hemocytometer for quantification (eight total counts).

Concentrations

	high	med	low	con
cells	1 ml	1 ml	1 ml	1 ml
media	4 ml	4 ml	4 ml	4.0 ml
Stock	0.05 ml @ 1% stock	0.05 ml @ .1 stock	0.05 ml @.01% stock	0 ml
	0.01%	0.001%	0.0001%	0%

Results (day 1)

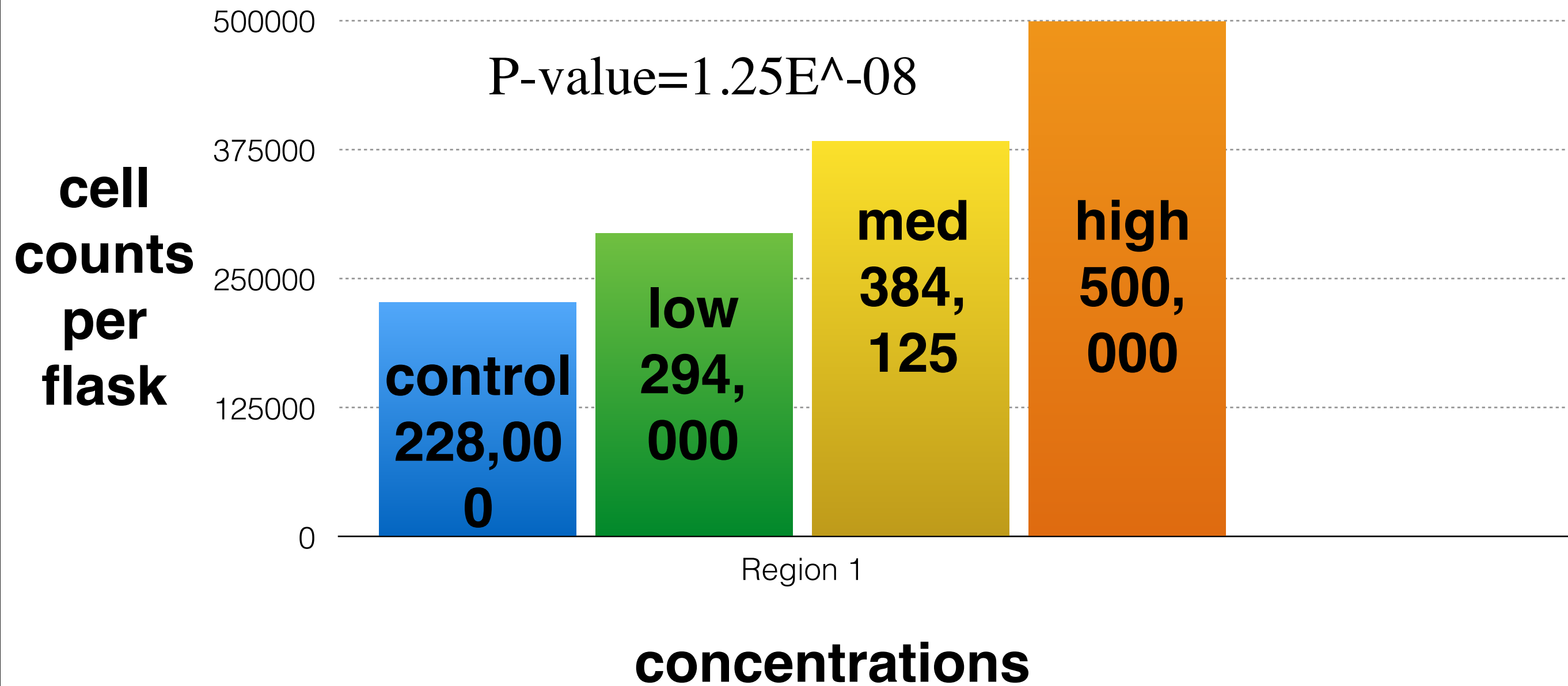


Dunnett's Test Results (day 1)

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

CONCENTRATIONS	T VALUE	T crit	Results
HIGH	14.597	2.88	Significant
MEDIUM	7.939	2.88	Significant
LOW	4.016	2.88	Significant

Results (day 3)



Dunnett's Test Results (day 3)

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

CONCENTRATION	T value	T crit	Results
HIGH	8.688	2.88	Significant
MEDIUM	4.987	2.88	Significant
LOW	2.124	2.88	Not Significant

Conclusion

- The null is rejected for all concentrations on day one and the medium and high concentrations on day 3. The data strongly suggests that the different concentrations did have a significant effect on the cell survivorship and proliferation for those concentrations on those days.
- the null is accepted for the low concentration on day 3.

Limitations

- cell counts can vary
- low number of flasks
- only one cell line used
- limited exposure time
- only one pesticide tested
- cell health not observed

Project Improvements

- more flasks
- use multiple cell lines
- more concentrations
- longer exposure time
- Different pesticides
- trypan blue assay

Citations/ Acknowledgements

- Mark Krotec, PTEI
- <http://www.epa.gov/espp/litstatus/effects/redleg-frog/2013/deltamethrin/appendix-j.pdf>
- <http://en.wikipedia.org/wiki/Deltamethrin>
- <http://www.raidkillsbugs.com/en-us/products/raid-max-bug-barrier>

Anova Day 1

Anova: Single Factor

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Column 1	8	1797000	224625	1.19E+09
Column 2	8	2396000	299500	1.2E+09
Column 3	8	2981000	372625	1.7E+09
Column 4	8	3974000	496750	1.46E+09

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3.22E+11	3	1.07E+11	77.56237	1.12E-13	2.946685
Within Groups	3.88E+10	28	1.39E+09			
Total	3.61E+11	31				

Anova Day 3

Anova: Single Factor

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Column 1	8	1824000	228000	3.99E+09
Column 2	8	2356000	294500	1.5E+09
Column 3	8	3073000	384125	4.46E+09
Column 4	8	4000000	500000	5.72E+09

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3.33E+11	3	1.11E+11	28.33081	1.25E-08	2.946685
Within Groups	1.1E+11	28	3.92E+09			
Total	4.43E+11	31				