TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of mutagenic effects of regular and environment friendly detergents on the Ames *Salmonella*/microsome mutagenicity assay.

Biology Extended Essay

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Abstract

The aim of this extended essay is to investigate the mutagenic effects of two different detergents (one is known to be environment friendly) by using a well standardized screening test; The Ames *Salmonella*/microsome mutagenicity assay.

The research question was "Is there a significant difference between regular detergent and environment friendly detergent in terms of promoting mutations in the Ames *Salmonella*/microsome mutagenicity assay?"

It was hypothesized that; there would be a significant mean difference in terms of promoting mutations between the groups. The regular detergent will show more mutagenic effect, followed by environment friendly detergent.

The Ames *Salmonella*/microsome mutagenicity plate incorporation assay was used for testing this hypothesis. In the first stage, toxicity assay was performed. Different dilutions of both detergents ($1x10^{-2}$, $1x10^{-3}$ and $1x10^{-4}$) were tested in the overnight *Salmonella* nutrient broth cultures. The *Salmonella* strain TA100 and, an enriched, non-selective agar medium was used in toxicity assay. The tested dilutions of two detergents were found to be non-toxic for tested strain. If planned testing dilutions of these detergents were found to be toxic for *Salmonella* strain TA100, it could not be possible to perform mutagenicity assay.

On the second stage of assay, these dilutions of two different detergents were tested according to the procedure defined in The Ames *Salmonella*/microsome mutagenicity plate incorporation assay using TA98 and TA100 *Salmonella* strains.

The ANOVA results revealed that there were no statistically significant difference between two detergents and between each detergent and negative control group in terms of mutagenicity. This finding was not supporting the hypothesis that environment friendly detergent would induce less mutagenic effects compared to regular detergent.

Word Count: 266

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I. Introduction/Background

Drastic effects of water pollution on ecological balance are a major problem for humanity. "Water pollution has been suggested to be the leading worldwide cause of deaths and diseases, and it accounts for the deaths of more than 14.000 people daily."[1] Chemicals and other contaminants are among the most important factors which contribute water pollution.[2] Detergents are among these substances polluting water supplies. Having worldwide usage, they are one of the major factors damaging ecological balances.

The answer for the question "Why do detergents pollute environment though they are being used as cleaning agents?" will be the "chemical ingredients of detergents". These chemicals include; wetting agents and emulsifiers, based on non-soap surfactants. Synthetic detergent powders consist of surface-active agents, builder and filters, also additives like anti re-deposition agents, whitening agents, bluing agents, bleaching agents, foam regulators, organic sequestering agents, enzymes, perfumes, and density regulators.[3] All these chemicals are harmful for both human health and environment. Inhalation or entrance of these chemicals through skin may cause irritations, allergies, asthma, long term health problems and rarely cancer.[4]

Detergents can be grouped into two as phosphate detergents and surfactant detergents. Phosphate based detergents are used to soften hard water and help suspend dirt. They contain large amount of phosphate which causes of water pollution via eutrophication. On the other hand surfactant detergents are highly toxic ones which provide wetting, foaming, dispersing.[5]

Detergents generally mix in water by sewage and, this affects aquatic life in several ways, the protecting mucus covering fish and protects them from bacteria is damaged by time. Water tension decreases so fish absorbs much more organic chemicals compared to

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[1] "Water pollution." *Wikipedia, The Free Encyclopedia.* 8 Nov 2009.
<http://en.wikipedia.org/wiki/Water_pollution>
[2] "Contaminants." *Wikipedia, The Free Encyclopedia.* 9 Nov 2009.
<http://en.wikipedia.org/wiki/Water_pollution>
[3] "Detergents." *Wikipedia, The Free Encyclopedia.*3 Nov 2009..
<http://en.wikipedia.org/wiki/Detergents>
[4] "Detergents." 3 Nov 2009
<http:www.indiatogether.org/environment/articles/tlink-1002.htm>
[5] "What are detergents." 3 Nov 2009
<http://www.lenntech.com/aquatic/detergents.htm>

non-polluted environment. Fifteen particles per million (ppm) of detergent composition in fresh water will make lots of fish die by the time, 5 ppm will cause the death of fish eggs.[6]

Considering these harmful effects of detergent some companies started to produce new detergents that they claimed to be environment friendly. A well known brand, "Frosch" is an example of these products. In Frosch's official site; it is stated that "In 1986, the newly created Frosch became an eco-pioneer by offering environment friendly products." Frosch claims that their detergent's ingredients are 98% environment friendly. [7]

On the other hand the ingredients of normal detergents and environment friendly detergents are inaccessible commercial secrets. There are many questions required to be answered regarding these issues; how does this change in ingredients affect aquatic habitats? Is there an important distinction in these habitats' life quality when environment friendly detergents are used?

The pollutants, including detergents are damaging the nature by killing different species or by causing damages in their genetic programs by changing their DNA composition. The acquired changes in the DNA composition are called mutation.[8,9] Chemicals that can induce mutations may cause fertility problems and cancer.[10,11,12]

There are different types of mutations. Point mutation is described as the modification, insertion or deletion of a single base. Large deletions or rearrangements of DNA such as chromosome breaks or rearrangements, or as gain or loss of whole chromosomes is called chromosomal mutations. Chromosome damage in mammalian cells can be demonstrated with the help of sophisticated molecular techniques, mainly based on observation of the cell's chromosomes under magnification. It is relatively easier to detect gene mutations in bacteria and other cell systems when they change the growth requirements of the cell.

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[6] "What occurs if detergents show up in fresh waters?." 3 Nov 2009
<http://www.lenntech.com/aquatic/detergents.htm>
[7] "Brandhistory of Frosh" 1 Nov 2009
<http://www.frosch.de/en/brand/brandhistory >
[8] "Damaging Effect of Detergents on Human Lymphocytes" Bulletin of Environmental Contamination and Toxicology
[9] "Mutagenesis of the Metabolite of Nonionic Detergents in Water" DC, Water Resources Research Center. Report No. 73
[10] "Biomarkers in population studies: environmental mutagenesis and risk for cancer". Rev Environ Health.
[11] Toxicological characteristics of endocrine-disrupting chemicals: developmental toxicity, carcinogenicity, and mutagenicity. J Toxicol Environ Health B Crit Rev.
[12] Papachristou F, Simopoulou M, Touloupidis S, Tsalikidis C, Sofikitis N, Lialiaris T. DNA damage and chromosomal aberrations in various types of male factor infertility. Fertil Steril. 2008;90:1774-81.

All this information and my curiosity of finding out if environment friendly detergents are beneficial as they are claimed to be, inspired me to plan and perform an essay involving the mutagenic capacity of detergents, in order to compare their harmful effects. A search of related literature revealed a relatively simple and reliable mutagenicity screening test[13] that can be used to compare the possible mutagenic effects of two different detergents; one is known to be environment friendly. The name of this test is "The Ames *Salmonella*/microsome mutagenicity assay". In this assay *Salmonella* strain TA98 and *Salmonella* strain TA100 were used. These two strains have relatively low colony formation rates, and the low colony formation rate makes the colony counting procedure easier. TA98 is sensitive for frameshift mutations and TA100 is sensitive for base-pair substitution mutations, and these two strains are accepted to be sufficient for a mutagenic screening.[13] I planned to compare the mutagenic effects of two different detergents on these *Salmonella* strains.

[13] "The Ames *Salmonella*/microsome mutagenicity assay" Mutation Research

II. Hypothesis

Detergents are one of the major pollutants of water. There are lots of studies done about the toxicity of the detergents and its effect on environment. One of these studies is about the toxic effect of detergents on Chalcal burnus tarichi and daphnia species in Van Lake which's results point out the mortal effects of detergents on water supply,[14] and it is practically impossible to avoid exposure to polluted water . Mutagenicity, i.e. damaging the genetic material (DNA) of the cells has always been a concern for scientists. Because mutations are dangerous, they may cause cancer or infertility. In the literature there is subtle evidence that; detergents or some of their ingredients may be mutagenic.[15,16]

Based on these facts, this study has been planned for comparing the possible harmful effects of two different detergents (one of them is known to be environment friendly) using a mutagenicity assay.

In this study it was hypothesized that, there would be a significant mean difference in terms of promoting mutations between the groups. The regular detergent will show more mutagenic effect, followed by environment friendly detergent.

[14] "Van Gölü'nde yaşayan inci kefali (Chalcal burnus tarichi) balığı ve su piresi (Daphnia sp.) için deterjan kirliliğinin etkisi" 4 Nov 2009 <http://www.ekolojidergisi.com.tr/resimler/10-5.pdf>

[15] "Damaging Effect of Detergents on Human Lymphocytes" Bulletin of Environmental Contamination and Toxicology

[16] "Mutagenesis of the Metabolite of Nonionic Detergents in Water" DC, Water Resources Research Center. Report No. 73

III. Method Development and Planning

Before deciding to perform The Ames *Salmonella*/microsome mutagenicity assay, I planned to use Daphnia for comparing the harmful effects of environment friendly and regular detergent, because It would be easier. Moreover, there are many studies performed on Daphnia which has an important place in the ecosystem. Placing different dilutions of two detergents into petri plates containing same numbers of Daphnia in 10 milliliters of water, number of live Daphnia was counted every five minutes. I planned to measure average survival time of Daphnia in different dilutions of both detergents. It was hypothesized that the more harmful detergent would kill more Daphnia in the same time period. Unfortunately this method didn't work since it was practically impossible to differentiate dead organisms from living ones by direct inspection. Therefore I decided to select another tool for testing my hypothesis, and after performing a literature search I reached The Ames *Salmonella*/microsome mutagenicity plate incorporation assay. It is a short-term and well standardized method, and some university laboratories perform this test routinely, and this makes this test suitable for my study. I had technical and laboratory support from the Molecular Biology Laboratory of Hacettepe University Faculty of Biology, for performing this assay.

Being one of the most cited tests for screening mutagenic effects of chemicals[13] in literature, this test is a bacterial reverse mutation assay specifically designed for detecting the damaging effects of various chemicals on genetic material that leads to gene mutations. Selected *Salmonella* strains developed by Dr.Ames[17,18] are used in this test. These are histidine dependent strains each carrying different mutations in various genes in the histidine operon (genes involved in histidine metabolism). These mutated strains are unable to synthesize the necessary aminoacid, histidine, and therefore unable to grow and form colonies in the absence of histidine.

If mutagenic substances cause new mutations at the site of preexisting mutations, or nearby in the genes, they can restore gene's function and the cells gain their ability to synthesize histidine. These mutant bacteria can grow and form colonies in the absence of

[17] "Carcinogens are mutagens: their detection and classification." Environ Health Perspect
[18] "An improved bacterial test system for the detection and classification of mutagens and carcinogens." Proc Natl Acad Sci USA.

histidine. This assay is also called “revision assay”, mutant bacteria is called revertant bacteria and the colonies formed by the revertant bacteria are called revertant colonies.[13]

These mutations act as hot spots for chemical mutagens. The meaning of growth of the *Salmonella* tester strains on a minimal media agar plate containing a trace of histidine is; “these bacteria acquire a new mutation that allows them to revert to histidine independence and to form colonies”. These strains also mutate in the absence of mutagens, and the number of spontaneously induced revertant colonies per plate is relatively constant. These numbers are given in the literature as 20-50 colonies and 75-200 colonies for TA98 and TA100 strains.[13]

Mutagenic substances usually increase the number of revertant colonies per plate in a dose-related manner. Special *Salmonella* strains used in this assay. These strains have different mutations in various genes involved in histidine metabolism; each of these mutations is designed to detect mutagens that act via different mechanisms.

Three plates per each dilution of detergents are used as it is recommended in the article[13] and the standards of the laboratory where the assay performed. Three different dilutions and three plates for each dilution is recommended because it is enough to obtain the desired data for the assay. The colony numbers obtained per plate is also found to be similar to data reported in literature

The bacteria used for assay:

In toxicity assay *Salmonella* strain TA100, in mutagenicity assay *Salmonella* strain TA98 and *Salmonella* strain TA100 were used. These two strains have relatively low colony formation rates, in the absence of mutagenic chemicals, the spontaneous formation rate of revertant colonies (spontaneous mutation rate of strains) per assay were reported to be 20-50 and 75-200 respectively. The low colony formation rate makes the colony counting procedure easier. TA98 is sensitive for frameshift mutations and TA100 is sensitive for base-pair substitution mutations, and these two strains are accepted to be sufficient for a mutagenic screening.[13]

Toxicity Assay

Mortelmans indicated that a preliminary toxic dose range experiment should be conducted to determine an appropriate dose range and the top dose for the mutagenicity assay in order to obtain successful results from mutgenicity assay.[13] In our assay different dilutions of both detergents, which are also used for the mutagenicity assay ($1x10^{-2}$, $1x10^{-3}$ and $1x10^{-4}$) were tested in the overnight *Salmonella* nutrient broth cultures. TA100 strain and an enriched, non-selective agar medium were used in toxicity assay. The tested dilutions of two detergents were found to be non-toxic for tested strain. If planned testing dilutions of these detergents were found to be toxic for *Salmonella* strain TA100, it could not be possible to perform mutagenicity assay.

Materials

- Autoclave
- Boiling water bath
- Centrifuge
- Freezer
- Gas line
- General laboratory glassware: flasks, bottles, graduated cylinders
- Gloves
- Incubator
- Mask
- Media and reagents
- Petri plates (100x15 mm)
- Pipets (1, 2, 5 and 10 ml)
- Positive control chemicals
- Refrigerator
- Sterile glass tubes
- Test tube racks
- Vortex
- Water bath (43^{o}C to 48^{o}C)
- Water purification system

IV. Method

Mask and gloves must be worn during the procedure at all times to minimize the effect of external contamination to agar plates.

Procedure:

A. *Salmonella* strains TA98 and TA100 meeting all of the criteria described in the article of Mortelmans, are obtained from the Molecular Biology Laboratory of Biology Faculty, Hacettepe University.

B. Toxicity assay performed

1. Nutrient broth (NB) is prepared

(Appendix 1)

2. Nutrient agar (NA) is prepared

(Appendix 2)

3. Top agar is prepared

(Appendix 3)

4. *Salmonella* TA100 strain provided by the laboratory inoculated in 10 ml NB
5. Inoculated NB is incubated in a shaking autoclave at 37°C for 24 hours
6. After incubation, bacteria obtained from this tube inoculated to a new 10 ml NB containing sterile tube for obtaining maximum number of bacteria
7. Inoculated NB is incubated in a shaking autoclave at 37°C for additional 5 hours
8. Serial dilutions of NB culture from 10^{-1} to 10^{-6} are performed using sterile %0.9 sodium chloride solution and vortex
9. Appropriate number of NA plates and sterile test tubes for each dilution of both detergents and negative controls are labeled

10. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4} (per volume) are prepared for both detergents
11. Top agar is melted and maintained at 45^{o}C
12. To the 13x100 mm sterile glass tubes maintained at 45^{o}C, following items are added in the following order with vortexing
    1. 2.5 ml of melted top agar
    2. 0.05 ml of the detergent dilution
    3. 0.1 ml overnight culture of the *Salmonella* strain TA100 (from 10^{-6} diluted NB culture)
    4. The contents of the test tubes are then mixed with vortex and poured onto the surface of NA plates
    5. When the top agar has hardened, the plates are inverted and placed in a 37°C incubator for 24 h
13. In the control plate of toxicity assay only 0.05 ml of sterile %0.9 sodium chloride solution is added to test tube instead of tested dilutions of detergents
14. Colonies are counted and the results are expressed as the number of colonies per plate.

C. Mutagenicity assay performed

1. Nutrient broth (NB) is prepared

(Appendix 2)

2. Glucose minimal agar (GMA) plate is prepared

(Appendix 4)

3. Top agar supplemented with histidine/biotin is prepared

(Appendix 5)

4. *Salmonella* TA98 and TA100 strains provided by the laboratory inoculated in 10 ml of NB separately
5. Steps 5-6-7 described in toxicity assay are performed for each strain.

6. Appropriate number of GMA plates and sterile test tubes for each *Salmonella* strain, each dilution of both detergents, negative and positive controls are labeled
7. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4} (per volume) are prepared for both detergents
8. Top agar supplemented with histidine/biotin is melted and maintained at $45^{o}C$
9. To the 13x100 mm sterile glass tubes maintained at $45^{o}C$, following items are added in the following order with vortexing
    1. 2.5 ml of melted top agar supplemented with histidine/biotin
    2. 0.05 ml of the detergent dilution
    3. 0.1 ml overnight culture of the *Salmonella* strain TA98 or TA100
    4. The contents of the test tubes are then mixed with vortex and poured onto the surface of GMA plates
    5. When the top agar has hardened, the plates are inverted and placed in a 37°C incubator for 48 h
10. In the negative control plates of mutagenicity assay only 0.05 ml of sterile %0.9 sodium chloride solution is added to test tubes instead of tested dilutions of detergents for each *Salmonella* strain
11. In the positive control plates of mutagenicity assay; 1.5 µgr sodium azide for TA100 strain, 6 µgr daunomycine for TA98 strain, are added to test tubes in a volume of 0.05 ml solution instead of tested dilutions of detergents
12. The colonies are then counted and the results are expressed as the number of colonies per plate

The assay procedure is summarized in Figure 1.

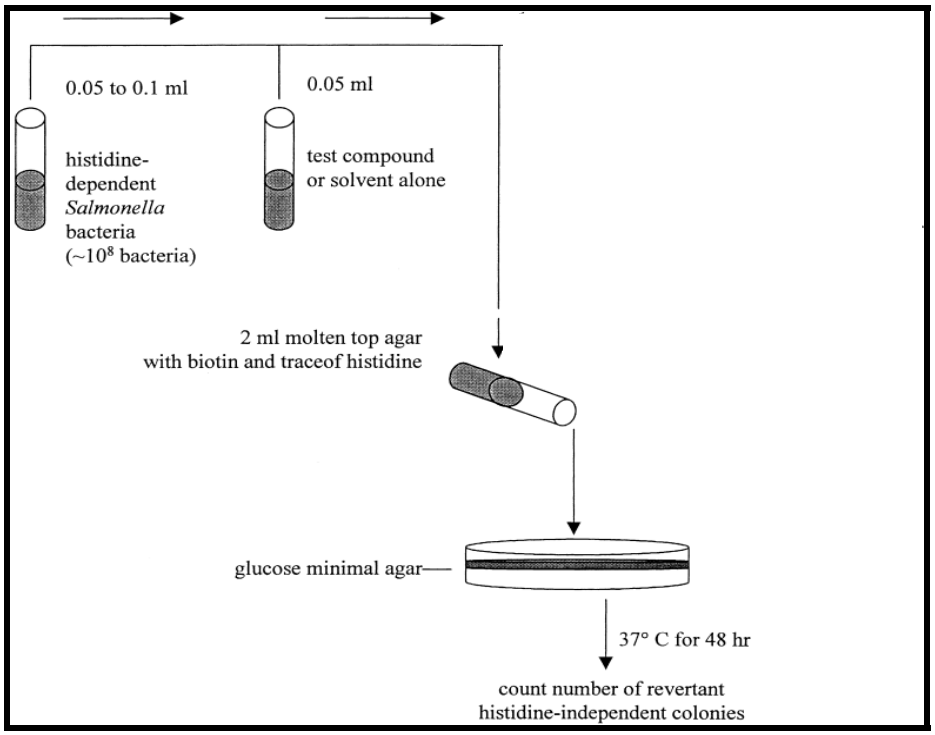


Figure 1. The Ames test procedure[13]

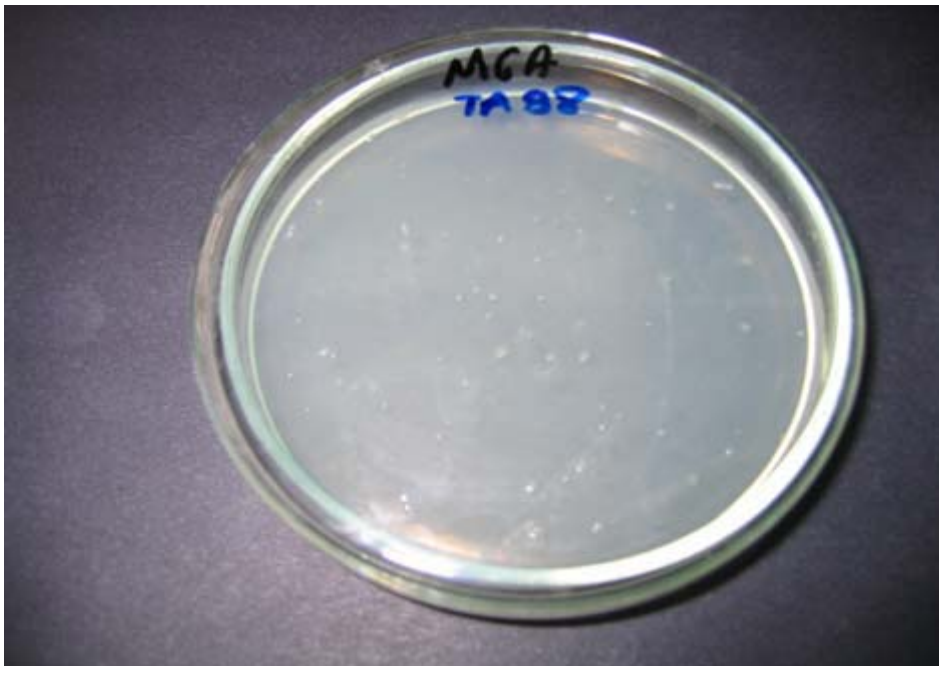


Figure 2. Spontaneous revertant colony formation of TA98 strain in control plate.

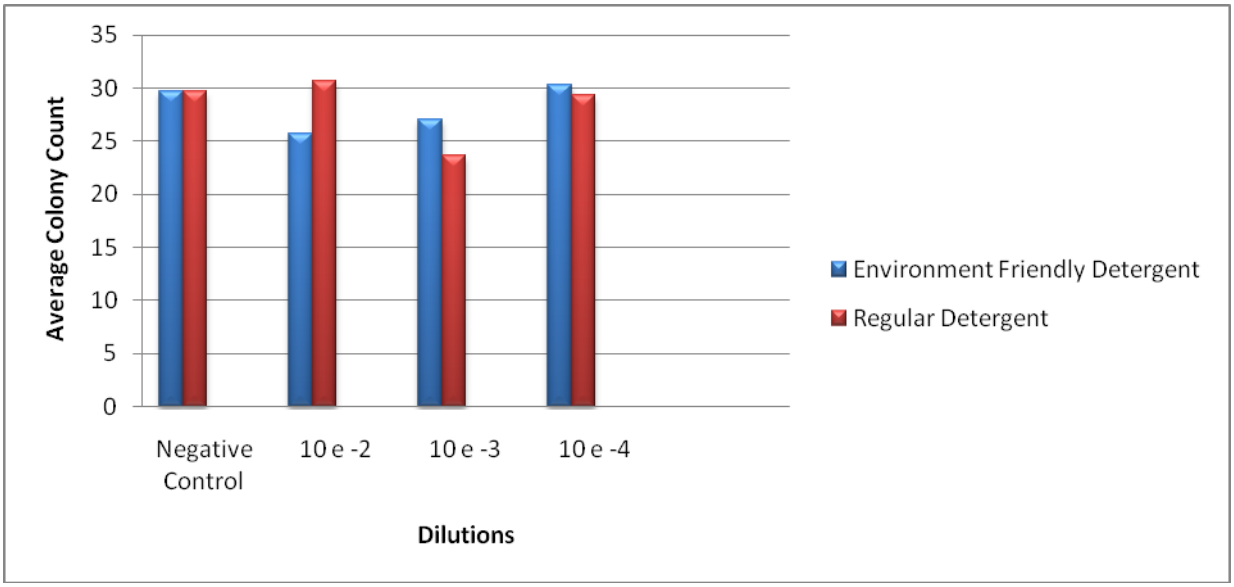
Results

Results obtained from the toxicity and mutagenicity assays are depicted in the following tables and graphs In Table 1 and Graph 1. Results given on columns are and standard deviations (SD) of *Salmonella* colonies obtained from three different petri plates for the same dilution of particular detergent.

Table 1. The average colony counts obtained for different dilutions of two different detergents in toxicity essay

Group	**Dilution**							
	10^{-2}		**10^{-3}**		**10^{-4}**		**Negative Control**	
	Average	SD	Average	SD	Average	SD	Average	SD
Environment Friendly Detergent	25,66	± 3,21	27,00	±3,60	30,33	±0,57	29,66	±1,52
Regular Detergent	30,66	±4,04	23,66	±2,51	29,33	±5,03	29,66	±1,52

Graph 1. The mean average colony counts obtained for different dilutions of two different detergents in toxicity essay



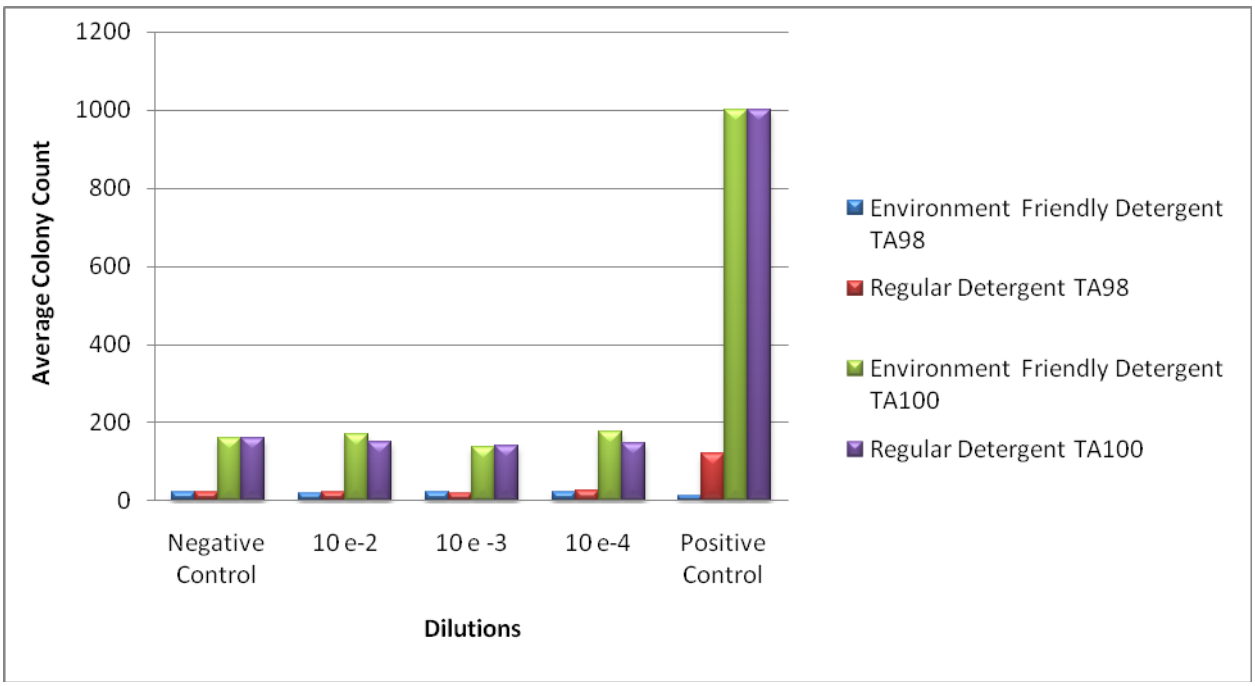
In Table 2 and Graph 2, the results given on columns are average and standard deviations of *Salmonella* colonies obtained from three different petri plates for the same dilution of particular detergent, and same particular *Salmonella* strain.

Table 2. The average colony counts obtained for different dilutions of two different detergents for TA98 and TA100 strains in mutagenicity essay

Group	**Dilution**									
	10^{-2}		**10^{-3}**		**10^{-4}**		**Negative Control**		**Positive Control**	
	Avrg* colony count	SD	Avrg colony count	SD	Avrg colony count	SD	Avrg colony count	SD	Avrg colony count	SD
Environment Friendly Detergent TA98	19,33	1,15	22,33	1,52	21,33	2,88	21,33	4,04	12,66	24,94
Regular Detergent TA98	21,33	2,88	19,66	1,52	27,33	0,57	21,33	4,04	121,66	24,94
Environment Friendly Detergent TA100	169,33	38,85	136	10	176	21,16	162	10	1000	0
Regular Detergent TA100	149,33	12,22	142	14	148	10	162	10	1000	0

*Avrg: Average

Graph 2. The average colony counts obtained for different dilutions of two different detergents for TA98 and TA100 strains in mutagenicity essay



The detailed results obtained from the toxicity and mutagenicity assays are depicted in the tables given in Appendix 6.

VI. Data Analysis

Statistical analysis is performed by SPSS w11 computer software. For detecting the difference between groups ANOVA analysis is used. In case of a difference between groups, Waller-Duncan[a,b] subgroup analysis performed to find out the groups which are causing the difference.

The results of ANOVA analysis of the data are given on the tables below. In Table 3, the results of toxicity essays are evaluated by using ANOVA test.

Table 3. The P values obtained by ANOVA analysis for the results of toxicity essay

Group	**Dilution**				
	10^{-2}	**10^{-3}**	**10^{-4}**	**Negative Control**	**P**
Environment Friendly Detergent	25,66 ± 3,21	27,00 ±3,60	30,33 ±0,57	29,66 ±1,52	0,161
Regular Detergent	30,66 ±4,04	23,66 ±2,51	29,33 ±5,03	29,66 ±1,52	0,145

P values for both detergents were found to be greater than 0.05. The different dilutions of those two detergents were found to be comparable to control, and these detergents are accepted to be non-toxic at these dilutions.

In Table 4, the results of mutagenicity essays are also evaluated by using ANOVA test.

Table 4. The P values obtained by ANOVA analysis for the results of mutagenicity essay

Group	Dilution				
	10^{-2}	**10^{-3}**	**10^{-4}**	**Negative Control**	**Positive Control**
Environment Friendly Detergent TA98	19,33 [a] ±1,15	22,33 [a] ±1,52	21,33 [a] ±2,88	21,33 [a] ±4,04	12,66 [b] ±24,94
Regular Detergent TA98	21,33 [a] ±2,88	19,66 [a] ±1,52	27,33 [a] ±0,57	21,33 [a] ±4,04	121,66 [b] ±24,94
Environment Friendly Detergent TA100	169,33 [a] ±38,85	136,00 [a] ±10	176,00 [a] ±21,16	162 [a] ±10	1000 [b] ±0
Regular Detergent TA100	149,33 [a] ±12,22	142,00 [a] ±14	148,00 [a] ±10	162,00 [a] ±10	1000 [b] ±0

[a] $p>0.05$; No statistically significant difference between dilutions according to ANOVA analysis for each individual detergent and individual *Salmonalla* strain (ie for each line)

[b] $p<0.05$; The positive control group which is found to be statistically different according to ANOVA analysis (for each line)

In all groups, only the positive control tests were found to be statistically different from control and different dilution tests.

The interpretation of these data clearly demonstrates us, our testing procedure is working as it is desired, because the positive controls for each essay found to induce mutations as expected.

On the other hand, no statistically significant differences observed between control and different dilution tests of two detergents, which mean the tested dilutions of these two detergents, are not mutagenic.

The detailed results of ANOVA analysis of the data are given on the tables listed in Appendix 7.

VII. Evaluation

The aim of this study was to find out whether there was a significant mean difference in terms of inducing mutations in *Salmonella* strains between a regular detergent and environmental friendly detergent, as an indicator of being harmful to the nature. It was hypothesized that there would be a significant mean difference in terms of promoting mutations between groups. Regular detergent will show more mutagenic effect, followed by environment friendly detergent.

Both of the detergents did not show mutagenic effect at the end of my experiment, while the positive control tests were found to be statistically different from control and different dilution tests. That means the essay is valid for detecting the mutagenic substances. The mutation values obtained were expressed as number of mutants (colonies)/plate.

My null hypothesis was that there was no significant mean difference between regular detergent and environment friendly detergent in terms of promoting mutations in *Salmonella* tester strains. As the statistical analysis performed by using Waller-Duncan[a,b] subgroup analysis for detecting the groups causing difference for subset for alpha=0.05, revealed that all tested dilutions of both detergents were not different from controls, i.e. they were not causing mutations, my null hypothesis was verified (see Appendix 7).

My hypothesis which was “the regular detergent will show more mutagenic effect, followed by environment friendly detergent” has been rejected by the results of experiments and data analysis (see Appendix 7).

During the experiment there were no unexpected occurrences that may have affected the results of the experiment.

In this essay the mutagenic effects of the metabolites of two different detergents have not been checked, only the mutagenic effects of the original compounds tested. To perform this essay for checking also the harmful effect of the metabolites of these detergents was exceeding the level of my knowledge and my abilities.

Another limitation was the limited technology of the laboratory I was studying in; as I am collecting data I had to count the colonies in each Petri with the help of marker, but in high-budget laboratories there are special microscopes for counting colonies in those Petri and the data collected by those microscope is more reliable, hence some colonies are too small to be recognized.

The results of my experiment could not be generalized because I have compared the effects of two specific brands of detergents; however, the data obtained is insufficient to generalize results.

Although the harmful effects of detergents on nature and on living organisms are well known, the role of mutagenicity as a component of this process could not be demonstrated.

For future repetitions, the mutagenic effects of metabolites of these detergents should also be investigated for gathering more information on this topic.

VIII. Conclusion

My research question: “Is there a significant mean difference between regular detergent and environment friendly detergent in terms of promoting mutations in the Ames *Salmonella*/microsome mutagenicity assay?” is answered in the light of the results of my study. There is not a significant difference between promoting mutations of these two detergents in the Ames *Salmonella*/microsome mutagenicity assay, both of the detergents are not causing mutations in Ames test, which is not expected nor hypothesized. Although my hypothesis is not confirmed, a conclusion is acquired after the evaluation of data. The conclusion is; “there is no difference between regular detergent and environment friendly detergent in terms of promoting mutations, so, there is no need to prefer environment friendly detergents which are claimed to be less harmful”

The reason I preferred doing my extended essay on this subject was the discussion whether the environment friendly detergents are less harmful to environment as they claimed or not. I chose to test the mutagenic capacity of detergents as a screening tool for their harmful effects. However the extent of this discussion was too large for my capabilities. So, I decided to limit my study to the mutagenic effects of environment friendly and regular detergents on TA98 and TA100 *Salmonella* strains on the Ames *Salmonella*/microsome mutagenicity plate incorporation assay. I only tested mutagenic effects of main compounds of these detergents. Although there are lots of studies using the Ames *Salmonella*/microsome mutagenicity assay, my essay differs from others; it has never been done and reported before.

It is worth noting that, the scope of this assay is limited, because only the mutagenic effects of main compounds have been tested. The mutagenic effects of their metabolites have not been tested; therefore although the hypothesis of this study is not supported by the data, this should not be interpreted as these detergents do not have mutagenic effects.

Contamination with chemicals and artificial substances (plastics, CFC, etc) is a big danger awaiting environment. Detergents which have been used for cleansing purposes since many decades are also a threat for environment. This observation and competition between the producers force the producers to come up with new detergents with new and

superior properties; such as being environment friendly. The question “are these detergents really environment friendly as their producers claim?” is still need to be investigated in a more detailed manner.

IX. Appendices

a. Appendix 1

Nutrient broth

Use: to grow the tester strains overnight

Ingredients Per liter

Distilled water	1000 ml
Oxoid nutrient broth #2	25 g
Ampicillin solution (0.8% w/v)	1.5 ml

Nutrient broth powder is added to the water and stirred to dissolve. The ampicillin solution is added and stired. The solution is dispensed as 10 ml aliquots in 100x16 mm test tubes. Test tubes are closed with a piece of cotton, then autoclaved for 20 min. When the tubes are cooled, store in the dark at room temperature.

a. Ampicillin solution

Ingredients Per 100 ml

Distilled water	100 ml
Ampicillin	8 mg

The ampicillin is dissolved in warm (65°C) water. Solution is filtered using a 0.45 mm filter and stored at 4°C.

b. Appendix 2

Nutrient agar plates

Use: to test toxicity of compounds (to test for viability of bacteria)

Ingredients Per liter:

Distilled water	1000 ml
Agar	15 g
Oxoid Nutrient Broth #2	25 g

The agar is added to the water in a flask and heated to dissolve. The nutrient broth powder is added and stirred until dissolved. The mixture is autoclaved for 20 min at 121°C., waited to cool to about 65°C., and dispensed 25 ml in sterile petri plates. The agar plates are stored upside down in sealed plastic bags at 4°C.

c. Appendix 3

Top agar

Use: to deliver the bacteria and chemical to the bottom agar

Ingredients Per liter

Distilled water	1000 ml
Agar	6 g
Sodium chloride	6 g

The agar and sodium chloride are added to a flask containing 1000 ml of distilled water. The mixture is heated for 10 min in an autoclave, to melt the agar. Then it is autoclaved for 30°C and stored at room temperature in the dark. When ready to use, the top agar is melted in boiling water.

d. Appendix 4

Glucose minimal (GM) agar plates

Use: bottom agar for mutagenicity assay

Ingredients Per liter

Distilled water	900 ml
Agar	15 g
VB salt solution (50x)	20 ml
Glucose solution (10% v/v)	50 ml

The agar is added to the water in a flask. The mixture is autoclaved for 30 min at 121°C., waited to cool for 45 min to about 65°C. 20-ml of sterile VB salts is added and mixed thoroughly. Then the 50 ml of a sterile glucose (10% v/v) solution is added and solution is mixed. The agar medium is dispensed in 100x15 mm petri dishes (approximately 25 ml/plate). The agar plates are stored upside down in sealed plastic bags at 4°C. (The agar should never be autoclaved together with the VB salts and glucose.)

a. Vogel–Bonner (VB salts) medium E (50x)

Use: salts for the GM agar plates

Ingredients Per liter

Warm distilled water (about 50°C)	650 ml
Magnesium sulfate ($MgSO_4 \cdot H_2O$)	10 g
Citric acid monohydrate	100 g
Potassium phosphate, dibasic, anhydrous (K_2HPO_4)	500 g
Sodium ammonium phosphate ($Na_2NH_2PO_4 \cdot 4H_2O$)	175 g

The above ingredients are added to warm water in a flask, in the order indicated above. Each salt is dissolved thoroughly by stirring well before adding the next salt. It takes about 1 h to dissolve all ingredients. The volume of the mixture is adjusted to 1 liter. The solution is distributed in 20-ml aliquots and autoclaved 30 min at 121°C. When the solutions have cooled, it is stored at room temperature in the dark with tightened caps.

b. Glucose solution (10% v/v)

Use: as carbon source for the GM agar plates

Ingredients Per liter

Distilled water	700 ml
Dextrose	100 g

The dextrose is added to the water in a flask. The solution is mixed well until mixture is clear. Additional water is added to bring the final volume to 1000 ml. The solution is dispensed as 50 ml aliquots into 250 ml screw-cap bottles, and autoclaved 121°C for 20 min. When it is cooled, stored at 4°C with tightened caps.

e. Appendix 5

Top agar supplemented with histidine/biotin

Use: to deliver the bacteria and chemical to the bottom agar

Ingredients Per liter

Distilled water	900 ml
Agar	6 g
Sodium chloride	6 g
Histidine/biotin solution (0.5 mM)	100 ml

The agar and sodium chloride are added to a flask containing 900 ml of distilled water. The mixture is heated for 10 min in an autoclave, to melt the agar. Then, 100 ml of limited histidine and biotion solution (0.5 mM) is added. The mixture is divided 200-ml aliquots in 500-ml screw-cap bottles, autoclaved for 30°C and stored at room temperature in the dark. When ready to use, it is melted in boiling water.

a. Histidine/biotin solution (0.5 mM)

Use: to supplement top agar with excess biotin and a trace amount of histidine

Ingredients Per liter

Distilled water	1000 ml
d-biotin (F.W. 247.)	124 mg
l-Histidine·HCl (F.W. 191.7)	96 mg

The biotin and histidine are added into boiling water. The solution may be sterilized by autoclaving for 20 min at 121°C. Than solution can be stored at 4°C in a glass bottle.

f. Appendix 6

The data obtained from the essay is given on the following tables. In all tables; the results given on petri columns are number of *Salmonella* colonies counted per plate.

SD means standard deviation for the colony counts obtained from three different petri plates for the same dilution of particular detergent, and same particular *Salmonella* strain .

Table 1. Toxicity Essay Results of Environment Friendly Detergent with TA100 Strain

Bacteria	Dilution	1st petri	2nd petri	3rd petri	Mean	SD
Negative control	--	30	28	31	29,66	1,52
TA100	10^{-2}	27	22	28	25,66	3,21
TA100	10^{-3}	31	24	26	27,00	3,60
TA100	10^{-4}	31	30	30	30,33	0,57

Table 2. Toxicity Essay Results of Regular Detergent with TA100 Strain

Bacteria	Dilution	1st petri	2nd petri	3 rd petri	Mean	SD
Negative control	--	30	28	31	29,66	1,52
TA100	10^{-2}	35	27	30	30,66	4,04
TA100	10^{-3}	26	21	24	23,66	2,51
TA100	10^{-4}	34	24	30	29,33	5,03

Table 3. Mutagenicity Essay Results of Environment Friendly Detergent with TA98 Strain

Bacteria	Dilution	1st petri	2nd petri	3rd petri	Mean	SD
TA98	10^{-2}	20	18	20	19,33	1,15
TA98	10^{-3}	24	21	22	22,33	1,52
TA98	10^{-4}	18	23	23	21,33	2,88
TA98	Negative control	17	25	22	21,33	4,04
TA98	Positive control	103	112	150	121,66	24,94

Table 4. Mutagenicity Essay Results of Environment Friendly Detergent with TA100 Strain

Bacteria	Dilution	1st petri	2nd petri	3 rd petri	Mean	SD
TA100	10^{-2}	160	212	136	169,33	38,85
TA100	10^{-3}	136	146	126	136,00	10
TA100	10^{-4}	168	160	200	176,00	21,16
TA100	Negative control	172	162	152	162,00	10
TA100	Positive control	>1000	>1000	>1000	1000,00	0

Table 5. Mutagenicity Essay Results of Regular Detergent with TA98 Strain

Bacteria	Dilution	1st petri	2nd petri	3 rd petri	Mean	SD
TA98	10^{-2}	23	23	18	21,33	2,88
TA98	10^{-3}	21	20	18	19,66	1,52
TA98	10^{-4}	27	28	27	27,33	0,57
TA98	Negative control	17	25	22	21,33	4,04
TA98	Positive control	103	112	150	121,66	24,94

Table 6. Mutagenicity Essay Results of Regular Detergent with TA100 Strain

Bacteria	Dilution	1st petri	2nd petri	3rd petri	Mean	SD
TA100	10^{-2}	160	152	136	149,33	12,22
TA100	10^{-3}	152	148	126	142,00	14
TA100	10^{-4}	158	148	138	148,00	10
TA100	Negative control	172	162	152	162,00	10
TA100	Positive control	>1000	>1000	>1000	1000,00	0

g. Appendix 7

Below is information on statistical analysis of the data obtained from study. Table 1-2 shows the statistical analysis data of toxicity essays. Table 4-6 depicts the statistical analysis data of mutagenicity essays.

Table 1. ANOVA Results of Toxicity Essay for Environment Friendly Detergent

	Sum of Squares	df	Mean Square	F	**Sig*.**
Between Groups	43,667	3	14,556	2,239	**,161**
Within Groups	52,000	8	6,500		
Total	95,667	11			

***p value=1.61, (>0.05)**

Table 2. ANOVA Results of Toxicity Essay for Regular Detergent

	Sum of Squares	df	Mean Square	F	**Sig*.**
Between Groups	90,000	3	30,000	2,384	**,145**
Within Groups	100,667	8	12,583		
Total	190,667	11			

***p value=1.45 (>0.05)**

Table 3. ANOVA Results of Mutagenicity Essay for Regular Detergent with TA98 Strain

	Sum of Squares	Df	Mean Square	F	**Sig*.**
Between Groups	23743,600	4	5935,900	45,684	**,000**
Within Groups	1299,333	10	129,933		
Total	25042,933	14			

***p value=0,000 (<0,05) indicates there is a difference between groups**

Waller-Duncan[a,b]

		Subset for alpha = 0.05	
Dilution	N	1	**2**
-3,00	3	19,6667	
-2,00	3	21,3333	
Negative control	3	21,3333	
-4,00	3	27,3333	
Positive control	3		**121,6667**

The groups causing the difference are shown in column 2
The positive control group is causing the difference

Table 4. ANOVA Results of Mutagenicity Essay for Regular Detergent with TA100 Strain

	Sum of Squares	Df	Mean Square	F	***Sig.**
Between Groups	1733276,267	4	433319,067	3972,974	**,000**
Within Groups	1090,667	10	109,067		
Total	1734366,933	14			

***p value=0,000 (<0,05) indicates there is a difference between groups**

Waller-Duncan[a,b]

		Subset for alpha = 0.05	
Dilution	N	1	**2**
-3,00	3	142,0000	
-4,00	3	148,0000	
-2,00	3	149,3333	
Negative control	3	162,0000	
Positive control	3		**1000,0000**

The groups causing the difference are shown in column 2
The positive control group is causing the difference

Table 5. ANOVA Results of Mutagenicity Essay for Environment Friendly Detergent with TA98 Strain

	Sum of Squares	Df	Mean Square	F	***Sig.**
Between Groups	24295,067	4	6073,767	46,673	**,000**
Within Groups	1301,333	10	130,133		
Total	25596,400	14			

***p value=0,000 (<0,05) indicates there is a difference between groups**

Waller-Duncan[a,b]

		Subset for alpha = 0.05	
Dilution	N	1	**2**
-2,00	3	19,3333	
-4,00	3	21,3333	
Negative control	3	21,3333	
-3,00	3	22,3333	
Positive control	3		**121,6667**

The groups causing the difference are shown in column 2
The positive control group is causing the difference

Table 6. ANOVA Results of Mutagenicity Essay for Environment Friendly Detergent with TA100 Strain

	Sum of Squares	Df	Mean Square	F	***Sig.**
Between Groups	1692842,667	4	423210,667	980,865	**,000**
Within Groups	4314,667	10	431,467		
Total	1697157,333	14			

***p value=0,000 (<0,05) indicates there is a difference between groups**

Waller-Duncan[a,b]

		Subset for alpha = 0.05	
Dilution	N	1	**2**
-3,00	3	136,0000	
Negative control	3	162,0000	
-2,00	3	169,3333	
-4,00	3	176,0000	
Positive control	3		**1000,0000**

The groups causing the difference are shown in column 2
The positive control group is causing the difference

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