Please make any changes to the department's purpose statement, goals, or assessment cycle chart. Remember each SLO must be assessed between program review cycles.

Natural Science Purpose Statement:

The Department of Natural Science provides:

- A career-oriented approach to pre-professional preparation in the health sciences
- A liberal arts and sciences approach to environmental health and sustainability

- A hands-on approach to education in the laboratory and through student research
- Preparation to meet the Kansas State Department of Education standards for licensure in biology and chemistry

Program Student Learning Outcomes	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022	2023-2024
A career-oriented approach to pre-professional preparation in the health sciences	Program Review	Assessment Planning		X			Program Review-Spring	X					Program Review-Spring
A liberal arts and sciences approach to environmental health and sustainability				X					X				
A hands-on approach to education in the laboratory and through student research				X						X			

Preparation to meet the Kansas State Department of Education standards for licensure in biology and chemistry				X							X		

Environmental Stewardship Major:

This program achieves its purposes when its graduates:

Program Student Learning Outcomes	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022	2023-2024
Demonstrate knowledge of contemporary theories in the natural sciences	Program Review	Assessment Planning	X				Program Review-Spring					X	Program Review-Spring
Demonstrate skill in the application of laboratory and field experimental techniques			X								X		
Demonstrate knowledge of contemporary theories of human social systems and behavior			X							X			
Demonstrate a critical understanding of their personal roles in history, culture, and community			X						X				

Evaluate the impacts of human society and Earth's natural systems on one another			X					X					
Differentiate between their personal belief system and societal belief systems			X						X				
Express a personal environmental ethic			X							X			

1. Select Your Department	Natural Science
2. Has the program's purpose, SLOs, or program review year changed from the above document?	No
Upload the edited Purpose/SLO doc here.	app.captainform.com/upload_dld.php?fileid=7b49591c93ba2d47
3. Will/did the department submit a program review this year? If yes, please attach the final review as a pdf document.	No
Upload program review pdf.	app.captainform.com/upload_dld.php?fileid=33776c418daa142
1. Please select the type of direct evidence of student learning that was gathered THIS YEAR.	
-Assignment/exam paper completed as part of regular coursework and used for program-level	yes
-Capstone work product (e.g. written paper,	yes
-Exam created by department or external agency	yes
-Oral performance (e.g. oral presentation,	yes
Please describe any other direct evidence gathered this year	The outcome chosen for assessment this year (see Natural Science Department 2016-2017 Assessment Plan.docx) is that "• Graduates will have demonstrated the ability to communicate scientific information in both written and oral form." Two senior students submitted manuscripts for publication in the journal BIOS, a publication of the National Biological Honor Society, Beta Beta Beta. All seniors presented their research at the annual Research Forum, and published their research in our journal Cantaurus. All of the juniors wrote and presented a formal Research Proposal in NS300, NS375.
2. Please select the type of indirect evidence of student learning that was gathered THIS YEAR.	
-Student surveys (course evaluation additional questions, program created, from institutional student survey data) contain reports of SLO achievement	yes
Please describe any other indirect evidence gathered this year:	
3. Please select how the evidence was evaluated, analyzed, or interpreted.	
-Used a rubric/scoring guide	yes
-External organization/person analyzed the data (e.g. external accrediting/licensure organizations, standardized exam company)	yes
Please describe any other methods not listed:	Course grades for NS300, NS375, and NS475
4. Enter the number of students assessed for each SLO.	23
5. Summarize the results of the assessment activities including the percentage of students that met or exceeded each SLO and a list of student learning strengths and weaknesses.	The student grade distribution in the Junior course sequence NS300, NS375 was: 6A:7B:3C:1F and in NS475 was: 6A, 1IP
6. Please upload any supporting documentation (i.e. rubrics, data analysis, charts/tables, department minutes, etc.)	app.captainform.com/upload_dld.php?fileid=a95063ae9d76315e8ad52b9d57434649
7. What describes how the program plans to use the results?	
-Assessment procedure changes (SLOs, curriculum map rubrics, evidence collected, sampling, communications with faculty, etc.)	yes

-Course changes (course content, pedagogy, courses offered, new course, pre-requisites, requirements)	yes
-Use is pending (typical reasons: insufficient number of students in population, evidence not evaluated or interpreted yet, faculty discussions continue)	yes
Please describe any other uses not listed:	The department faculty will complete our curriculum mapping in Fall 2018. Minor revisions will be made to the syllabi of NS300, NS375, and NS475 for 2018-19. Faculty discussion of those changes should conclude by the start of Fall 2018.
8. What program changes or modifications to improve student learning were made this year based on last year's assessment results?	Last year we submitted our Program Review/Departmental Growth Plan, and so did not have assessment results, per se. There has been a significant amount of work done on the admissions and development portions of the plan, and we've increased the capacity of our courses to accommodate our growth. Two new courses have been added to our schedule for 2018-19: Bioinformatics, and Green Chemistry.
If you have supporting data please include it. (Previous department reports can be found at Step 01. Use the Previous button below.)	
1. The department will submit a program review NEXT YEAR.	No
2. SLO(s) the department will assess NEXT YEAR is/are:	The department has written SLOs for each of the majors we offer, and the assessment reporting is based on SLOs for the department that were extracted from the department's Purpose Statement as reported in the college's Catalog. The department and assessment office need to in sync about at what level we should be focusing our efforts.
3. Please select the type of direct evidence of student learning the department plans to use NEXT YEAR.	
-Capstone work product (e.g. written paper, presentation, research)	yes
-Exam created by department or external agency	yes
-Oral performance (e.g. oral presentation, conference presentation)	yes
Please describe any other direct evidence planned for next year:	
4. Please select the type of indirect evidence of student learning that was gathered NEXT YEAR.	
-Student surveys (course evaluation additional questions, program created, from institutional student survey data) contain reports of SLO achievement	yes
Please describe any other indirect evidence planned for next year:	
5. Based on previous assessment data, what percentage of students does the department expect to meet or exceed the SLO(s)?	90
6. Is the department interested in learning how to apply for an assessment grant?	No

Guidelines for Grading Formal Writing

[*The following set of guidelines for grading writing is taken from* Language Across the Curriculum at McPherson College: A Handbook, *which credits the guidelines to Barbara Walvoord in* Helping Students Write Well: A Guide for Teachers in All Disciplines, 2/e (MLA, 1982), p.152.]

A: The paper is well organized, even at the paragraph level. Sentences are smooth and carefully crafted. There are virtually no errors in punctuation or spelling, grammar or usage. Words are chosen with precision. Informal language or dialect is used only when appropriate. The paper avoids triteness and generalizations; the language is fresh and vivid. The paper is tight, not wordy.

B: The work is well organized, but the paragraph structure may sometimes be disjointed. The paper may have a few awkward passages and some errors in punctuation, spelling, grammar, or usage. The language may at times be too general; it may lack the freshness or precision of the A paper, but none of these errors is glaring or highly distracting.

C: The paper is basically well organized, though individual paragraphs may be disunified or misplaced. Generally, however, the paper shows that the writer has followed a logical plan. The writing is competent but wordy, general, imprecise, or trite. Sentences may at times be awkwardly constructed, but their meaning is clear. Grammar, punctuation, and spelling are not highly distracting, but there may be some errors.

D: The paper is poorly organized, though there is a recognizable thesis. Some of the sentences or passages may be so confused that their meaning does not clearly emerge. Words may be imprecise, incorrect, trite, or vague. In general, however, the paper is understandable.

F: The paper lacks a clear thesis, the language is so muddled as to be unclear in several spots, or the errors in punctuation, spelling, grammar and usage are highly distracting.

Guidelines for Grading Formal Speech

[The following set of Guidelines for Grading Formal Speech is based on the Guidelines for Grading Formal Writing *taken from* Language Across the Curriculum at McPherson College: A Handbook, *which credits the guidelines to Barbara Walvoord in* Helping Students Write Well: A Guide for Teachers in All Disciplines, 2/e (MLA, 1982), p.152.]

A: The oral presentation is well organized, indicating knowledge of the full range of issues relevant to the topic. Sentences are smooth and carefully crafted. Words are chosen with precision. Informal language or dialect is used only when appropriate. The speech avoids triteness and generalizations; the language is fresh and vivid. The speech time is fully and fruitfully used.

B: The oral presentation is generally well organized, indicating knowledge of the major issues relevant to the topic. The presentation may sometimes be disjointed, with a few awkward passages and some errors in grammar or usage. The language may at times be too general; it may lack the freshness or precision of the A speech, but none of these errors is glaring or highly distracting.

C: The oral presentation is basically well organized, indicating knowledge of some issues relevant to the topic, though some crucial gaps in knowledge are evident. Generally, however, the speech shows that the student has followed a logical plan. The speech is competent but wordy, general, imprecise, or trite. Sentences may at times be awkwardly constructed, but their meaning is clear. Grammar and usage are not highly distracting, but there may be some errors.

D: The speech is poorly organized, and though there is a recognizable thesis, gaps in knowledge and lapses in reasoning indicate insufficient preparation. Some of the sentences or passages may be so confused that their meaning does not clearly emerge. Words may be imprecise, incorrect, trite, or vague. In general, however, the speech is understandable, but contributes little to scientific discourse.

F: The speech lacks a clear thesis, the language is so muddled as to be unclear in several spots, and errors in grammar and usage are highly distracting. The student's limited verbal contribution provides insufficient evidence of their preparation for or substantive contributions to scientific discourse.

McPherson College

Natural Science Research Forum

Division of Science and Technology

1:00pm, April 20, 2018
Melhorn Science Hall, Rm 112

McPherson College

The 35th Natural Science Research Forum

1:00 – 1:05	**Welcome and Introductions**
1:05 – 1:20	**Nora Grosbach:** *Mycoplasma pulmonis* in *Rattus norvegicus*: isolation and microbial diversity
1:20 – 1:35	**Andrea Kadeba:** The effects of stress-induced mutation using EMS on ethanol tolerance in *Saccharomyces cerevisiae*
1:35 – 1:50	**Yann Kadeba:** The synthesis of an oxytetracycline derivative
1:50 – 2:05	**Break**
2:05 – 2:20	**Amy Makovec:** Epigenetic effects of stress on survival in *Caenorhabditis elegans*
2:20 – 2:35	**Chantelle Theron:** Colorimetric determination of acetaminophen degradation in blood samples stored at 24°C and 40°C
2:35 – 2:50	**Evan Willow:** Efficacy of intravenous aspirin on blood clotting after *Vipera russelli* injection

Mycoplasma pulmonis in *Rattus norvegicus*: isolation and microbial diversity

Nora Grosbach

The presence of *Mycoplasma pulmonis* is very common in domestic and laboratory rats. *Mycoplasma* is the smallest bacterial cell species that was been discovered thus far. It can survive without oxygen, comes in various shapes, and lacks a cell wall. It is penicillin resistant and can be parasitic or saprotrophic. Depending on where the bacteria is located, genitalia or in the upper respiratory system, it will cause different disorders and diseases. The presence of these bacteria can cause many problems in the health of the person or animal. It poses large problems in laboratory settings and any impending experiments. This article observes the diversity of the microbiota in the throat and nose of rats, as well as the change in the ratio of *M. pulmonis* to the other microbiota at different levels of health; healthy, sick, and dead. The results of this study conclude that there is not a significant difference between the mean ratios of *M. pulmonis* to other bacteria along the decline in health of the rat.

Keywords*: Isolation, microbial diversity, Murine Respiratory Disease, Mycoplasma pulmonis, Rattus norvegicus*

The effects of stress-induced mutation using EMS on ethanol tolerance in *Saccharomyces cerevisiae*

Andrea Kadeba

Ethanol is a multifunctional compound that has many uses and can be made naturally by sugar-fermenting yeast such as *Saccharomyces cerevisiae*. However, ethanol is toxic to yeast, so the process is not as efficient as it could be. This research aims to identify an efficient mechanism to increase the ethanol tolerance in *Saccharomyces cerevisiae* using various techniques such as ethanol as a stressor, artificial selection, and ethyl methanesulfonate (EMS) as a chemical mutagen. *Saccharomyces cerevisiae* cells were repeatedly exposed to increasing levels of ethanol from 9%-27% and EMS. The parental strain was defined to have an ethanol tolerance of 13%; the artificial selection strain (only exposed to ethanol as a stressor and artificially selected after each round) was defined to have an ethanol tolerance of 16%; the EMS-exposed strain (also exposed to ethanol as a stressor and artificial selection) was defined to have an ethanol tolerance limit at 27%. To test differences between strains, the parental strain, selection strain, and the EMS-exposed strain were separately plated on ten 27% ethanol plates and ten 0% ethanol plates and growth was checked after 24 hours. The EMS-exposed strain was the only strain that grew at 27% ethanol; all strains grew at 0% ethanol. These results show that EMS, artificial selection, and ethanol as a stressor might be effective in producing strains of *Saccharomyces cerevisiae* that are able to produce greater amounts of ethanol before toxicity sets in.

Keywords: *Saccharomyces cerevisiae, Ethyl methanesulfonate, artificial selection, ethanol tolerance, chemical mutagenesis, molecular genetics, microbiology*

The synthesis of an oxytetracycline derivative

Yann Kadeba

Antibiotics have been used for the past few centuries, and their misuse and overuse has contributed to the rise of antibiotic-resistant pathogens. Therefore, it is of great importance to synthesize novel antibiotics that may be effective against these new superbugs, which is the aim of this research. For this research project, my objective was to take a known antibiotic and change one or more of its functional groups to synthesize a novel antibiotic. Oxytetracycline was used as the starting material for the reaction with hydrochloric acid and n-chlorosuccinimide as reagents. The product of the reaction was characterized using analytical techniques such as NMR and IR spectroscopy.

Keywords: *antibiotics, organic synthesis, oxytetracyline, spectroscopy*

Epigenetic effects of stress on survival in *Caenorhabditis elegans*

Amy Makovec

Caenorhabditis elegans are members of the nematode family and have been model organisms for epigenetic studies for many years due to their small size, transparent body, short life cycle, and easy maintenance. For this study, *C. elegans* were used to explore the epigenetic effects of stress on the survival of the worms. The animals in this study were subjected to the environmental stressor starvation and showed an increase in overall lifespan based on the statistical analysis of a Kaplan-Meier Survivorship test. This increased survival rate was also found to be passed to at least three subsequent generations who were grown under unstressed conditions. These results showcase the hormetic epigenetic effects of starvation in *Caenorhabditis elegans.*

Keywords: *Epigenetics, Stress, Caenorhabditis elegans*

Colorimetric determination of acetaminophen degradation in blood samples stored at 24˚C and 40˚C

Chantelle Theron

Acetaminophen (APAP) overdose fatalities have decreased slightly over the last few years but still remain ranked in the top 10 by the American Association of Poison Control. Due to APAPs chemical composition it can undergo various possible degradation pathways. Previous studies utilized thermal stressing and measured the rate of degradation by High Performance Liquid Chromatography, however the degradation of APAP within biological samples has not been well established. In this study the degradation of APAP was studied in bovine blood at 24°C and 40°C over a four week period. When comparing the initial and final concentration of APAP in the blood samples, the final measurement was 14.41% higher for the 240C and 11.79% higher for the 400C samples. However, results obtained were not statistically significant enough to conclude that the change was due to the storage of the blood samples at 24°C and 40°C. There was a slight increase in the first measurement recorded for both the 24°C and 40°C samples which could suggest that there was a loss of volume upon storage.

Keywords: *Acetaminophen, Overdose, Blood, Colorimetric*

Efficacy of intravenous aspirin on blood clotting after *Vipera russelli* injection

Evan Willow

Vipera russelli, more commonly known as the Russell's Pit Viper, is one of the deadliest snakes in all of South East Asia. The venom causes coagulation by activating the factor X enzyme causing the Factor XA enzyme to initiate the coagulation cascade. The antivenin used to counteract the effects of the venom is readily available in hospitals or wealthier households or businesses. However, the majority of the population in South East Asia are low income families and cannot afford the antivenin. A cheap alternative to the antivenin is intravenous aspirin, it can be easily purchased by the majority of population for a fraction of the cost. In this experiment, six trial groups were constructed, three control groups, and three different concentrations of intravenous aspirin were used test the efficacy against the coagulation effects of the Vipera russelli venom. The viscosity of the pig blood was measured by doing timed tilt trials which were recorded digitally. The result showed statistically that the .13 mg/ml and .26 mg/ml intravenous aspirin groups showed no significant change between the control group. However, qualitatively those two aspirin groups did have some clotting in the vials during their tilt period even though statistically they had no significant change from the control. This concludes that .13 mg/ml aspirin and .26 mg/ml aspirin can prolonging the coagulation effects of the *Vipera russelli* venom.

Keywords: *Antivenin, Coagulation, Non-Parametric ANOVA, Vipera Russelli, 2-Acetoxybenzoic Acid*

Natural Science Research Awards

Awards are bestowed on students who demonstrate excellence in research. The highest honor, the **Burkholder Research Award**, honors outstanding achievement. The **Merit Research Award** distinguishes significant achievement. For each award, the Natural Sciences faculty selects one or more candidates based on three criteria: 1) selection and design of a senior research project; 2) quality of the research, including technique, observations, and data analysis; and 3) presentation of the research, comprising preparation of a scientific research paper and an oral presentation to students and faculty. A Certificate is granted to each winner. Persons qualifying for the Burkholder Research Award have their name inscribed on a plaque and receive a year membership in the American Association for the Advancement of Science and a subscription to *Science*.

Year	Burkholder Award	Merit Award
2017	Nathan Finch	Sheryl Evans, Lucas Giesey
2016	Tiffany Fraser, Ashley Long	Alia Khalidi, Kaley Kinnamon
2015	Nathaniel Schowegerdt	Shannon Coldren, Sydney Lipton, Jordan Stewart
2014	Yi Qun Chai, Sean DeYoung	Lori Crain, Alejandro Esparza, Christian Rodriguez
2013	Amanda Baxter, Emily James Taylor Roop	Torey Fry, Kasey Miller
2012	Audrey McTaggart	Savannah Sievers, Andrew Skinner
2011		Karissa Ferrell, Kelley Green, Ashley Zodrow
2010	Ashlee Jost, David Miller	
2009	Adam Horinek	Amanda Pangburn, Nicole Sampson, Lezli Warkentin
2008	Joel Grosbach, Landon Snell, W. Brett Whitenack	Alan Grosbach
2007	Callie Crist	Rhonda Hoffert, Jamie Rodriguez
2006	Travis Allen	Lisa Sader
2005	Joseph Blas	David Cockriel, Jenny Harper, Danielle Lucore
2004	Robert Ullom	
2003	Michelle Schulz	Adeline Cripe
2002	Elizabeth Stover	Renata Lichty
2001	Genelle Wine	Jonas Lichty
2000	Nathan McLaughlin	Jeffrey McPherson
1999	Roy Johnson, Jr.	Jennifer M. Amiot, Janet Bowen, Eric D. Putnam, Anna Katharina Schenk
1998		Rebecca Standafer, Cameron Mahler
1997	Kerri Kobbeman	Rod Samuelson
1996		Stasi Zirkel, Wes Sechler, Chris Owens
1995	Heather Hughbanks, Monica Embers	Erik Harmon
1994	Adam Smith	Paula Worley, Adeola Grillo, Sherry Coopple, Susan Blubaugh
1993	Tyson Burden	Robin Morgan
1992	Pete Hanson	
1991		Shannon Hull, Thomas Champion
1990	James Dechand	David Maxey
1989		Michelle Roesch
1988	David Lehmen	Sandra Ashbaugh, Cynthia Aeschacher
1987	Marla Ullom, David Krehbiel	Jay Nicholson, Marsha Morley, Cassandra Clark

Cantaurus

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Cover: The cover illustration is by artist Brenda Tejero, a McPherson College alumna (Sociology, 2017).

The quotation is taken from Wendell Berry's essay, *The Loss of the University*, (p. 82 in *Home Economics*, North Point Press, 1987) in which he references the book *Samuel Johnson*, by W. Jackson Bate (Harcourt Brace Jovanovich, 1977, p. 51) as follows: "Dr. Johnson told Mrs. Thrale that his cousin, Cornelius Ford, `advised him to study the Principles of everything, that a general Acquaintance with Life might be the Consequence of his Enquiries - Learn said he the leading Precognita of all things ... grasp the Trunk hard only, and you will shake all the Branches.'"

Cantaurus is an official publication of the Division of Science and Technology, McPherson College, McPherson, KS. The purpose of this journal is to publish the results of original research conducted by students majoring in the natural sciences at McPherson College. The research published herein represents partial fulfillment of the requirements for the B.S. degree at McPherson College.

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Mycoplasma pulmonis in *Rattus norvegicus*: isolation and microbial diversity

Nora Grosbach

ABSTRACT

The presence of *Mycoplasma pulmonis* is very common in domestic and laboratory rats. *Mycoplasma* is the smallest bacterial cell species that was been discovered thus far. It can survive without oxygen, comes in various shapes, and lacks a cell wall. It is penicillin resistant and can be parasitic or saprotrophic. Depending on where the bacteria is located, genitalia or in the upper respiratory system, it will cause different disorders and diseases. The presence of these bacteria can cause many problems in the health of the person or animal. It poses large problems in laboratory settings and any impending experiments. This article observes the diversity of the microbiota in the throat and nose of rats, as well as the change in the ratio of *M. pulmonis* to the other microbiota at different levels of health; healthy, sick, and dead. The results of this study conclude that there is not a significant difference between the mean ratios of *M. pulmonis* to other bacteria along the decline in health of the rat.

Keywords: *Isolation, microbial diversity, Murine Respiratory Disease, Mycoplasma pulmonis, Rattus norvegicus*

INTRODUCTION

Respiratory diseases are a major health problem for people in the United States, resulting in the fifth most common cause of death (Yancey et al. 2001). Respiratory and chronic obstructive pulmonary diseases include: chronic bronchitis, chronic asthma, emphysema, and Mycoplasma Respiratory Disease. The disease is the result from an infection of *Mycoplasma pneumoniae* in humans. This infection is most commonly seen in children, and young adults (Yancey et al. 2001). Similar forms that cause similar effects can be found in other animals, such as murine animals. Mice and rats infected with *Mycoplasma pulmonis* develop Murine Respiratory Disease or MRM (Davis et al. 1985).

Mycoplasma pulmonis is part of the *Mycoplasmatacea* bacterial family which are pleomorphic bacteria and lack cell walls (McAuliffe et al. 2006). This species of bacteria is the smallest bacterial cell species discovered thus far (0.2-0.3 μm) (Madigan et al. 2017). This species of bacteria possess a very limited genome and thus little is known about its virulence mechanism and methods. *Mycoplasmas* are thought to have undergone reductive evolution resulting in its small size (McAuliffe et al. 2006). This is thought because it is a type of obligate intercellular parasite. *Mycoplasmas* lack many genes including those for cell wall synthesis, the production of all 20 amino acids, genes encoding enzymes of the citric acid cycle, and many other biosynthetic genes. The cells are capable of surviving because they are able to acquire essential resources from the host in vivo (McAuliffe et al. 2006).

Commonly affected species include mice, rats, guinea pigs, and hamsters. Transmission occurs by direct contact, aerosol, and through transplant. Typical sites for colonization are in the middle ear and the nasopharynx. Clinical signs of MRM are weight loss, ruffled coat, loud breathing, hunched posture, porphyrin, lethargy, and reproductive effects. Reproductive effects include infertility, pup infection, low birthweight, abortions, or fetal death (Charles River Laboratories 2009). The severity of this disease causes a large problem, especially in laboratory environments. Approximately 60% of all barrier-maintained murine animals are known to be infected with *M. pulmonis* (van Kuppeveld et al. 1992). The animal may be infected for months before antibodies may begin to develop. As a result the infection can decimate the host quite rapidly. Subjects that do survive the acute phase, approximately seven days post infection, develop many of the conditions previously stated (Davis et al. 1985). These conditions can only be treated symptomatically and once a subject is infected the *M. pulmonis* cannot be eradicated. There are multiple methods in preventing and purging infected populations including immunization, and the use of enzyme-linked immunosorbent assay, also known as ELISA. However, ELISA is not practiced in populations that are in the process of being studied because it requires the elimination of infected animals (Cassell et al. 1981).

There is no cure for MRM, but there are treatments that can be used to suppress the chronic symptoms. The treatment drug used in this descriptive analysis is enrofloxacin. The purpose of this study is to observe the microbiota of the nose and throat as well as the ratio of *M. pulmonis* to the rest of the microbiota over the progression of health of *Rattus norvegicus*.

MATERIALS AND METHODS

Rattus norvegicus, commonly known as the Norway rat, is used as the primary testing subject for this

experiment. These rats are optimally maintained at temperatures between 21±2°C, and at a relative humidity of 60%±10% (van Kuppeveld et al. 1992). A total of sixteen feeder rats, which are most often used as feed for other animals, were purchased from multiple Wichita, KS Scales and Tails pet store locations. Each rat was then placed in isolation. Every 24 hours the rat's health were observed along with their food and water intakes. All of the subjects were treated with antimicrobial water. The first round of treatment was for seven days. The second round lasted fourteen days and began two days after the first round ended. The antimicrobial water was a solution of $2.7E^{-3}$M enrofloxacin. Three rats were treated with a solution of 22.7 mg/mL enrofloxacin subcutaneously. During this time a series of tests were done to aid in the determination of the type of bacteria that was making the rats sick. In this small test, one live and one dead rat were used. All media used for this testing and analysis was manufactured by Carolina Biological Supply. The throat of the rats were swabbed using a sterile swab dipped in sterile Tryptic Soy Broth (TSB). The sample was then streaked on a sterile Tryptic Soy Agar (TSA) plate and incubated at 37°C for 24 hours. A hemolysis test and a Glucose test were then ran. The hemolysis test was performed by taking a sample of the microbes grown on the TSA plates and streaking the sample on a Muller Hinton Agar plate containing 5% sheep's blood. The glucose test took samples of the microbes grown on the TSA plates and homogenized them in previously sterile glucose broth containing phenol red. Both tests were incubated at 37°C for 24 hours. The results from this side study proved that there were bacteria present in both the live and dead rats that are capable of fermenting glucose and bacteria that is hemolysing in the live rat.

Microbial diversity

In order to observe the microbial diversity in the nose and throats of the rats a series of sampling and gram staining was completed. The remaining live rats are swabbed for microbes every two days for 10 days. The procedure of the swab was performed the same way as in the side study. The nose of the rat is also swabbed using the same procedure. After 24 hours of incubation a sample was taken from the growth and gram stained. The gram positive and gram negative bacteria were then counted and the diversity was compared.

Ratio and Isolation

A complex media was made to observe the microbiotic ratio. This media consisted of egg yolk and nutrient agar outlined by Atlas (1995). The isolating media contained 0.208 IU/L of penicillin. Samples were taken from the throat of all subjects. The swab sample was diluted 100 fold then 100μL of the diluted solution was used to inoculate two plates, one of egg yolk agar without penicillin and one of egg yolk agar with penicillin. The plates were then incubated at 37°C for 24 hours. The number of colonies present were then counted on each type of plate and the number of colonies were compared to each other to calculate a ratio. From the calculated ratio a One-Way ANOVA was used to analyze the differences in means.

RESULTS

The subjects were treated with an antimicrobial water containing enrofloxacin for one week at the beginning of containment. After being removed from the treatment for 24 hours the symptoms reappeared, and two subjects died. The remaining subjects were then given the treatment for another two weeks. Overall 37% of the subjects died due to symptoms caused by the *M. pulmonis*.

The bacteria *M. pulmonis* was isolated and confirmed in 100% of the rats in the study. The ratio of *M. pulmonis* to the rest of the throat microbiota showed a correlation between the ratio and the health status of the rat. There are three groups of health; healthy, sick, and dead. The healthy group consisted of rats who showed symptoms less than or equal to 10% of the duration of captivity. Specifically, less than eight days during the 77 observational duration. The sick rats were those who presented with symptoms eight or more days during the observational period. The rats in the dead category consisted of rats that died during the observational period. This was determined by the number of days each individual presented unhealthy symptoms. These symptoms included ruffled coat, loud breathing, hunched posture, porphyrin, and lethargy.

As can be seen in figure 1, the healthy rat group the average ratio of other bacteria to *M. pulmonis* was 3.65:1 with a standard deviation of 1.14. The ratio of the sick group was 3.16:1 with a standard deviation of 1.83, and the ratio of the dead group was 1.78:1 with a standard deviation of 1.20.

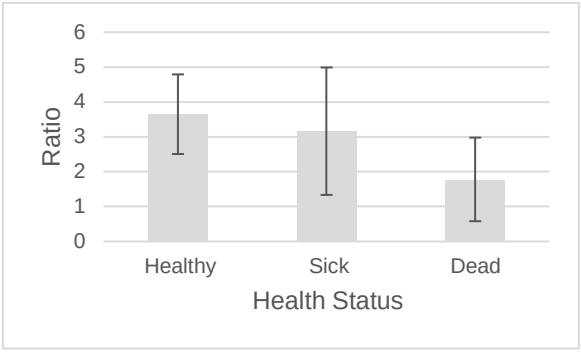

Figure 1: Ratio of *M. pulmonis* to other microbiota in the throat of the rat. Sample sizes are as follows: Healthy (*N=3*), Sick (*N=7*), Dead (*N=6*).

A One-Way ANOVA was used to test for differences in ratios. The α-value was set to 0.05, the resultant p-value was 0.16. It can be concluded that there is no significant difference between the means.

Microbial Diversity

The diversity study observed the phenotypic qualities of the microbiota in the throats of the rats using the gram staining method. Each rat was swabbed five times from the nose and throat. Each swab was used to innaculate one plate of TSB agar. In total 15 plates were innaculated for both the nose samples in the healthy group, 35 total plates in the sick group, and 6 total plates in the dead group. The same number of plates were used to innaculate the throat samples. From this the innaculated plates were incubated for 24 hours then a random colony was selected form each plate and gram stained. The frequencies of each type of general bacteria was recorded and the average frequencies of each type of cell in each group calculated. This can be seen in Table 1. This table shows only the types of bacteria that were observed, it is not a complete list of all bacteria that can be found in the throat of the rat. *M. pulmonis* is a gram negative cocci bacteria. As it can be seen in the table, gram negative bacteria were seen among all the groups. This does not conclude that *M. pulmonis* was what was seen every time a gram negative cocci was seen.

Table 1: The average frequencies of bacteria observed in the nose and throat of rats. The values are the frequency per plate observed.

Type of Cell	Nose			Throat		
	Healthy n=15	Sick n=35	Dead n=6	Healthy n=15	Sick n=35	Dead n=6
(+) cocci	0.27	0.69	0.17	0.33	0.57	0.50
(+) cocci chained	0.00	0.03	0.00	0.07	0.34	0.33
(-) cocci	0.07	0.11	0.17	0.13	0.03	0.33
(-) cocci chained	0.00	0.00	0.17	0.00	0.00	0.00
(+) rod	0.47	0.34	0.33	0.20	0.34	0.00
(+) rod chained	0.07	0.03	0.00	0.07	0.00	0.00
(-) rod	0.20	0.00	0.17	0.00	0.03	0.00
(-) rod chained	0.00	0.00	0.17	0.00	0.00	0.00
(+) coccobacilli	0.07	0.00	0.00	0.00	0.00	0.00

DISCUSSION

The study observed the diversity of the microbiota in the throat and nose of rats, and observed the change in the ratio of *M. pulmonis* to the other microbiota at different levels of health in the rat. A One-Way ANOVA was run in order to observe the difference in means. The p-value was 0.16 which is greater than the 0.05 α-value. Based on these results it can be concluded that there is no significant difference between the means. Meaning that the ratio of *M. pulmonis* to other bacteria does not significantly change as the health of the rat changes. Research does suggest that gender is a major factor in the severity of the disease. Males with MRM are consistently more severe clinical disease and have a higher mortality rate than females. The study observed more fatal shock-like syndrome in males and more of a chronic wasting syndrome in females (Yancey et al. 2001). In my observational study of this 40% of the females that died presented with a fatal shock-like symptom then death as opposed to the chronic wasting syndrome. The one male that died showed chronic wasting syndrome, and had more severe symptoms than the female rats. To obtain more conclusive results a much larger sample size is needed with a more even number of males and females.

Another area that I would do differently is in the diversity of the microbiome. More samples could have been taken to give a much larger view of what is present. To have a thorough understanding of the entire microbiome all of the colonies that were present should have been gram stained. The microbial diversity should have also been done under the same conditions as the ratio to *M. pulmonis* study, this would have allowed for more streamlined results. Though TSB is another broad spectrum media, like nutrient agar, it is highly likely that there are certain types of bacteria that are not capable of growing on TSB that can grow on the egg yolk nutrient agar.

Despite these various shortcomings the goal to see if there is a change in microbiota with the change in the rat's health was achieved. This descriptive study can help to understand how *M. pulmonis* evolves over time in chronic cases. These implications can also be considered in other *Mycoplasma* causing diseases like *Mycoplasma pneumonia* in humans as this bacteria acts in humans in a very similar way as *M. pulmonis* does in murine animals.

ACKNOWLEDGEMENTS

I would first like to thank Paul Prose for providing the antimicrobial enrofloxacin as well as preforming the subcutaneous injections. Dustin Wilgers I would like to thank for being my advisor and aiding in all areas of the study. My co-advisor, Jonathan Frye, I would like to thank for his exceptional help in the microbiological portion of this observational study. I would also like to thank Allan Ayella for aiding in this portion of the observational study as well. I would like to thank the McPherson College

science department for the facilities and funding to perform a research study as well as the rest of the natural sciences faculty for their academic knowledge and overview.

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The effects of stress-induced mutation using EMS on ethanol tolerance in *Saccharomyces cerevisiae*

Andrea Kadeba

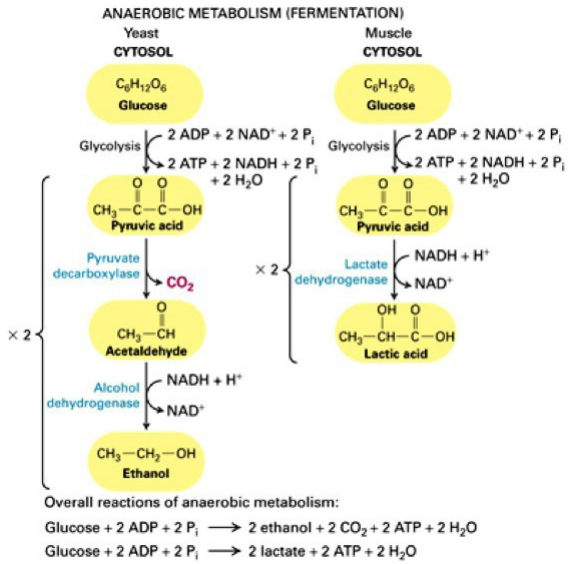
ABSTRACT

Ethanol is a multifunctional compound that has many uses and can be made naturally by sugar-fermenting yeast such as *Saccharomyces cerevisiae*. However, ethanol is toxic to yeast, so the process is not as efficient as it could be. This research aims to identify an efficient mechanism to increase the ethanol tolerance in *Saccharomyces cerevisiae* using various techniques such as ethanol as a stressor, artificial selection, and ethyl methanesulfonate (EMS) as a chemical mutagen. *Saccharomyces cerevisiae* cells were repeatedly exposed to increasing levels of ethanol from 9%-27% and EMS. The parental strain was defined to have an ethanol tolerance of 13%; the artificial selection strain (only exposed to ethanol as a stressor and artificially selected after each round) was defined to have an ethanol tolerance of 16%; the EMS-exposed strain (also exposed to ethanol as a stressor and artificial selection) was defined to have an ethanol tolerance limit at 27%. To test differences between strains, the parental strain, selection strain, and the EMS-exposed strain were separately plated on ten 27% ethanol plates and ten 0% ethanol plates and growth was checked after 24 hours. The EMS-exposed strain was the only strain that grew at 27% ethanol; all strains grew at 0% ethanol. These results show that EMS, artificial selection, and ethanol as a stressor might be effective in producing strains of *Saccharomyces cerevisiae* that are able to produce greater amounts of ethanol before toxicity sets in.

Keywords: *Saccharomyces cerevisiae,* Ethyl methanesulfonate, artificial selection, ethanol tolerance, chemical mutagenesis, molecular genetics, microbiology

INTRODUCTION

Saccharomyces cerevisiae is a yeast species that is capable of fermenting simple sugars such as glucose into ethanol. The pathway used to produce ethanol in yeast follows the glycolysis pathway in anaerobic conditions and transforms glucose into pyruvate. From pyruvate, humans can produce lactic acid in anaerobic muscle tissues, but yeast turn pyruvate into acetaldehyde and then into ethanol following the pathway below:



http://fhs-bio-wiki.pbworks.com/w/page/12145772/Fermentation

Although *Saccharomyces cerevisiae* can produce ethanol from many different sugars, the ethanol produced is lethal to the yeast cells in higher concentrations (Ma & Liu, 2010). Accumulation of ethanol can wreak havoc on the cells by inhibiting glucose uptake, deteriorating cell membrane functions, causing protein denaturation and conformation issues, and a host of other detrimental effects (Stanley et. al., 2009).

Although ethanol can be detrimental to yeast in higher concentrations, there are many advantageous uses of bioethanol, and perhaps, the most well-known use is the fermentation process in fruit and grains to produce alcoholic beverages. Another, more recent, beneficial use is the production of bioethanol from cellulosic starches for use as a biofuel that is considered clean and renewable (Ma & Liu, 2010). One breakthrough for this area of science was the use of ethanol to make E85 (85% ethanol) as a fuel to power automobiles. It is important to the future of our planet to continue to seek out renewable energy sources such as biofuels that don't attribute to greenhouse gases, don't focus on using precious natural resources, and don't contribute to global warming. Other uses for ethanol include antiseptics, antidotes to methanol and ethylene glycol poisoning, solvents for water-insoluble agents, and household heating.

Ethanol tolerance in *S. cerevisiae* can vary depending on the strain, but the average upper-limit tolerance is around 15-16%. Researchers have recently been interested in determining the

mechanisms behind ethanol tolerance in yeast cells so that they can be modified to produce higher concentrations of the substance and still survive. One way of doing this is introducing positive mutations in genes responsible for ethanol tolerance, however, there isn't enough evidence to see exactly which genes are affecting ethanol tolerance the most. It is likely due to an effort of multiple genes and regulators (Ding et. al., 2009). Over 400 genes have been linked to the process (Ma & Liu, 2010 (quantitative)). A few of the main family of genes identified in ethanol tolerance in yeast are the heat shock protein genes and cell membrane protein genes (Ma & Liu, 2010 (quantitative)). Instead of performing direct mutagenesis within a specific gene using genome editing techniques that can be costly, EMS (ethyl methanesulfonate) can be used as a mutagenic agent that is capable of producing genome-wide SNP's (single nucleotide polymorphisms) that is relatively inexpensive (Shirasawa et. al., 2016). EMS is capable of producing transition, insertion and deletion mutations and works by acting as an ethylating agent that attacks nitrogen positions in the bases in DNA, and it is also capable of alkylation of oxygens in guanine bases (Sega, 1984). By subjecting yeast cells to a mutagen and increasing levels of ethanol, the cells will become stressed out and will be readier to adapt and turn on stress-response mechanisms to induce higher ethanol tolerance (Hemmati et. al., 2012).

I expect the selective engineering techniques along with the EMS exposure will be an effective mechanism to produce a genetic change at the molecular level in the *S. cerevisiae* treatment strain that enables the cells to have increased ethanol tolerance, and it will be able to produce more ethanol per gram of glucose consumed so the process will become more efficient.

MATERIALS AND METHODS

Strain and Media

The parental strain used was a *Saccharomyces cerevisiae* haploid wild-type strain purchased from Carolina Biological. Fresh stock cultures were grown in Yeast Extract/Peptone/Dextrose (YPD) broth at 30ºC for 24 hours. The parental stock was kept at 4ºC for later use. YPD agar with varying concentrations of ethanol was used for plating techniques.

Treatment Groups

Three different treatment groups were used to determine which method to increase tolerance would be the most effective. The control group was the parental strain with natural variation. The selection strain was exposed to artificial selection and ethanol as a stressor only. And the EMS-exposed strain was exposed to artificial selection, ethanol as a stressor, and EMS as a chemical mutagen.

EMS Exposure

I added 1 mL of the stock culture and 1 mL 4% EMS to 10 mL of fresh broth. This mixture was vortexed for 10 seconds and placed in a shaker incubator at 30ºC with gentle agitation for 40 minutes. 1 mL of 5% Sodium Thiosulfate was added to the mixture and vortexed to stop the effects of EMS. The mixture was then centrifuged and washed again with 5% Sodium Thiosulfate. The cells were pelleted and resuspended in 10 mL of fresh broth.

Plating

I spread 20 microliters of the EMS exposed cells on YPD agar plates with varying concentrations of ethanol. For the first round of plating, I used ethanol in 0%, 9%, 12%, and 15% concentrations. The parental strain was also plated on those concentrations as a control. Plates were placed in a 30ºC incubator and growth was checked after 24 hours.

Repeat Rounds

Surviving EMS-exposed cells from the highest ethanol concentration plate were selected and resuspended in 50 mL of fresh broth and grown overnight in a shaker incubator at 30ºC. EMS exposure was repeated, and the second-round concentration agar plates were 0%, 13%,16%, and 19%. Growth was checked after 24 hours. Selection of cells, resuspension and growth, and EMS exposure were repeated, and third-round concentration agar plates were 0%, 17%, 20%, and 23%. Growth was checked after 24 hours. Selection of cells, resuspension and growth, and EMS exposure was repeated, and fourth-round concentration agar plates were 0%, 21%, 24%, and 27%.

Parental strain cells from the highest ethanol concentration plate were selected and resuspended in 50mL of fresh broth and grown overnight in a shaker incubator at 30ºC. This strain became the new artificial selection strain that was only exposed to increasing ethanol as a stressor, but not the EMS. Percentages for each round of increased ethanol was the same as described above in EMS rounds.

Testing Differences

EMS exposure ceased after four rounds, and an upper limit concentration tolerance for the mutated cells was defined at 27%. Parent cells, artificial selection cells, and EMS-exposed cells were plated separately on ten YPD agar plates each at the defined upper-limit concentration of 27% and ten 0% plates. The cells grew at 30ºC for 24 hours and growth was checked.

RESULTS

The upper-limit tolerance of the parental strain was defined at 13%. The upper-limit tolerance of the artificial selection strain exposed only to ethanol as a stressor was 16%. The upper-limit tolerance of the EMS-exposed cells was defined at 27%. The parental strain did not grow on any of the 10 plates at 27%; the selection strain did not grow on any of the 10 plates at 27%; the EMS-exposed strain grew on 10 of the 10 plates at 27% (χ^2=30.00, df=2, p=<.001). All three of the strains grew on all plates at 0% concentration (χ^2=30.00, df=2, p=1.00). These results show that the combining effects of ethanol as a stressor and EMS as a chemical mutagen altered the parental strain at the molecular level to allow for increased ethanol tolerance in the environment. Ethanol as a stressor alone also showed to increase the tolerance slightly.

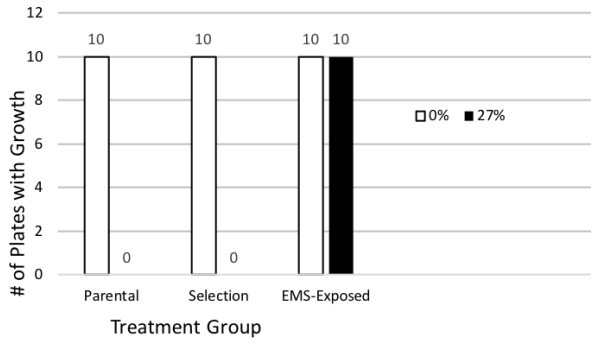


Figure 1: This figure shows how many plates each of the treatment group strains grew on at either 0% ethanol or 27% ethanol.

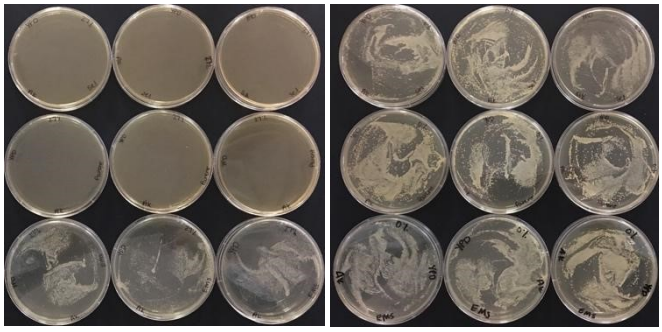


Figure 2: The image on the left shows all three strains at 27% ethanol (Top: selection, Middle: parent, Bottom: EMS-exposed) with only the EMS-exposed strain showing growth. The image on the right shows all three strains at 0% ethanol (Top: selection, Middle: parent, Bottom: EMS-exposed) with all three strains showing growth.

DISCUSSION

Saccharomyces cerevisiae naturally ferment sugars into ethanol, but due to ethanol toxicity, the process is not very efficient. If S. cerevisiae could tolerate higher levels of ethanol, then they could produce more of the product to be harvested for various uses. My research aimed to identify an efficient mechanism to increase the ethanol tolerance using artificial selection techniques, EMS as a chemical mutagen and increasing levels of ethanol as a stressor.

The process produced significant increases to ethanol tolerance in the new strain. However, the new strain was not tested to see precisely how much ethanol it could produce, only how much it could tolerate in its environment. Also, based on methods used to add ethanol to agar plates, it was difficult to determine final concentrations on plates after growth had taken place. There may have been various amounts of ethanol that evaporated from the plates that altered final concentrations throughout the growth period. Further research would need to be done on the strain to detect how much ethanol it could produce compared to the parental strain.

Regardless of the shortcomings of this paper, I found that the combining effects of artificial selection, ethanol as a stressor, and EMS as a chemical mutagen may be an effective method for increasing tolerance in *Saccharomyces cerevisiae.*

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The synthesis of an oxytetracycline derivative

Yann Kadeba

ABSTRACT
Antibiotics have been used for the past few centuries, and their misuse and overuse has contributed to the rise of antibiotic-resistant pathogens. Therefore, it is of great importance to synthesize novel antibiotics that may be effective against these new superbugs, which is the aim of this research. For this research project, my objective was to take a known antibiotic and change one or more of its functional groups to synthesize a novel antibiotic. Oxytetracycline was used as the starting material for the reaction with hydrochloric acid and n-chlorosuccinimide as reagents. The product of the reaction was characterized using analytical techniques such as NMR and IR spectroscopy.

Keywords: *antibiotics, organic synthesis, oxytetracyline, spectroscopy.*

INTRODUCTION

From the beginning of our existence on earth to today, we have been living with an infinite number of microorganisms. Those microorganisms are found all over the place and are considered omnipotent. Some of them are good for us to consume and work to assist us, while others are harmful and can cause an individual to get sick and even die. Bacteria are a type of the many microorganisms which can fall in both groups. Some bacteria are harmful and can cause infections which are the development of an organisms where they do not usually grow (Madigan et al. 2015). In order to deal with those infections various researchers observed diverse antimicrobial agents. Antibiotics are substances that are used to inhibit the growth of or destroy bacteria (Madigan et al. 2015). Without antibiotics, our life expectancies would have been greatly reduced. It is important to use the proper antibiotics to deal with the specific bacterial infections since out of all the antibiotics produced in nature, only about one percent can be used (Madigan et al. 2015), the rest of the antibiotic are potent to human consumption (Kuperman and Koren 2016).

There are multiple families of antibacterial drugs with various purposes. Some of the antibiotics are able to disrupt the bacterial DNA or affect its protein synthesis which leads to the bacteria not being able to properly reproduce (Getino et al. 2015). The main antibiotic family that I am planning on working with is Tetracycline, specifically the derivative Oxytetracyline. The structure of Oxytetracycline is composed of four rings containing different substituents such as numerous hydroxyl groups, amides. While looking to modify that structure, I observed most of the alterations happening at the location of the carbon C_6, C_7, C_8 and C_9.

Tetracycline and its derivatives are a part of a family known as broad-spectrum antibiotics because they are usable against both gram positive and gram negative bacteria, as well as Chlamydia and Rickettsia (Madigan et al. 2015). Gram-negative bacteria possess a peptidoglycan wall unlike gram positive. It

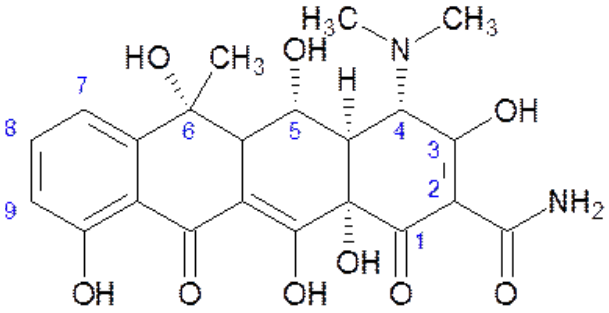

Figure 1. Oxytetracylcine

is currently known that bacteria have two different ways to resist Tetracycline, the efflux pump and the ribosomal protection (Chopra and Roberts 2001). The efflux pump is used by some bacteria to pump the antibiotics out of the cell and the ribosomal protection consists of the bacteria making a protein which it uses to stop the Tetracycline from biding to the 30s ribosomal site causing the cells to have both the antibacterial to become ineffective. The family of tetracycline's were named that way because they are made out of four six membered rings with different substituents attached to them (Salyers and Dixie 2005). Tetracycline derivatives include Doxycycline, Methylcycline, Democlocycline, and Oxytetracycline (Salyers and Dixie 2005).

It is important that researchers are able to come up with new derivatives of antibiotics. When they were first discovered in the 40's, as well as today in developing countries, they were not properly monitored (Mensah and Ansah 2016). Today, one big issue that the world is dealing with is the fact that the bacteria became resistant to many antibiotics which are overused to get rid of infections. This is the reason why today we have resistant bacteria such as MRSA which stands for Methicillin-Resistant *Staphylococcus aureus*.

MATERIALS AND METHODS

For this research, Oxytetracycline, which is a derivative of Tetracycline, was used, while adapting the method from United States Patent (Blackwood and Stephens 1978)

Synthesis

Oxytretracycline hydrochloride, 40g was dissolved in a mixture of 4 ml of concentrated hydrochloric acid and 2000 ml of water while stirring in a 3000 ml round bottom flask. 14 g of N-chlorosuccinimide was added to the flask and stirred for 4 hours at room temperature. After stirring for 4 hours, the mixture changed color from a black to a brick red color. This was placed in a centrifuge for 10 mins. The solid was pelleted at the bottom, and the supernatant was poured off and discarded. The solid was then washed with DI water and placed in a Buchner funnel attached to a filter flask and was attached to an air pump and left to air dry overnight.

Purification

To purify the product, 5.0 g. of crude was dissolved in water in a 250 ml beaker while stirring at room temperature. After being dissolved, it was placed in a separatory funnel and 320 ml of ether was added. The water layer was discarded, and the ether layer was washed with 60 ml x 5 of DI water which was decanted. The ether solution was placed in a 125 ml Erlenmeyer flask and Sodium sulfate was added to dry the solution and the ether layer was decanted in a round bottom flak where a simple distillation was set up and pure ether was collected leaving the product in the round bottom flask. A 100 ml of water was added to the product to remove it from the round bottom flask, which was placed in a 125 vml Erlenmeyer flask and left to stir for 2 hours and filtered using a Buchner funnel and a filter paper. The product was left to air dried overnight.

The product was characterized using IR Nicolet Avatar 320 FT-IR and NMR Varian Inova 400 MHz spectroscopy.

RESULTS

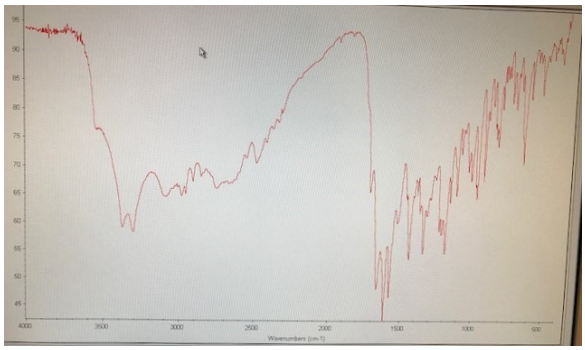

Figure 2. IR of Starting Material

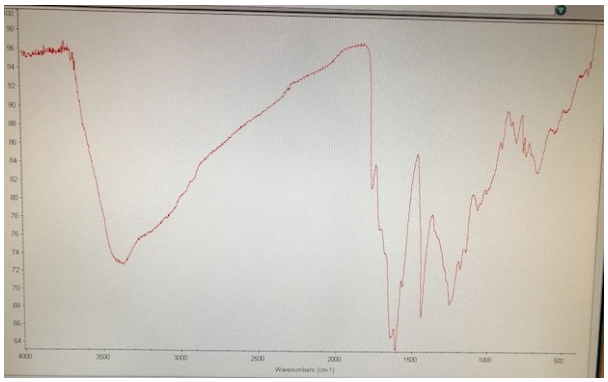

Figure 3. IR of Product

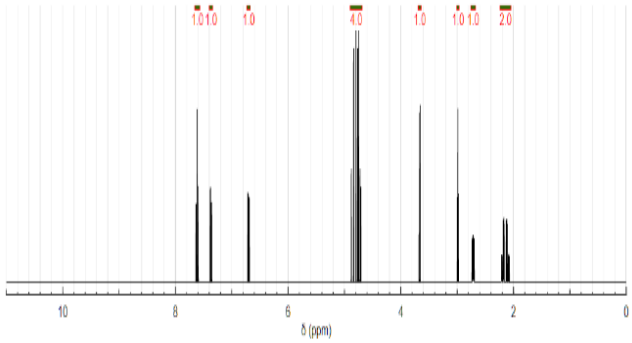

Figure 4. Predicted ^{1}H NMR of Starting Material using the website nmr.org

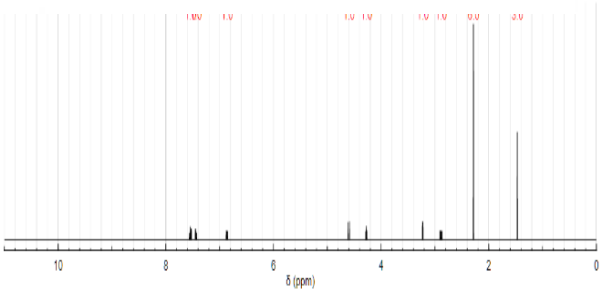

Figure 5. ^{1}H NMR of Starting Material

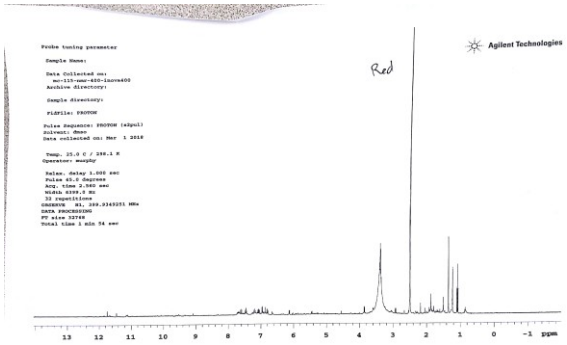

Figure 6. ^{1}H NMR of Product

DISCUSSION

A similar procedure was performed by Blackwood and Stephens (1978) with Tetracycline as a starting material instead of Oxytetracycline.

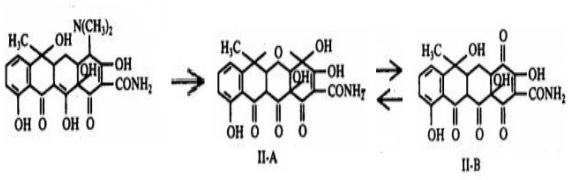

Figure 7. Tetracycline reaction leading to the formation of products identified as II-A and II-B.

Their results show: Found: C,51.96;H, 3.83;N, 3.22; K, 8.18; H_2O, 1.71, Calcd. For $C_{20}H_{16}O_9NK.O.5H_2O$: C, 52.0% H, 3.8; N, 3.2; K, 8.2; H_2O, 1.7

The reaction resulted in the structure 4-hydro-4-dedimethylaminotetracyline (Blackwood and Stephens 1978). Also, the reaction done in the patent showed a mixture of product but the structure of the tetracycline was mostly conserved, and the product collected in the patent contained the four rings which. On the other hand, my product did not show any of the similar peaks. After the NMR analysis, I found that the product NMR was different from the predicated and it looked like it was not a pure compound. I ran a TLC to see how many compounds I was observing, but it was not successful on silica gel column chromatography.

The quest to discover or synthesize new antibiotics is at an all-time high. Related to this project, further research needs to be done in order to properly evaluate and study the effectiveness of this novel compound.

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I would like to thank my advisors Dr. Manjula Koralegedara and Dr. Jonathan Frye for their guidance and expertise. In addition, I would like to thank the McPherson College Science Department for funding my project and allowing me access to supplies and equipment.To my family, thank you for putting up with me during this stressful period.

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Epigenetic effects of stress on survival in *Caenorhabditis elegans*

Amy Makovec

ABSTRACT

Caenorhabditis elegans are members of the nematode family and have been model organisms for epigenetic studies for many years due to their small size, transparent body, short life cycle, and easy maintenance. For this study, *C. elegans* were used to explore the epigenetic effects of stress on the survival of the worms. The animals in this study were subjected to the environmental stressor starvation and showed an increase in overall lifespan based on the statistical analysis of a Kaplan-Meier Survivorship test. This increased survival rate was also found to be passed to at least three subsequent generations who were grown under unstressed conditions. These results showcase the hormetic epigenetic effects of starvation in *Caenorhabditis elegans.*

Keywords: *Epigenetics, Stress, Caenorhabditis elegans*

INTRODUCTION

Epigenetic changes within the environment, whether initiated by DNA methylation, histone modification, or non-coding RNA-associated gene silencing, can lead to modifications in gene expression by switching certain genes on or off, controlling which proteins will be transcribed (Simmons, 2008). These changes in gene expression may cause little to no effect on an organism, however, it is also possible that these changes may cause more damaging effects that could lead to the acquisition of diseases such as cancer. Understanding the epigenetic effects of certain environmental factors could help show the role that epigenetics plays in the health field and could even allow for the early diagnoses and better medical treatment of many disorders.

There are many environmental factors that may be potential modifiers of gene expression including a factor of recent interest, stress caused by a restriction of diet. For instance, an article published by Rechavi *et al.* in July of 2014 titled "Starvation-Induced Transgenerational Inheritance of Small RNAs in *C. elegans*" found that the experience of a specific environmental factor (in this case starvation) by an ancestor is enough to cause genetic modifications in the descendants of that ancestor for up to three consecutive generations. Other recent studies have also concluded that these gene modifications, caused by the environmental factors, can create what is known as an epigenetic memory, which explains why the effects of an event experienced by an ancestor are present in the subsequent generations (Gaydos *et. al.,* 2014).

Another topic of recent interest is hormesis. Hormesis effects are thought to be possible beneficial effects that are brought on by low-dose exposure to specific conditions and stressors. This low-dose exposure to stressors early in an organism's life has even been found to go as far as extending the organism's overall lifespan (Yanase, S. *et al*., 1999).

For this research project, I will attempt to address the research question "Does stress have a transgenerational effect on survival in *Caenorhabditis elegans*?"

MATERIALS AND METHODS

For this experiment, I will be using the methods laid out in the paper "Environmental stresses induce transgenerationally inheritable survival advantages via germline-to-soma communication in *Caenorhabditis elegans*" (Kishimoto, 2017).

These methods are as follows: All nematodes will be cultured using standard *C. elegans* methods. The experimental and the control group studies were performed at 25 °C. The N2 wild type strain of *C. elegans* will be used in this experiment.

Starvation:

For this experiment, I obtained one NGM plate to be used for my control groups. Then, I seeded the plate with 0.05 mL *E. coli* OP50 liquid culture and spread the *E. coli* to create a lawn. Next, I transferred L1 stage N2 worms onto the *E. coli* seeded NGM plate, allowing the worms on the plate to grow under normal conditions for three days. Then, I transferred fifteen of the L4 worms onto new *E. coli* containing NGM plates, without inducing starvation, and allowed the animals to lay eggs for twenty-four hours. After the worms reproduced, the parents were removed from the plates to obtain the F1 generation which I kept incubated for four days. I then repeated this process to obtain F2 and F3 generations.

For the experimental groups, I began by obtaining one NGM plate. Then, I seeded the plate with 0.05 mL *E. coli* OP50 liquid culture and spread the *E. coli* to create a lawn. Next, I transferred L1 stage N2 worms onto the *E. coli* seeded NGM plate allowed the worms to grow under normal conditions for three days. Then, I transferred fifteen of the L4 stage animals onto a new NGM plate without *E. coli* OP50 to induce starvation. I then incubated the animals for twenty-four hours. Next, I transferred the L4 animals onto new plates that

once again contained *E. coli* OP50 and allowed the worms on each plate to grow under normal conditions for three days. Next, I transferred fifteen of the adults onto new *E. coli* containing NGM plates without inducing starvation and allowed the animals to lay eggs for twenty-four hours. After the worms reproduced, the parents were removed from the plates to obtain the F1 generation which I kept incubated for four days. This process was then repeated to obtain F2 and F3 generations.

The F1-3 generations of both the control and experimental groups were not subjected to any type of environmental stressors during their lifespan.

Assessing epigenetic effects:

In order to determine the epigenetic effects caused by the starvation environmental stressor I assessed the lifespan of the F1-3 generations.

To assess lifespan, the P0 (parental) generation animals were raised under the indicated environmental stress condition for four days while the F1-F3 generation animals were raised without environmental stressors for four days. Fifteen animals from each generation were then transferred to separate 5' flurodeoxyuridine-containing NGM plates seeded with ultraviolet-treated *E. coli* to inhibit progeny growth. The day the worms were transferred to the FUdR-containing NGM plates was defined as *t*=1 day. Deaths were recorded every four days. Animals were scored as dead if they fail to respond to touch by a platinum wire picker.

Microsoft Excel was used to organize the collected data and SigmaPlot was used to conduct a Kaplan-Meier Log Rank Test on the data. The Log Rank Test was chosen due to its ability to compare the survival distributions of the two groups (experimental and control) for each of the four generations (P0, F1, F2, & F3). For this test, statistical significance was defined as a P-value < 0.05 and the data was censored to ensure the removal of worms from the remaining data once they were defined as dead.

RESULTS

For this experiment, I attempted to recreate or replicate certain aspects of a study published by Saya Kishimoto, Masaharu, Emiko Okabe, Masanori Nono, & Eisuke Nishida published in January of 2017 titled "Environmental Stresses Induce Transgenerationally Inheritable Survival Advantages via Germline-to-Soma Communication in *Caenorhabditis elegans.*" In their experiment Kishimoto *et al.* looked at hormesis effects caused by environmental stressors as well as the resulting germ-to-soma communication.

For my experiment, I focused solely on the epigenetic effects of the starvation stressor on survival in the *C. elegans.* I grew hermaphroditic L4 stage worms under fasting conditions (for this experiment we shall define fasting/starvation as a highly limited supply of E. coli food) for a total of four days. After the four days, the worms were transferred onto plates that contained an unlimited supply of E. coli and remained on plates such as these for the remainder of their lifespan. Subsequent generations were produced and transferred onto new plates without being subjected to the stressor to determine if there was a transgenerational effect taking place. To compare the experimental and control groups I used the data collected from the experiment to conduct a Kaplan-Meier Survival Analysis.

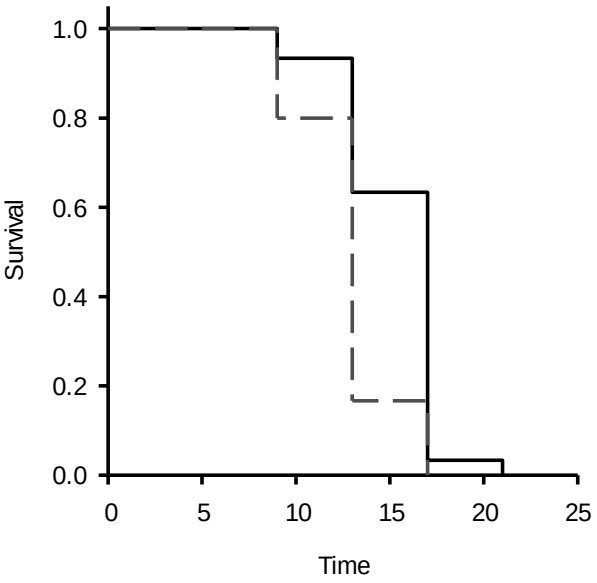

Figure 1. Comparison between control (---) and experimental (—) groups of parental generation.

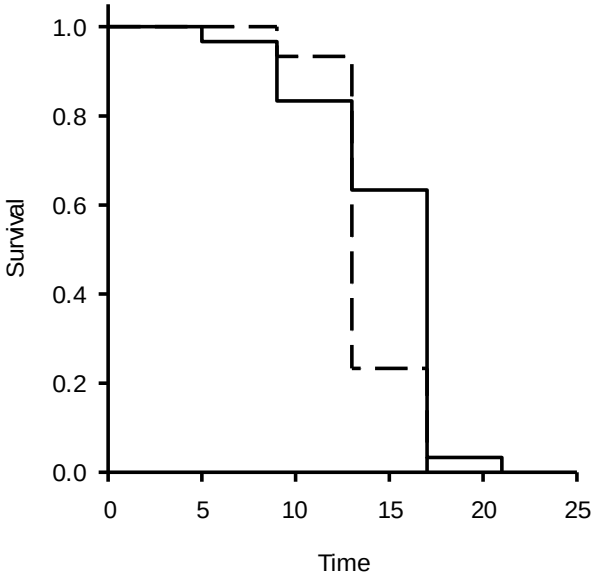

Figure 2. Comparison between control (---) and experimental (—) groups of F1 generation**.**

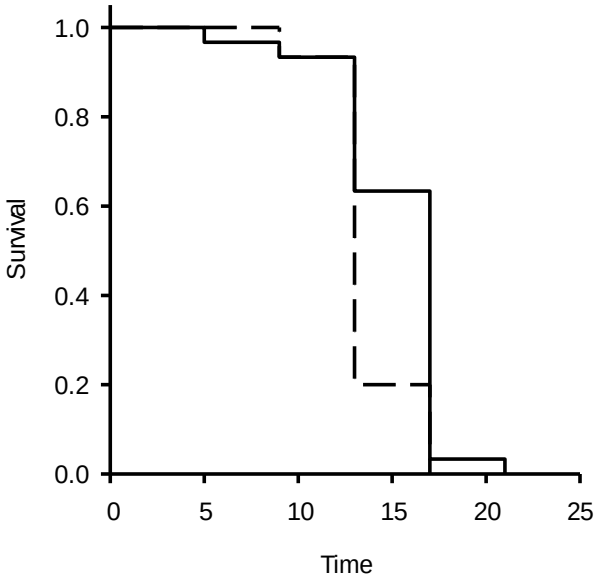

Figure 3. Comparison between control (---) and experimental (—) groups of F2 generation.

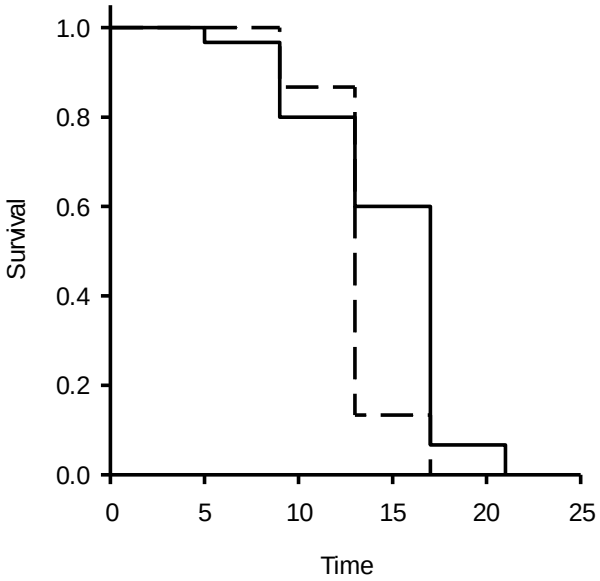

Figure 4. Comparison between control (---) and experimental (—) groups of F3 generation.

Table 1. LogRank test statistics for the comparisons between the experimental and control generations.

	Statistic	DF	P-Value
P0	13.195	1	<0.001
F1	5.742	1	0.017
F2	9.636	1	0.002
F3	8.067	1	0.005

DISCUSSION

The main focus of this experiment was to determine whether exposure to an environmental stressor during developmental stages of *C. elegan* worms would induce a transgenerational effect through three subsequent generations in the form of an increased lifespan. To determine the presence of an epigenetic effect such as this, a graphical and statistical comparison between each experimental and control group was done.

The comparisons between the four experimental and control generations resulted in P-values of <0.001, 0.017, 0.002, and 0.005 respectively. The cutoff for significance in this experiment was defined as a P-value of 0.05 therefore, these P-values allow for the rejection of the null hypothesis and the conclusion that a significant difference does exist between the experimental and control groups of each generation. The LogRank test statistics for the comparisons were found to be 13.195, 5.742, 9.636, and 8.067. Each LogRank statistic is greater than what would be expected by chance again proving that there is a statistically significant difference between the survival curves.

Based on these calculated statistical results from the Kaplan-Meier survivorship LogRank test, I can conclude that stress exposure during the developmental period of the parental generation of *C. elegan* worms can produce beneficial effects, such as increased longevity in the subsequent generations. These findings agree with the results published by Saya Kishimoto, Masaharu, Emiko Okabe, Masanori Nono, & Eisuke Nishida in which they found that subjecting the worms to various stressors provides beneficial effects as well as phenotypic effects in the unstressed generations that follow. This study revealed that not only do the three subsequent generations experience a greater longevity, but also that the experimental parental generation, which was the only generation subjected to the environmental stressor, also experienced an increase in overall lifespan when compared to the control parental generation.

It is thought that the production of small-RNAs are behind these epigenetic changes within the worms and that the mechanism by which they function is a method the parents use to help prepare their future generations to face hardships like the ones they themselves experienced (Rechavi *et. al.*, 2014). This theory reverts to Darwin's original thinking of survival of the fittest where only individuals who are equipped with certain traits will be able to survive and, more importantly, reproduce.

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2010 and 33.41% in 2005 of fatalities in the cases year period (Gilpin and Zhou, 2004). This research

Colorimetric determination of acetaminophen degradation in blood samples stored at 24ºC and 40ºC

Chantelle Theron

ABSTRACT

Acetaminophen (APAP) overdose fatalities have decreased slightly over the last few years but still remain ranked in the top 10 by the American Association of Poison Control. Due to APAPs chemical composition it can undergo various possible degradation pathways. Previous studies utilized thermal stressing and measured the rate of degradation by High Performance Liquid Chromatography, however the degradation of APAP within biological samples has not been well established. In this study the degradation of APAP was studied in bovine blood at 24ºC and 40ºC over a four week period. When comparing the initial and final concentration of APAP in the blood samples, the final measurement was 14.41% higher for the 24^0C and 11.79% higher for the 40^0C samples. However, results obtained were not statistically significant enough to conclude that the change was due to the storage of the blood samples at 24ºC and 40ºC. There was a slight increase in the first measurement recorded for both the 24°C and 40^oC samples which could suggest that there was a loss of volume upon storage.

Keywords: *Acetaminophen, Overdose, Blood, Colorimetric*

INTRODUCTION

Acetaminophen or N-acetyl-para-aminophenol (APAP) is a commonly used analgesic and antipyretic drug that is prescribed as an alternative long-term treatment to aspirin in order to decrease the risk of gastrointestinal bleeding (Diamond 2000). The prescribed single dose of APAP for adults is 500-1000 mg, and may be repeated every 4 to 6 hours with a maximum combined dosage of 4000 mg in 24 hours (Tylenol 2016). APAP is expediently available over the counter without a prescription, which has resulted in the drug becoming misused in the United States (Mowry et al. 2016). APAP overdose is defined as the ingestion of a minimum concentration of 140 mg/kg at which toxicity is initiated (Kolambabe and Soysa, 2010), dosages greater than 350 mg/kg result in hepatic damage, and in severe cases hepatic failure and death (Larson 2005). APAP is metabolically activated by the cytochrome P450 by a two electron oxidation process to form a reactive metabolite N-acetyl-p-benzo-quione imine (NAPQI). At a toxic dose NAPQI binds to cysteine groups causing centrilobular hepatic necrosis (Davern 2006).

APAP fatalities have decreased slightly over the past 10 years, however APAP still ranks yearly among the top 10 largest number of fatalities out of the cases studied by the American Association of Poison Control Centers (AAPCC) (Mowry et al. 2016). Figures from the 2015 Annual Report of the AAPC indicated that out of 1256 cases studied 143 APAP (alone) and 135 APAP (combination) deaths occurred in 2015 (Mowry et al. 2016), compared to 130 (alone) and 240 (combination) deaths in 2010 out of 1366 cases studied (Bronstein, et al., 2011) and 47 (alone) and 92 (combination) deaths in 2005 out of 416 studied (Lai, et al., 2006). Resulting in 22.13% in 2015, 27.09% in studied are due to APAP. Other research states that APAP overdose was the leading cause of acute liver failure between 1998 and 2003 (Larson 2005).

Numerous cases of APAP overdose are accidental or unintentional due to a rapid increase in APAP/ opioid combination medications manufactured in recent years (Bunchorntavakul and Reddy 2013). As a result, the FDA released compliance statements aimed at APAP manufacturers to include a warning to patients on the packaging of any APAP combination medications and to distinctly state the concentration of APAP in the medication and the danger associated with using the drug in combination with another source of APAP (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) 2017). Currently there are 48 prescription and non-prescription APAP containing drugs available. Some commonly known prescription drugs containing APAP are: Endocet®, Fioricet®, HycotabHydrocet®, Hydrocodone, Bitartrate, Lortab®, Percocet®, Phenaphen®, Sedapap®, Tapanol®, Tylenol® with Codeine, Tylox®, Ultracet®, Vicodin®, Zydone® (KnowYourDose.org, 2015).

Due to the fact that APAP is not considered as a high-importance abuse drug, such as heroin or cocaine, information regarding the stability of APAP within biological samples over a period of time is not yet well established. It is suspected that due to APAP's chemical structure it is subjected to chemical degradation by a variety of pathways. Previous studies utilized conventional thermal stressing and measured the rate of decomposition by high-performance liquid chromatography (HPLC) and provided a predicted rate of decomposition over a five-

was not conducted within biological samples.

This is of high importance as incorrect analysis and the unreliability of the analytical results can severely affect the development and outcome of forensic services. The standard procedure of drug testing is to establish a chain of custody and analyze samples using an accurate and reliable technological procedure (James 2012). If the reliability of the evidence is questionable, then the evidence is inadmissible in a court of law.

The goal of my study is to understand the effect that temperature has on the stability of APAP contained in the samples. Therefore, this study aims to determine the rate of degradation of APAP within the blood using a colorimetric assay. This method is based on the formation of 2-nitro-5-acetamidophenol produced through the reaction of APAP with nitrous acid which produces a yellow colored compound. The color produced is measured at a wavelength of 430nm and is proportional to the concentration of APAP in the sample (Hale and Polkis, 1983).

MATERIALS AND METHODS

Method altered from Hale and Polkis (1983).

Apparatus

A Spectrotroninc Genesys 2 UV spectrometer was used for all of the colourimetric determinations. A Isotemp 215 water bath was used.

Reagents

4-Acetamindophenol and sodium hydroxide obtained from Acros Organics was used to make 2g/L and 8M solutions respectively. 3% Trichloroacetic acid (w/v) was obtained from Sigma Aldrich, 0.07M sodium nitrite was obtained from Fisher Scientific, and 3.8% sodium citrate (w/v) was obtained from Merck. All chemicals used were supplied by McPherson College.

Standards

200mg of APAP was weighed out and dissolved in 100mL of deionized water to obtain a 2g/L stock APAP solution. A series of dilutions were then made up at 1.5g/L, 1g/L, 0.5g/L and 0.25g/L solutions.

Calibration Curve

200uL aliquots of the APAP solutions were added to 1mL of fresh citrated bovine blood which was obtained through Carolina Biologicals, to obtain 50, 100, 200, 300 and 400 mg/L samples used to construct the calibration curve.

Storage

Blood samples spiked with APAP were stored in 20mL centrifuge tubes, in an incubator at 24^{o}C and 40^{o}C.

Serum

25mL of fresh citrated bovine blood was spiked with 5mL of 1.5g/L APAP stock solution. 1mL aliquots of spiked blood as well as non-spiked blood was then placed in 1mL micro centrifuge tubes for initial testing and the remaining blood was stored. 1mL blood aliquots were centrifuged at 10 000g for 10min initially as well as after storage time in order to obtain serum.

Sodium Citrate

3.8g of sodium citrate was added to 100mL of deionized water to obtain at 3.8% (w/v) solution. 0.1mL of solution was added to 0.9mL of deionized water to form a 1:9 ratio contained in blood samples. A sodium citrate APAP blank sample was analyzed with each run.

Colorimetric determination

3mL of 3% Trichloroacetic acid solution was added to a Pyrex No. 9826 13x10cm test tube containing 0.3mL of serum as well as a test tube containing 0.3mL of sodium citrate/ APAP sample. The solutions were then vortex mixed for approximately five seconds and centrifuged at 3500g for five minutes.

2mL of the supernatant was transferred to clean test tubes and 0.5mL of 0.07M sodium nitrite solution was added. The solutions were vortex mixed again for approximately five seconds and placed in a water bath set at 37^{o}C for 10 minutes.

After this period two drops of 8M sodium hydroxide was added which initiated the color change and the solutions were vortexed for the last time for 5 seconds. The absorbance of the colored solution was then measured at a wavelength of 430nm about a minute after the sodium hydroxide addition.

Statistical Analysis

A paired-samples t-test was conducted to compare the degradation of APAP in samples stored at 24°C and 40°C

RESULTS

The purpose of this experiment was to measure the concentration of APAP in blood samples stored at 240C and 40^{o}C, in order to detect a change or degradation in the concentration.

Calibration Curve

An APAP serum calibration curve was constructed for the method by using concentrations between 50mg/L and 300mg/L. Figure 1 represents the calibration curve for APAP in serum, each point is representative of the mean of three values obtained. The trend-line equation obtained was: $y=0.0005x+0.0263$ with an $R^2=0.9979$. Values obtained were corrected for a serum blank.

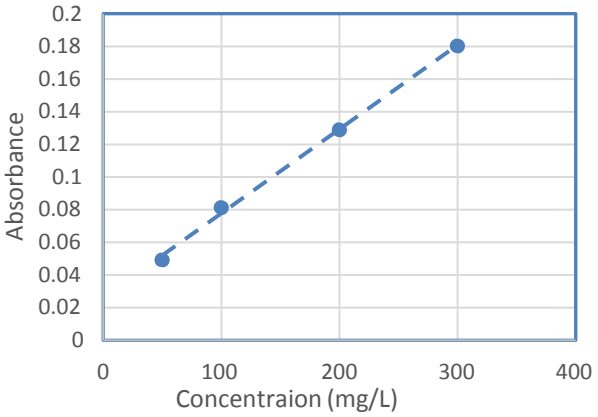

Figure 1. Calibration curve constructed in bovine serum with varying APAP concentrations.

Blood Samples

The absorbance of the spiked blood samples was measured weekly over four weeks and corrected for a serum blank as well as sodium citrate sample, which was subjected to the same experimental conditions.

Using the trend-line obtained from the calibration curve, the absorbance obtained from the samples was converted to the concentration of APAP in the serum. The concentrations obtained for each measurement are shown in Figure 2. The total amount of blood samples analyzed was 24. APAP concentrations increased over 592 for both the 24ºC and 40ºC by 30.67mg/L and 30.67mg/L respectively.

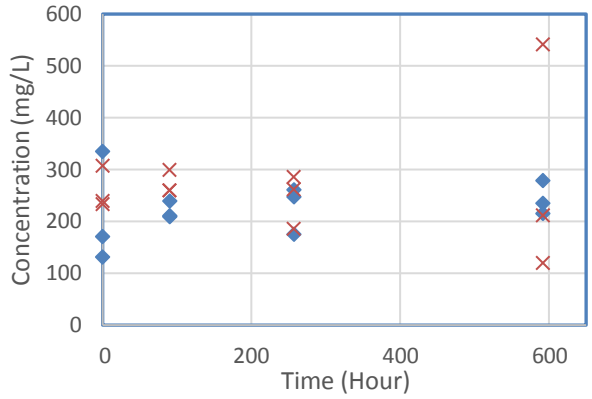

Figure 2. Concentration of 2-nitro-4-Acetamidophenol in 24 samples stored at 24ºC (represented as diamonds) and 40ºC (represented as crosses) over a period of four weeks.

The concentration of APAP measured in samples is also represented as a percentage of the initial APAP concentration shown in Figure 3. There was an overall percentage increase of 14.41% for the 24ºC series and 11.79% for the 40ºC series.

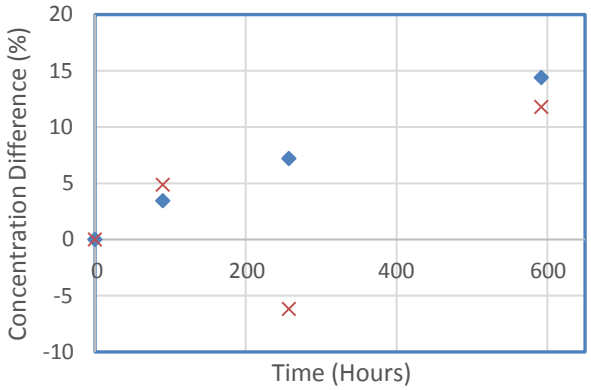

Figure 3. Change in APAP concentration represented as a percentage of the initial concentration measured. Samples stored at 24ºC are represented as diamonds and 40ºC represented as crosses.

DISCUSSION

The method used in this study is a very cost effective and easily accessible method to analyze the concentration of APAP in serum, when compared to the cost and availability of analysis done by HPLC.

Previous studies, that utilized thermal stressing to degrade APAP over a five-year period, provided a relative APAP degradation percentage at 35ºC and pH 6 and 8 (Gilpin and Zhou, 2004). These degradation rates were used as a guide for the current study since blood pH is around 7.4. The aim of our work was to determine if Gilpin and Zhou's (2004) degradation rates apply in a biological matrix at 24ºC and 40ºC.

A paired-samples t-test was conducted for the current study, to compare the degradation of APAP in samples stored at 24ºC and 40ºC over a period of 592 hours. There was not a significant difference in degradation for samples stored at 24ºC and 40ºC respectively, with t=-2.81 and P=0.068. I am therefore unable to conclude that the change in concentration observed between the 24ºC and 40ºC series was due to their storage conditions.

A previous study focused on the postmortem pharmacokinetics of APAP and was conducted on New Zealand white rabbits (Gomez et al. 1995). This study saw an overall blood APAP concentration increase and concluded that post-mortem drug concentrations do not reflect pre-mortem values. Which is consistent with the overall percentage increase observed in the current study.

A possible explanation for the slight increase in concentration observed in my study for both the samples stored at 24ºC and 40ºC, would be a decrease in sample volume. This would be misrepresented as a concentration increase since concentration is a measure of mass per unit volume (mg/L). However, samples were stored in capped

20mL centrifuge tubes.

For future studies I would increase the sample size as n=12 for each temperature group was not a statistically sufficient number of samples (P>0.05). However, a sample size of n=33 for each temperature group would provide a power of 0.8 and detect a 10mg/L change in concentration.

This analytical method is very cost effective and would therefore allow for the repetition of this study by increasing storage temperature, storage time and sample size which would allow for a better understanding of degradation of APAP in bovine blood.

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Efficacy of intravenous aspirin on blood clotting after *Vipera russelli* injection

Evan Willow

ABSTRACT

Vipera russelli, more commonly known as the Russell's Pit Viper, is one of the deadliest snakes in all of South East Asia. The venom causes coagulation by activating the factor X enzyme causing the Factor XA enzyme to initiate the coagulation cascade. The antivenin used to counteract the effects of the venom is readily available in hospitals or wealthier households or businesses. However, the majority of the population in South East Asia are low income families and cannot afford the antivenin. A cheap alternative to the antivenin is intravenous aspirin, it can be easily purchased by the majority of population for a fraction of the cost. In this experiment, six trial groups were constructed, three control groups, and three different concentrations of intravenous aspirin were used test the efficacy against the coagulation effects of the *Vipera russelli* venom. The viscosity of the pig blood was measured by doing timed tilt trials which were recorded digitally. The result showed statistically that the .13 mg/ml and .26 mg/ml intravenous aspirin groups showed no significant change between the control group. However, qualitatively those two aspirin groups did have some clotting in the vials during their tilt period even though statistically they had no significant change from the control. This concludes that .13 mg/ml aspirin and .26 mg/ml aspirin can prolonging the coagulation effects of the *Vipera russelli* venom.

Keywords: *Antivenin*, *Coagulation, Non-Parametric ANOVA, Vipera Russelli, 2-Acetoxybenzoic Acid.*

INTRODUCTION

Vipera russelli has become an overwhelming problem in certain areas in South East Asia. Morbidity and mortality following envenomation are frequent in Pakistan, Sri Lanka and India (Tripathy, S., et al., 2010). *Vipera Russelli's* envenomation activates factors V, X, VIII and induces quicker clotting times inside the blood vessels (Isbister, et al., 2015). The coagulant causes prothrombin to turn into thrombin when there is a presence of factor V and a phospholipid (Tun PE et al., 1995). The venom also induces, bleeding from the gums and urine, kidney failure, and necrosis.

Vipera russelli antivenin is prepared from a horse serum and used to neutralize 0.8mg/ml venom injection in a human. It is readily available in hospitals in Asia. Side effects of the antivenin include: fever, vomiting, hypotension, vertigo, allergic reactions, and shock. Considering the severity of the allergic reactions patients may have after injection it is recommended that skin tests are performed before the administration of the antivenin (Red Cross Antivenin. 2018). Patients may develop an immune complex mediated demyelination (Tripathy, S, et al., 2010) which causes damage to be done to the myelin sheath of neurons casuing signal responses between nerves to be impaired. Antivenin is available in hospitals or for 120 euros online (243 USD or 3,331,295 Indonesian Rupiah) (Red Cross Antivenin). These are two major conflicts to the general population of an underdeveloped country.

An alternative to antivenin that could slow the progression of symptoms could increase survival is a self-induced blood thinner; aspirin. The antivenin may be readily available in hospitals or large income institutions however to the lower class population the antivenin costs are to high to have this readily available.

2-Acetoxybenzoic acid is a common blood thinner used in many applications to prevent blood clotting such as long-term prevention for heart attacks, strokes, and blood clots. It can be found over the counter at a much cheaper cost than the antivenin, which may be a more affordable choice for the lower class populations of South East Asia. The use of daily aspirin for primary prevention reduced cardiovascular events by 15%, myocardial infarctions by 30% and deaths by 6% (Sanmuganathan, et al.). Previous research dosed pigs with 2-20 times the regular dosage of ibuprofen after *Vipera Russelli* envenomation. This study concluded that ibuprofen did not effect PT time until 16 times the recommended dose (Martini, 2015) which may harm the host. Because of aspirins effectiveness at thinning blood, with the right dose, aspirin could possibly counteract the effects of the viper's venom. Aspirin tablets take a long time to get absorbed through the digestive process, therefore liquid aspirin is a better alternative. Liquid aspirin can be injected intravenously which allows the aspirin to directly inserted into the blood stream.

This experiment can help us understand the coagulation effects of the *Vipera russelli* venom on blood, and also how to quickly counteract a *Vipera russelli* bite using an over the counter blood thinner.

MATERIALS AND METHODS

This experimental design involves a quantitative analysis of the coagulation properties of pig blood when affected by *Vipera russelli* venom and aspirin. Each individual trial was performed under the same conditions to properly understand how intravenous aspirin affects the coagulation times of envenomed blood.

Six different sample groups were performed all including ten samples per group. Sample groups include: blood control (pig blood with CaCl2), venom control (Bovine blood, CaCl2, Venom), 0.0162 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.0162 mg/ml aspirin solution), 0.13 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.13 mg/ml aspirin solution) and 0.26 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.26 mg/ml aspirin solution).

It is crucial to this experiment to compare multiple experimental groups to multiple control groups to determine if the independent variable affects the dependent variable. Multiple intravenous aspirin samples were included to show the efficacy of low and high concentrations in effect to the venom and if the more concentrated aspirin will counteract the venom more successfully than the lower doses.

Whole pig blood with sodium citrate was purchased to represent human blood in this experiment. The sodium citrate additive is readily applied to blood samples because of its anticoagulant properties. Sodium citrate binds to the calcium in the blood therefore creating a non-clotting whole blood sample. Injecting a 1.5 ml .10 mM sample of calcium chloride dihydrate can reverse the effects of the sodium citrate to enable use of this blood in coagulation tests. Other products such as the *Vipera russelli* venom, non-additive 10ml blood vials were purchased from Sigma-Aldrich.

The 0.10 mM calcium chloride solution was made by dissolving 0.736 g of calcium chloride dihydrate into one liter of water (Heather Liwang et al., 2010 Each vial of blood used in this experiment will be injected with 1.5 ml of the 0.10 mM calcium chloride dihydrate solution.

Three 250 ml aspirin samples were made with different concentrations (Martini et al., 2015) by dissolving ground aspirin in isotonic saline solution (Erin Larowe et al., 2013) corresponding with amounts of intravenous aspirin that could possibly be taken by a 74.1 kg human. Concentrations include 0.0162 mg/ml (1 baby aspirin), 0.13 mg/ml (2 325mg aspirin) and 0.26 mg/ml (4 325mg aspirin).

An adult *Vipera russelii* (111 +/- 1.8 cm) averages an injection of 144.5 mg of venom per bite (Tun Pe et al., 1986). To make the solution equivalent to the normal injection amount of an adult snake *Vipera russelli* venom was dissolved into an isotonic saline solution to a concentration of 2.89e-5 g/ml. All solutions were made quantitatively using a four-place analytical balance and precise liquid measurements using pipets.

Each sample group's blood was incubated for ten minutes at 37 Celsius before adding any other solutions to simulate human body conditions. The incubation period was then followed by the addition of 1.5 ml of 0.10 mM calcium chloride dihydrate, 1.0 ml of *Vipera russelii* venom, and 1.0 ml of aspirin depending on the sample group. Each individual vial of blood was tilted twice using a viscosity apparatus made out of a pulley system and hardware parts to simulate a constant tilt speed and angle to observe the liquidity of the blood after each sample Every vial tilt was recorded in seconds from when the blood started to move until all 6 ml of it reached the bottom of the vial. Vials were tilted at two different intervals: 0 minutes which occurred directly after venom and or aspirin were injected into the sample, and 5 minutes after the additives were injected into the sample.

Analysis

I calculated and averaged the difference of the 0-minute and 5 minute intervals for each trial group.I used JASP (version 0.8.5.1) to conduct a non-parametric ANOVA test which was used to test for significant differences between the six different trial groups. This is represented by figure 1.

RESULTS

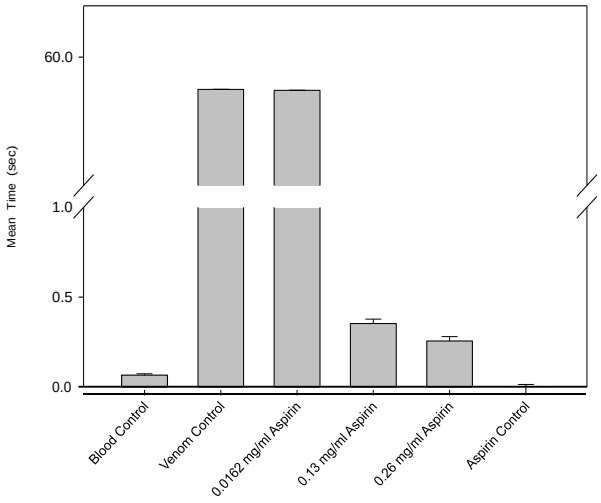

Figure 1 Showing the comparison of mean times in seconds between experimental groups with standard error bars.

In Figure 1 the columns represent the average difference of times of the ten trials for each trial group. The groups which showed significant change from the control group was the venom control (p value of 0.0) and the 0.0162 mg/ml (p value of 0.002) aspirin

sample. The 0.13 mg/ml aspirin sample (p value of 0.272), 0.26 mg/ml aspirin sample (p value of 1.000), and aspirin control (p value of 1.000) showed no significant change in their average difference times when compared to the blood control.

I did not observe a significant difference between the control group and aspirin control. This most likely occurred because the 0-minute time interval was almost exactly the same for both groups. The 5-minute interval trail of both control groups showed a 0.115 second decrease in the tilt time in the aspirin group proving that the intravenous aspirin did positively affect the viscosity of the blood in comparison to the blood control.

There was no significant change between the control group, and both the 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group. This proves that injecting both these concentrations of intravenous aspirin directly after getting envenomed will slow the clotting time of blood.

The 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group displayed slight clotting during the experiment even though the statistics proved no significant change between the trials and the blood control. The clot was not thick enough to stop the sliding of blood down the vile, which resulted in an adequate time difference in comparison to the control groups.

The statistical tests show that the efficacy of the 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group were observed to slightly reduce clotting times in effect to *Vipera russelli* venom.

DISCUSSION

After examination of the statistical analysis of the efficacy of intravenous aspirin on *Vipera russelli* venom, I observed a slight reduction in clotting times in the 0.13 mg/ml aspirin group and the 0.26 mg/ml aspirin group. These results were expected.

This experiment was conducted outside of the human body which accounts for many variables that are difficult to recreate in a laboratory setting. These variables include blood temperature, amount of oxygen to enter the blood, counteracting the sodium citrate to recreate normal clotting conditions, and expiration of the swine blood. Precautions were taken to recreate a normal human blood scenario such as refrigeration to preserve the blood and incubation to warm the blood to body temperature during the experimental period.

The venom controls showed that the venom when interacting with the blood completely clotted in the vials not allowing it to slide during the recording times. The 0.13 mg/ml and 0.26 mg/ml intravenous aspirin groups cause the blood to not combine into one congealed clot but multiple small clots. Thus, these concentrations of aspirin allowed the blood to slide down the tube but did not completely counteract the coagulation effects of the venom.

The statistical data demonstrates that the 0.13 mg/ml and 0.26 mg/ml aspirin groups successfully reduced the clotting times of the viper venom. However, qualitatively the aspirin only moderately succeeded. This may be because the aspirin did not completely counteract the activation of the factor XA enzyme which is formed by the venom and deceive the human body that a clot needs to form. The higher concentration of intravenous aspirin used may result in a more positive qualitative result.

Similar to the Martini (2015) study on ibuprofen, which recorded partial positive results at 16 times the recommended dose, we might see optimal qualitative results if we increase the concentration of intravenous aspirin to high above the recommended amounts. Increasing the concentration of aspirin can cause harmful side effects to the human body. Aspirin overdose can cause: alkalemia or acidemia, alkaluria or aciduria, hypoglycemia or hyperglycemia, water and electrolyte imbalances may occur, nausea, vomiting, tinnitus, hyperpnea, hyperpyrexia, disorientation, coma, and/or convulsions (Arch Interm Med. 1981).

Another possible replacement for the antivenin is a coagulation cascade blocker. Argatroban which is a competitive inhibitor and bivalirudin a bivalent oligopeptide that blocks the active site of protein substrates both act as thrombin inhibitors (Ustinov, N et al., 2016). This could possibly delay or reverse the coagulation cascade that is falsely brought on by the *Vipera russelli* venom.

From the statistical tests and observational data, I conclude that intravenous aspirin when injected into the blood stream right after envenomation slightly reduces coagulation effects brought on by the *Vipera russelli* venom. This is not adequate substitution to the antivenin, however it may allow the patient who has been bitten increased time to get to a hospital where the antivenin is available.

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Research Awards in the Natural Sciences

The awards are sponsored by the Natural Sciences of McPherson College and Midwest Oilseeds of Adel, Iowa.

The **Burkholder Research Award**, the highest award, is presented in recognition of outstanding achievement in student research. The **Merit Research Award** is presented in recognition of achievement in student research.

Each student completing a senior research project in the Natural Sciences is a candidate for an award. The Natural Science Faculty select the winners of the awards. Three criteria are used to judge the quality of the research and in selection of student award winners: (1) Selection and planning of a research project; (2) Quality of the research work, including techniques, observations made, and the analysis of data; and (3) Reporting the research, consisting of preparation of the research paper, and a poster or oral presentation to students and faculty.

Each student receiving an award will receive a Certificate of Award. Those receiving the Burkholder Award will have their name inscribed on a plaque, and will receive a year membership in the American Association for the Advancement of Science and a subscription to the journal *Science*.

Year	Burkholder Award	Merit Award
2018		Nora Grosbach, Amy Makovec
2017	Nathan Finch	Sheryl Evans, Lucas Giesey
2016	Tiffany Fraser, Ashley Long	Alia Khalidi, Kaley Kinnamon
2015	Nathaniel Schowengerdt	Shannon Coldren, Sydney Lipton, Jordan Stewart
2014	Yi Qun Chai, Sean DeYoung	Lori Crain, Alejandro Esparza, Christian Rodriguez
2013	Amanda Baxter, Emily James, Taylor Roop	Torey Fry, Kasey Miller
2012	Audrey McTaggart	Savannah Sievers, Andrew Skinner
2011		Karissa Ferrell, Kelley Green, Ashley Zodrow
2010	Ashlee Jost, David Miller	
2009	Adam Horinek	Amanda Pangburn, Nicole Sampson, Lezli Warkentin
2008	Joel Grosbach, Landon Snell, W. Brett Whitenack	Alan Grosbach
2007	Callie Crist	Rhonda Hoffert, Jamie Rodriguez
2006	Travis Allen	Lisa Sader
2005	Joseph Blas	David Cockriel, Jenny Harper, Danielle Lucore
2004	Robert Ullom	
2003	Michelle Schulz	Adeline Cripe
2002	Elizabeth Stover	Renata Lichty
2001	Genelle Wine	Jonas Lichty
2000	Nathan McLaughlin	Jeffrey McPherson
1999	Roy Johnson, Jr.	Jennifer M. Amiot, Janet Bowen, Eric D. Putnam, Anna Katharina Schenk
1998		Rebecca Standafer, Cameron Mahler
1997	Kerri Kobbeman	Rod Samuelson
1996		Chris Owens, Wes Seckler, Stasi Zirkel
1995	Monica Embers, Heather Hughbanks	Erik Harmon
1994	Adam Smith	Susan Blubaugh, Sherry Coopple, Adeola Grillo, Paula Worley
1993	Tyson Burden	Robin Morgan
1992	Pete Hanson	
1991		Thomas Champion, Shannon Hull
1990	James Dechand	David Maxey
1989		Michelle Roesch
1988	David Lehmen	Cynthia Aeschacher, Sandra Ashbaugh
1987	David Krehbiel, Marla Ullom	Cassandra Clark, Marsha Morley, Jay Nicholson

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