

How Does Nicotine Affect the Burrowing Time of an Earthworm?

Marcus Wentzel
Lori Voelker
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Lake Country School

Abstract

This article is a detailed and explained procedure of how a group of scientists decided that they wanted to see what the effects of nicotine are on earthworms burrowing time. And then they made a hypothesis of what they thought the contaminant was going to do to the earthworms burrowing time. They thought that nicotine was a relatable and adequate contaminant in the world today due to the harmful effects it has on the human body when inhaled or even when exposed to. The scientists were able to observe the effects of nicotine up close. For this experiment, the scientists decided to expose the earthworm to different nicotine concentrations by placing it in a red solo cup containing the solution and placing a coffee filter and another cup over the earthworm so it was completely enclosed by the nicotine. The scientists would then place the worm in a cup of soil and record the time the earthworm took to burrow. Using the collected data the scientists made charts and graphs, and either accepted or rejected their hypothesis based on the information. After testing the scientists record that nicotine made the earthworm's burrowing time increase.

Introduction

The scientists decided to test how the earthworm would react to nicotine. But in order to test that, they needed to learn about this contaminant. The field the scientists' study is biology, and the area of this field is environmental health. First, they decided to learn what kind of chemicals are in nicotine. A cigarette contains, "Nicotine, Tar, Carbon

Monoxide, Arsenic, Ammonia, Acetone, Toluene, Methylamine, Pesticides, Polonium - 210, and Methanol.¹

Nicotine is as addictive as heroin. It's used in cigarettes, juuls, chewing tobacco, sniffing tobacco, cigars, bidis, kreteks, pipes, hookahs, snuff, dip, and snus.²

Approximately one-fourth of the population uses tobacco products. Also, an estimated 63.4 million people ages 12 or older have used tobacco products this past month. In addition, 51.3 million people reportedly smoked cigarettes. The smoking rate has decreased from 20.9% in 2005 to 15.8% in 2016. The 25% of Americans with mental disorders, including addiction, account for 40% of the cigarettes smoked in the U.S.A. More than 40% of people with a GED smoke cigarettes.³

Some of the short term effects of nicotine include feeling relaxed, increased blood pressure and heart rate, decreased skin temperature, decreased appetite, dizziness, nausea, abdominal cramps, vomiting, headaches, and coughing. The long term effects of nicotine include lung cancer, throat cancer, risk of stroke, brain damage, loss of smell and taste, teeth decay, mouth cancer, shortness of breath, asthma, emphysema, heart disease, early wrinkles, slower healing process of wounds, and addiction.⁴

¹ "What's in a cigarette? | Irish Cancer Society." <https://www.cancer.ie/reduce-your-risk/smoking/health-risks/whats-in-cigarettes>. Accessed 28 Jan. 2020.

² "DrugFacts: Cigarettes and Other Tobacco Products | National ..." <https://www.drugabuse.gov/publications/drugfacts/cigarettes-other-tobacco-products>. Accessed 28 Jan. 2020.

³ "What is the scope of tobacco use and its cost to society" <https://www.drugabuse.gov/publications/research-reports/tobacco-nicotine-e-cigarettes/what-scope-tobacco-use-its-cost-to-society>. Accessed 28 Jan. 2020.

⁴ "Smoking: what are the effects? - myDr.com.au." <https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 28 Jan. 2020.

Smoking while pregnant can increase the chances of going into labor early, resulting in the baby having a lower than normal weight. The addiction to nicotine will cause the human body to develop a tolerance, which causes the body to crave more nicotine to get the same effect. Smokers may become dependent on nicotine, this can either be a physical dependence, psychological dependence, or both. This means the drug becomes far more important than other activities in their life. They crave the drug and will find it very hard to stop using it. Cigarettes also contain carbon monoxide, which reduces the amount of oxygen available to the muscles, brain and blood.⁵

When tobacco is smoked, nicotine rapidly reaches peak levels in the bloodstream and enters the brain. When nicotine reaches the brain it has been proven to slow down the thought process. In 2004-2005, roughly 3 quarters of a million people were hospitalized due to the consumption of nicotine.⁶ This hospitalization was most likely due to changes in heart rate and rhythm, an increase in blood pressure, and constrictions and diseases of the coronary artery, all after the consumption of nicotine.⁷

Some of the results resemble typical symptoms of nicotine overdose, but 1–4 mg of oral nicotine will certainly not evoke the severe adverse effects described, such as chronic seizures and loss of consciousness. Five cigarettes or 10 ml of a dilute nicotine-containing solution could kill an adult. Standard textbooks, databases, and safety sheets consistently state that the lethal dose for adults is 60 mg or less. Smoking a cigarette results in uptake of approximately 2 mg of nicotine and gives rise to mean arterial

⁵ "Smoking: what are the effects? - myDr.com.au." <https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 28 Jan. 2020.

⁶ "Smoking: what are the effects? - myDr.com.au." <https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 28 Jan. 2020.

⁷ "Nicotine: Facts, effects, and addiction - Medical News Today." 11 Jan. 2018, <https://www.medicalnewstoday.com/articles/240820>. Accessed 3 Mar. 2020.

plasma concentrations of about 0.03 mg/L.⁸ Arterial Plasma Concentrations mean, The apparent volume in which a drug is distributed in a human's artery or arteries.

The nicotine and additives in cigarettes is the leading cause of preventable cancers. There are an estimated 1.27 billion cigarette smokers world-wide. Tobacco consumption alone accounts for nearly 5.4 million deaths per year and one billion people may die in this century if global tobacco consumption remains at the current levels. In normal cells, nicotine can stimulate properties consistent with cell transformation and the early stages of cancer formation, such as increased cell proliferation, decreased cellular dependence on the extracellular matrix for survival, and decreased contact inhibition. Nicotine is the fundamental cause of addiction among tobacco users. Nicotine adversely affects many organs as shown in human and animal studies. It's biological effects are widespread and extend to all systems of the body including cardiovascular, respiratory, renal and reproductive systems.⁹

Knowing the effects that nicotine can cause on humans, the scientists decided that it was important to follow a procedure to test if nicotine affected the burrowing time of an earthworm. This is because the scientists wanted to see if the effects on an earthworm were similar to effects of nicotine on a human. If the scientists expose the earthworm to nicotine, then the earthworm will have a slower burrowing time after being completely enclosed by nicotine, because earthworms breathe through their skin.¹⁰

⁸ "How much nicotine kills a human? Tracing back the ... - NCBI." 4 Oct. 2013, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3880486/>. Accessed 28 Jan. 2020.

⁹ "Harmful effects of nicotine - NCBI." <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4363846/>. Accessed 28 Jan. 2020.

¹⁰ "10 Interesting Facts About Earthworms - EcoWatch." 8 Mar. 2014, <https://www.ecowatch.com/10-interesting-facts-about-earthworms-1881871982.html>. Accessed 29 Jan. 2020.

Materials and Methods

Materials

- 18 fl oz. red solo cup
- Adult earthworm/redworm from NASCO
- Liquid nicotine
- 1 - 40 ml beaker
- 2 - 250 ml beakers
- Spray bottle
- 3 droppers
- Coffee coffee filter
- Potting soil
- Tray with worms inside
- Goggles
- Medical gloves
- Timer
- Stopwatch

Methods

1. Put on safety goggles
2. Put on medical glove
3. Fill the 18 oz. solo cup $\frac{3}{4}$ of the way of up with potting soil
4. Get 3 earthworms from the soil
5. Place the earthworms in the burrowing chamber
6. Start the stopwatch
7. When one of the earthworms has completely burrowed, stop the stopwatch
8. Record this time as the control in the experiment
9. Cut the paper coffee filters to where they are just the inner circle of the filter

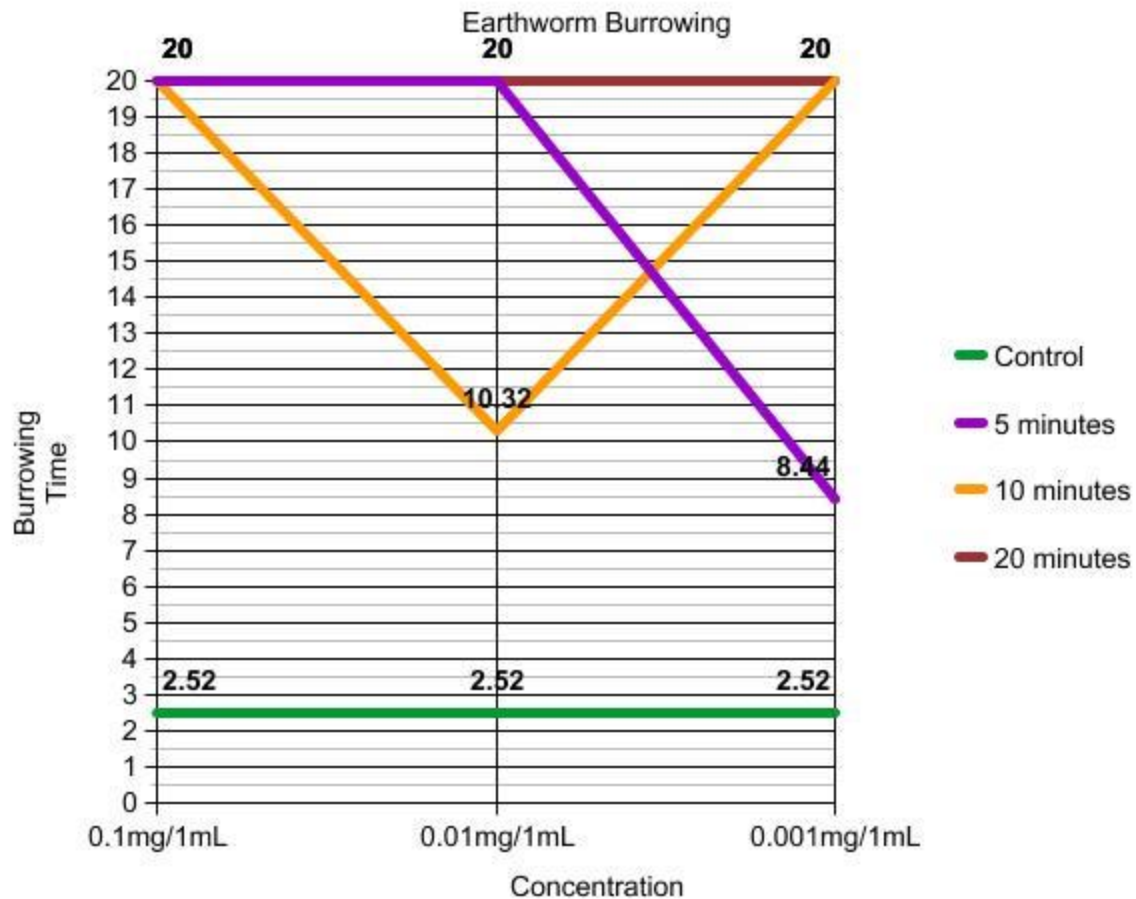
10. Using the dropper, make three samples of the same solution, 0.1 mg/ 1 mL of nicotine, by putting 12 drops of the stock solution with was 1 mg/ 1mL, to every 108 drops of water
11. Carefully place the three worms inside the cup
12. Start the timer for 5 minutes
13. Place the coffee filter over the three earthworms
14. Put another red solo cup over the filter and the earthworms, so the worm can not escape
15. Once the timer is done, take out the cup and the filter, carefully but quickly remove the three earthworms and place them in the burrowing chamber
16. Start the stopwatch
17. Once one of the earthworms is completely burrowed, stop the stopwatch and record the time it took for the earthworm to completely burrow. If the earthworm has not burrowed in at least 20 minutes, stop the timer and record 20+ minutes
18. Record this data
19. Select 3 new earthworms
20. Using one of the other two sample solutions of 0.1mg/ 1mL place the earthworms in the cup containing the solution.
21. Start the timer for now 10 minutes
22. Place the coffee filter over the earthworms
23. Place the red solo cup over the earthworms
24. After 10 minutes, remove the cup, the coffee filter, and carefully but quickly remove the earthworms and put them in the burrowing chamber

25. Start the stopwatch
26. Once one of the earthworms is completely burrowed stop the stopwatch and record the time it took for the earthworm to completely burrow. If the earthworm has not burrowed in at least 20 minutes stop the timer and record 20+ minutes
27. Record this data
28. Get 3 new earthworms
29. Using the last solution of 0.1mg/ 1mL place the earthworms in the cup with the solution in it
30. Start the timer for 20 minutes
31. Place the coffee filter over the earthworms
32. Place the red solo cup over the earthworms
33. After 20 minutes remove the cup, the coffee filter, then carefully but quickly remove the earthworms and put them in the burrowing chamber
34. Start the stopwatch
35. Once one of the Earthworms is completely burrowed stop the stopwatch and record the time it took for the earthworm to completely burrow. If the earthworm has not burrowed in at least 20 minutes stop the timer and record 20+ minutes
36. Record this data
37. Repeat this process for 0.01mg/ 1mL and 0.001mg/ 1mL

Some safety precautions that the scientists observed included wearing goggles at all times and wearing medical gloves when touching the contaminant.

Results

The scientists decided to test an earthworm's burrowing time after being exposed to nicotine because, about one fourth of the world's population uses tobacco products which include nicotine and many people have been harmed and some have even died from these products. The scientists wanted to see if the effects of nicotine would apply to earthworms because earthworms have similar bodies and features of the body. The hypothesis made by the scientists was if an earthworm is exposed to nicotine then the burrowing time of the earthworm will increase because the nicotine will affect the earthworm's movement. First the scientists gathered all the materials needed. Then they filled an 18oz. red solo cup with moist dirt $\frac{3}{4}$ of the way up, placed the worms inside and recorded their burrowing time. This recorded time was the control variable. Then the scientists placed a new set of earthworms into a cup filled with a 0.1mg/1mL concentration of nicotine which is the independent variable, and placed a coffee filter then another red solo cup over the worm so the worm was completely enclosed. The earthworms were then placed in the burrowing chamber. Then the scientists recorded the burrowing time of the earthworm which was the dependent variable, but stopped the timer the time reached 20 minutes. They then repeated this procedure again with the new concentrations of 0.01mg/1mL and 0.001mg/1mL of nicotine.



This graph shows that as the concentrations become more dilute, the burrowing time of an earthworm slightly decreases. Also the majority of the data points are 20+ minutes. Except for one of the 10 minute exposure times with is an outlier in the graph. But otherwise the data is very consistent and the earthworms reactions to the nicotine were very similar. The earthworms showed little movement and were in a paralysis state.

0.1mg/1mL

Five Minute Exposure Time	Statistically Significant
Ten Minute Exposure Time	Statistically Significant
Twenty Minute Exposure Time	Statistically Significant

0.01mg/1mL

Five Minute Exposure Time	Statistically Significant
Ten Minute Exposure Time	Statistically Significant
Twenty Minute Exposure Time	Statistically Significant

0.001mg/1mL

Five Minute Exposure Time	Statistically Significant
Ten Minute Exposure Time	Statistically Significant
Twenty Minute Exposure Time	Statistically Significant

All of the data and exposure times are statistically significant, meaning the results of the tests were not statistically close to the control time. The results of all the T-tests were large enough to be statistically significant. In order to be statistically significant the T-test has to be greater than 1.

Discussion

The results section showed that the data accepted the scientists' hypothesis. The scientists' data and tests were all statistically significant. The first test of 0.1mg/1mL of nicotine had a pattern of all being 20+ minutes because the concentration of the nicotine

was so strong it prevented the worms from being able to burrow. The second test of 0.01mg/1mL of nicotine showed that the exposure times of 5 and 20 minutes were consistent with a time of 20+ minutes. But the 10 minute exposure time was only 10 minutes and 32 seconds. This results of the test were statistically significant, however, one T-test did not have as great of a value compared to the first test, nor did it have a consistent pattern. This is because the concentration was lower, and had a lesser effect on the earthworm, but was still a very strong impact on their burrowing time. The final test solution had a concentration of 0.001mg/1mL of nicotine and had a reduced test time for when the earthworm was exposed to the nicotine, which was for 5 minutes. The finding was that the earthworm took less time to burrow. However, after 10 minutes of being exposed to the nicotine, the burrowing time was much longer and went over our stoppage time. This pattern in the data showed that the earthworm's tolerance time that reached over 20 minutes of 0.001mg/1mL is between 5 and 10 minutes. This same pattern occurred for the 20 minute exposure time of the concentration.

One of the potential errors that occurred in the experiment was the scientists did not have enough time to complete multiple trials. This could have been changed and prevented if the scientists had done multiple trials at once with multiple timers and people watching over each test. Also, the scientists could have repeated some of the trials, if the data was unlike the others and could have possibly been wrong. Or an error could have been the solution. The scientists may have miscounted or added too much water to the solution, causing the solution to have a greater or lesser effect on the earthworm. These errors can be found in the graph and it's pattern in the Results section above.

The earthworm was affected by the nicotine. This happened because when nicotine reaches the brain, it has an effect on the body, and will slow the thought process down.¹¹ The earthworm could not move because the nicotine was very harmful to its body and brain because it's thought process was slowed down to a point where the earthworm could not burrow properly. This was likely due to the fact that the earthworm was experiencing pain because nicotine causes tremors and pain in the muscles, and joint pains.¹² Therefore the earthworm would be in pain when the earthworm moves and tries to burrow, also for moving and burrowing the earthworm uses its muscles. For example according to "Earthworms!!!" *Earthworms!!!*, 2014. WinStep SEPA Program, "Worms make tunnels by wedging their muscular pharynx through the dirt and eats it." Also according to "Earthworms!!!" *Earthworms!!!*, 2014. WinStep SEPA Program, "Earthworms move by pulling themselves forward, when the long muscle contracts the body becomes short and thick, and when the ring-shaped muscle contracts the body gets long and thin." The earthworm's health could have also been affected because there are over 7,000 chemicals in tobacco products including nicotine. Also hundreds of compounds are added to tobacco to enhance its flavor and absorption of nicotine.¹³ Nicotine adversely affects many organs as shown in human and animal studies. Its biological effects are widespread and extend to all systems of the

¹¹ "Smoking: what are the effects? - myDr.com.au."
<https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 6 Feb. 2020.

¹² "Nicotine: Facts, effects, and addiction - Medical News Today." 11 Jan. 2018,
<https://www.medicalnewstoday.com/articles/240820.php>. Accessed 6 Feb. 2020.

¹³ "What is the scope of tobacco use and its cost to society"
<https://www.drugabuse.gov/publications/research-reports/tobacco-nicotine-e-cigarettes/what-scope-tobacco-use-its-cost-to-society>. Accessed 6 Feb. 2020.

body including cardiovascular, respiratory, renal and reproductive systems.¹⁴ In 2004-2005, roughly 3 quarters of a million people were hospitalized due to the use of nicotine.¹⁵ This is important because the human body is much larger than the body of an earthworm, so humans can handle a larger concentration of nicotine than earthworms, so earthworms will have a much larger reaction and effect. There also is carbon monoxide in cigarettes, which reduces the amount of oxygen available to the muscles, brain and blood.¹⁶ This is important because when your body doesn't have enough oxygen, you could get hypoxemia or hypoxia. These are dangerous conditions. Without oxygen, your brain, liver, and other organs can be damaged just minutes after symptoms start.¹⁷ So this explained why the earthworm was almost in a paralyse state, there was little oxygen getting through the earthworm's body so the earthworm could barely move and burrow itself and this explains why the earthworm had a slower burrowing time than when not exposed to nicotine.

This research has advanced the field of study of nicotine and its products by showing what the effects on an earthworm are and why this is important to human health because earthworms and humans have very similar nervous systems and body systems. And this could finally prove the point that nicotine can truly harm people in many ways. In the real world, millions of people use nicotine related products everyday and do not consider the harmful effects of nicotine. The information provided in this

¹⁴ "Harmful effects of nicotine - NCBI." <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4363846/>. Accessed 6 Feb. 2020.

¹⁵ "Smoking: what are the effects? - myDr.com.au." <https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 6 Feb. 2020.

¹⁶ "Smoking: what are the effects? - myDr.com.au." <https://www.mydr.com.au/addictions/smoking-what-are-the-effects>. Accessed 7 Feb. 2020.

¹⁷ "Hypoxia and Hypoxemia: Symptoms, Treatment, Causes." 30 Jul. 2018, <https://www.webmd.com/asthma/guide/hypoxia-hypoxemia>. Accessed 7 Feb. 2020.

paper could possibly decrease the number of people suffering and even dying from these harmful products.

To advance and gain more information and knowledge on this investigation, the scientists could perform more trials of observing the effects of different nicotine concentrations. Also the scientists could conduct a more advanced study of investigating and testing what the long term effects of nicotine have on an earthworm's burrowing time after being exposed to nicotine for various amounts of time.

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