Worms as a Model Organism to Determine Effects of Different Types of Sweeteners

Owen Antholine & Kaeley Kroenke

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UNIVERSITY of WISCONSIN UWMILWAUKEE

Introduction

Toxicology is the study of how certain substances affect organisms. This area of study is important to our society so we can lean more about the effects that chemicals and other substances have on our environment and the creatures that live in it. The substances we used in this investigation were sugar, honey, corn syrup, sucralose, and saccharin. The sugar served as a control in our experiment. We chose earthworms for our model organism because they are easy to care for and small enough to run multiple experiments in the same place at once, but big enough to clearly see the results of our tests. The worms were convenient for our experiment because, like a human, a worm has a nervous system. (Petering, David H., et al. 21-22) We were trying to which natural and artificial sweeteners have effects on worms, and used both circle and burrowing tests to study this. We chose to do both circle and burrowing tests to discover if the worms experienced physical or neurological effects (or both). This will help us prove whether or not the sweeteners we tested have an effect on the worms. In our circle experiments, we used a dropper to make a small circle of drops of the solution we were testing. We then set the worm inside and recorded the response when the worm touched the substance (None, mild, or severe). In our burrowing experiments, we set up a plastic container with a piece of paper towel damp with our solution on the bottom, and set the worms in it for 1 or 2 minutes, depending on the experiment. We then set the worms in our burrowing cups, and timed the worms on how long it takes to burrow. In our hypothesis, we stated that if we conduct multiple various ring (to see if our stimulus elicits a response) and burrowing (to see which sugars they react to neurologically) tests with a few diluted solutions of natural vs artificial sweeteners, then the worms will show a negative response to saccharin the most, because in our research, we found that saccharin may possibly be dangerous, (K. Tandel, 15-17).

Methods

To test the worms sensitivities to the dilutions of the sweeteners we created rings of droplets of the dilutions we were testing. These rings were about 2.5 inches in diameter, and we put one worm in each of these. Then when the worm touched and broke one of the droplets we recorded its reaction as either severe (lots of thrashing and/or releasing a mucus like substance), mild (some thrashing, but not a lot and no mucus being released by the worm, or visible pull back from the worm away from the substance), or no reaction (no thrashing and exhibiting no change). To test the possible neurological effects of the sweeteners of worms we tested the times it takes the worms to burrow after being exposed to the sweeteners. The worms are exposed to either 10 ppm, 100 ppm, or 1000 ppm for either 1 or 2 minutes in a tupperware container. 2 5 inch by 5 inch pieces of paper towels are damped by the concentration of the sweetener, with one piece of dampened paper towel under the worms and one piece of dampened paper towel under the worms. After the worms are done in the paper towel chamber they are each put in a plastic cup with soil 3 inches from the top of the cup, and how long it takes for them to burrow is timed and recorded in a data table. This is repeated for 4 worms total. First, create serial dilutions of 10, 100, and 1,000 ppm of honey, corn sugar, cane sugar, sugar substitute with saccharin in it, sugar substitute with aspartame in it, and sugar substitute with sucralose in it. Then create 4 circles of about 2.5 inches diameter of drops of honey on a plastic raceway at 10 ppm, each with a worm in it. When the worms touches one of the drops and reacts, record its reaction in the data table as well as any extra notes. If the worm thrashes wildly and/or begins to release a mucus-like substance, it is a severe reaction, if the worm thrashes a bit but not as much, with no mucus like substance it is a mild reaction, if it does not thrashes and exhibits no reaction, it is no reaction. Do this for all of the substances and dilutions and also a control of plain water until you have done all of them. When each worm is done being experimented on put it in a designated “resting chamber” with damp soil, and do not use it for the rest of the experiments. Wipe off and wash the raceway. (Figure 4) Create two pieces of 5 inch by 5 inch paper towel, and make them damp (wet with the substance being tested but not so wet the substance is dripping from it). Put one of these on the bottom of a Tupperware container, put four worms above it, and put the other piece of paper towel on top of the worms. Keep the worms in the container for either 1 or 2 minutes, depending on the experiment. When the time is up put each of the four worms in their own plastic cup, filled with soil about 3 inches from the top. (Figure 5) Time the amount of time it takes for each worm to burrow completely under the dirt, and record the times in the data table. Complete this for every other dilution, substance, and time (1 and 2 minutes for each dilution and substance). Make sure that when the worms are done being tested they are put in a “resting chamber” with damp soil, and do not use them for other experiments. To analyze our data we used the P values taken from t tests. By using this comparison of two data sets we compared all of the results for our tests with sweeteners with the control test of sugar at the same concentration. The p value would then tell us if they were the same or they were different enough to be able to create a conclusion from our data. We also used the statistics of standard error and standard deviation, as well as mean and median. This helped us evaluate our data quickly and see what our general results were form the experiment. The graph that we used to represent our actual data is a bar graph. Using error bars taken from our standard error, we are able to see if data sets are the same by seeing if the error bars overlap.

Abstract

As a group we did an experiment of the effects of sweeteners on worms and we tested using both the circle and burrowing tests to study this. We used sugar, honey, corn syrup, sucralose, and saccharin as a part of this investigation. To test the worms sensitivities to the dilutions of the sweeteners we created rings of droplets of the dilutions we were testing. We also wanted to test the possible neurological effects of the sweeteners of worms, so we tested how long (in minutes) it takes for the worms to burrow after being exposed to the sweeteners. When there are different exposures of a variety of substances, the worms reaction will be drastically different. While looking at our data, you can see the standard error bars overlap in some sections/areas, but when looking at a overview you can see that they aren’t in the same range. While conducting these experiments, we also noticed, when introduced to saccharin during the one and two minute exposures, the worms reacted negatively- they thrashed around and sometimes expelled yellow pus. When analyzing our experiment the results clearly show that different types of sugars stimulate a worm to react to different concentrations of sugars, and affect worms neurologically toward different sugar concentrations. Data collected showed one outlier during the ring test for a stimulate response, where honey had no stimulus for 1,00 ppm or 100 ppm and in a concentration of 10 ppm had a 1 point reaction average. Worms are living creatures like humans and were affected by a stimulus and a neurological test, we can conclude that different sweeteners can affect humans and other living things.

Table 1: TTests of PPM (1000, 100, 10) with P-Value

TTests (Compared to Sugar)

	PPM	Minutes Soaked	P-Value
Corn Syrup	1000	1	0.3919454486
Honey	1000	1	0.6036320593
Saccarin	1000	1	0.3018334231
Sucralose	1000	1	0.7390060509
Corn Syrup	1000	2	0.0916454903
Honey	1000	2	0.870174614
Saccarin	1000	2	0.0427308778
Sucralose	1000	2	0.1529380668
Corn Syrup	100	1	0.1123604234
Honey	100	1	0.7553738826
Saccarin	100	1	0.2788130442
Sucralose	100	1	0.4078220465
Corn Syrup	100	2	0.596157426
Honey	100	2	0.1156534111
Saccarin	100	2	0.0029131092?3
Sucralose	100	2	0.3186442418
Corn Syrup	10	1	0.3163214445
Honey	10	1	0.2765642623
Saccarin	10	1	0.0003821737
Sucralose	10	1	0.272215068
Corn Syrup	10	2	0.6624747375
Honey	10	2	0.5923737357
Saccarin	10	2	0.7290751522
Sucralose	10	2	0.6458729278

Figure 1: Reaction Averages 1000 PPM Solutions Graph

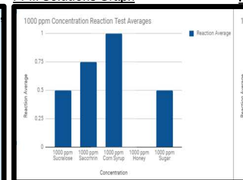


Figure 2: Reaction Averages 100 PPM Solutions Graph

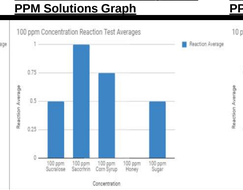


Figure 3 : Reaction Averages 10 PPM Solutions Graph

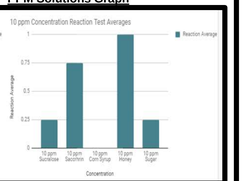


Table 2: ANOVA Test of Variance of Data

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8754388.63	14	625313.4736	2.490477877	0.010436558628	1.918248556
Within Groups	11298677.49	45	251081.7219			
Total	20053066.12	59				

Figure 4: A “Ring Test” being cleaned up.

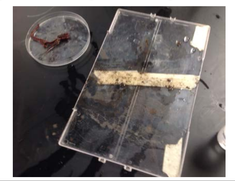


Figure 5: A “Burrowing Text” being completed.

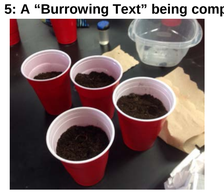


Figure 6: 1 Minute Exposure Burrowing Test Results Graph

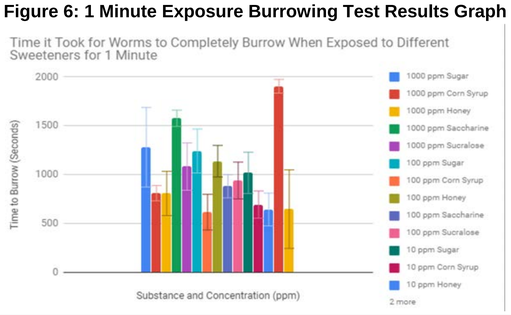
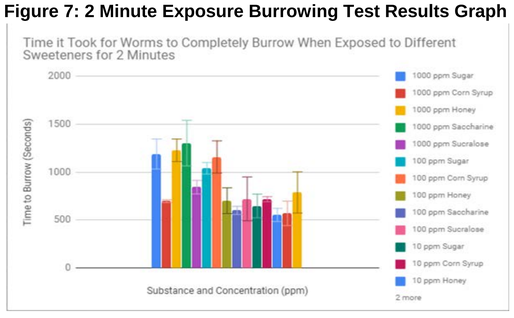


Figure 7: 2 Minute Exposure Burrowing Test Results Graph



Results

As mentioned in our introduction and methods sections, we conducted two types of experiments- circle and burrowing. Our circle test aimed to show the direct physical consequences the substances had on the worms, and the results are displayed in figures 1, 2 and 3. After conducting our second type of experiment, however, this data ended up becoming essentially obsolete in comparison to our burrowing tests, which not only showed the physical stresses of our solutions on worms, but also the psychological issues it created for our test subjects. In our 1 minute exposure burrowing experiments, the longest average time it took for our worms to completely disappear was 1280.1725 seconds, and the substance that holds this time was 10 ppm saccharine. The standard deviation, which shows the spread of data in a set, (“The Standard Deviation”) for this substance is 180.903501 seconds, and the standard error, which helps show the accuracy of our data, (Weisstein), is 78.33351377 seconds. On the flipside, the substance that caused the worms to burrow the fastest was 100 ppm corn syrup, with 525.905 seconds. The standard deviation for this substance is 162.0787636 seconds, and the standard error is 70.18216337 seconds. We had a huge outlier in our 1 minute experiments, which was the 10 ppm saccharine. Compared to the data on the other substances, saccharine at this concentration caused the worms to burrow significantly slower. This data/information can be found in our 1 minute exposure burrowing graph (Figure 6). The difference between these two times (range of the data) is 754.265, which is over 12 minutes. In our 2 minute exposure tests, we found that exposing the worms to 1000 ppm saccharin for this amount of time yielded the slowest burrowing time at an average of 1303.6 seconds. The standard error for this substance is 550.7777606 seconds, and the standard error is 238.4937662 seconds. 10 ppm honey caused the worms to burrow the fastest, at an average of 553.15 seconds. The standard deviation for this substance is 60.59480746 seconds, and the standard error is 26.2383213. This data can be found in our 2 minute exposure burrowing graph (Figure 7). The difference between these two times (range of the data) is 750.45, which is also over 12 minutes. In these experiments, we calculated the P-value and found it to be 0.0104365582779079. In both sets of experiments, we used cane sugar solutions as our controls, as we know this substance is safe. While conducting these experiments, we also noticed that, when introduced to saccharin during the one and two minute exposures, the worms reacted negatively- they thrashed around and would sometimes ooze yellow pus.

Discussion

When analyzing our experiment the results clearly show that different types of sugars stimulate a worm to react to different concentrations of sugars, and affect worms neurologically toward different sugar concentrations. Worms were ranked with different reaction toward 1,000, 100, and 10 parts per million (ppm) with saccharin, sucralose, aspartame, honey, corn syrup, and sugar as a control. Worms were found to be most stimulated by 1,000 ppm of corn syrup. At the concentration of 100 ppm of each substance, saccharine proved to stimulate the worms most, more than others by 0.25 reaction average. Concentrations of honey tested at at 10 ppm stimulated the worms the most. Now, neurologically, worms were affected by 10 ppm saccharine the most and, 100 ppm corn syrup the least. Our Hypothesis was correct, in saying that worms will be affected by sweeteners in a negative way. It took worms longer to burrow, meaning that they did not have full strength to burrow faster. Data collected showed one outlier during the ring test for a stimulate response, where honey had no stimulus for 1,00 ppm or 100 ppm and in a concentration of 10 ppm had a 1 point reaction average. The lowest concentration of saccharine affected the worms the most neurologically, which was interesting because it was lower for 100 ppm of saccharine. If we were to change our experiment, we would continue to go further with 1 ppm of the solutions to have a full range of results. We know that the worms are affected by a stimulus because the ring test caused the worms to become irritated, shown when they released yellow liquid. We know that the worms were affected neurologically when the burrowing test proved that concentrations of sweeteners affect the amount of time it takes to burrow in a negative way. Because worms are living creatures like humans and were affected by a stimulus and a neurological test, we can conclude that different sweeteners can affect humans and other living things.

Sources:

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