The Effectiveness of Lithium Chloride in Eliciting a Taste Aversion Response in *Blaptica dubia*

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Despite taste-aversion being a well-established concept in the field of psychology, little is known about the prevalence of the quality in invertebrates. Taste-aversions protect organisms from toxins through associations between tastes and sickening agents. The purpose of this study was to determine whether LiCl evokes a food aversion in *Blaptica dubia*, a species of cockroach, and to establish whether the invertebrates possess the capacity to demonstrate taste aversion. It was hypothesized that if the roaches were injected with a LiCl solution following exposure to a novel food, then the amount of food consumed upon the second exposure would decrease due to an association between adverse symptoms of LiCl and the novel food. The insects were exposed to novel food, and the amount consumed was recorded. After the remaining food was collected, the insects in the experimental group were injected with a LiCl solution. 24 hours later, the *Blaptica dubia* were again exposed to the food, and the mass was recorded. The results of a paired t-test, with an alpha value of 0.05, suggest that there were no significant differences between the mass of food eaten by the test group, $t(4)=0.078$, $p=0.942$, and control group, $t(4)=-0.318$, $p=0.771$. Thus, it was concluded that there is not sufficient evidence to suggest that there is a difference between the mass of the food consumed by the *Blaptica dubia* exposed to the LiCl and those not exposed to the LiCl.

Introduction

The development of an adaptive trait or characteristic as a result of environmental factors can give a species an advantage in survival and evolution. Scientists, philosophers, psychologists, and geneticists alike, including widely-known Charles Darwin, have long explored the theory that the genes most suited to survival are passed down through generations. John Garcia is credited with the discovery of taste aversion, one trait that is highly conducive to survival[1]. A conditioned taste aversion (CTA) is the avoidance of a specific taste or food that follows a negative experience associated with that taste or food. Traditionally, a CTA is formed by presenting a conditioned stimulus (CS), such as a novel food source, followed by an unconditioned stimulus (US), such as a sickening agent[2]. The formation of a learned taste aversion is attributed to the formation of an association between adverse symptoms caused by the unconditioned stimulus and the taste of the conditioned stimulus. Single-trial learning is a phenomenon somewhat unique to food aversion learning. The development of CTAs after only one exposure is paramount in survival, as it prevents continued exposure to a harmful substance[2]. The concept of conditioned taste aversions has been well established in the field of psychology, and it has been explored extensively in both humans and vertebrate animals. This being the case, there is a lack of extensive research regarding the prevalence of learned taste aversions in insects and other invertebrates.

The unconditioned stimulus in this taste aversion study was lithium chloride (LiCl), an ionic compound formed from the elements lithium and chlorine. As a salt and ionic compound, LiCl is soluble in water and when combined with distilled water forms a solution that can be injected. LiCl is a common unconditioned stimulus used in taste aversion studies, known to produce symptoms of hypothermia and a decreased heart rate in rats[2]. In invertebrates, such symptoms are neither expressed nor observed in the same manner, and their effects may only be reflected through a dramatic change in behavior, such as in the formation of a food aversion[2]. The LiCl solution was injected with a microliter syringe (Figure A1), following the procedure in a study involving the injection of Madagascar hissing cockroaches, *Gromphadorhina portentosa*[3]. The solution was formed at 0.24 M, as was the case in a study of associative learning in the grasshopper *Schistocerca americana*[4].

The species *Blaptica dubia*, commonly known as the dubia roach, was selected as the model organism due to the evolutionary significance of its order, Blattodea, as well as its widespread accessibility. It is believed that close ancestors of Blattodea evolved during the early Cretaceous period, and their continued presence in the modern age suggests that they possess certain characteristics that are conducive to survival[5]. *Blaptica dubia* were chosen with the intention of discovering whether the somewhat simplistic insect species is capable of developing CTAs. Some invertebrates, such as *Schistocerca americana*, a species of grasshopper, and *Procambarus clarkii*, the Louisiana crayfish, have demonstrated taste aversion in past studies, while other species, such as *Manduca sexta*, the tobacco hornworm, have not demonstrated this behavior. The formation of a taste aversion between the unconditioned stimulus and novel food was measured by comparing the mass of the novel food consumed prior to the LiCl injection to the mass of novel food consumed following the injection. It was hoped that the results of this study would determine whether the capacity for conditioned taste aversion could be a factor contributing to the survival and extensive evolutionary history of the order Blattodea, as well as establish what factors might contribute to the capacity for taste aversions.

While certain invertebrates, such as *Manduca sexta*, the tobacco hornworm, have been shown to develop odor aversions resulting from negative associations, taste aversions have not been widely observed, despite the known evolutionary advantages of CTAs[6]. Despite the absence of widespread evidence of conditioned taste aversions in invertebrates, some studies do suggest that certain species are capable of forming CTAs. Arzuffi et al. used lithium chloride to induce food aversion learning in *Procambarus clarkii*. The researchers injected the lithium chloride into the model organisms at varying dosages of 50-750 mg/kg, and recorded the presence of a CTA by gauging the mass of the remaining food after a set length of time[7]. The *Procambarus clarkii* exposed to LiCl were found to decrease their consumption of the novel food, chicken, suggesting that a conditioned taste aversion was formed[7]. In a similar study, researchers tested for the prevalence of CTAs in *Schistocerca americana* with a variety of both conditioned and unconditioned stimuli. One such unconditioned stimuli was lithium chloride; a solution of 50 µg of LiCl in 5 µL of distilled water was injected into each of the model organisms[4]. The *Schistocerca americana* exposed to the LiCl was found to reject the novel foods, behaving in a manner that suggests the presence of a conditioned taste aversion[4]. Although there is little current research regarding the possibility of learned taste aversions in insects belonging to the order Blattodea, Chua et al. do provide insight on the appropriate methodology for injecting *Gromphadorhina portentosa*, a species anatomically similar to *Blaptica dubia*. Held in a manner that restricts movement, the Madagascar hissing cockroaches are injected with a microliter syringe that pierces the skin in the same manner detailed in Figure A1[3].

The purpose of testing the effect of a LiCl injection on the feeding behavior of *Blaptica dubia* was to determine whether LiCl evokes a food aversion in the organisms and to establish whether or not such organisms possess the complexities required to demonstrate taste aversion. In prior literature, some invertebrate species, such as *Schistocerca americana* and *Procambarus clarkii*, have demonstrated taste aversion, while other species, such as *Manduca sexta*, have not demonstrated this behavior. The results of this study were hoped to provide insight as to why some invertebrate species seem capable of developing CTAs, while others do not. It was hypothesized that if the *Blaptica dubia* were injected with a LiCl solution following exposure to a novel food, then the amount of food consumed upon the second exposure would decrease due to the formation of an association between adverse symptoms of LiCl and the novel food. This hypothesis is supported by multiple studies that suggest that invertebrates, including certain species of insects, are capable of forming taste aversions.

Following a seven-day acclimation period, 80 *Blaptica dubia*, divided into eight groups of 10 organisms, were exposed to a novel food, the conditioned stimulus. After 24 hours of exposure, the remaining food was taken up, a change in the mass of the food was recorded, and four of the eight groups were injected with a LiCl solution formed from 50 µg of LiCl in 5 µL of distilled water. 24 hours later, the *Blaptica dubia* were exposed to the same quantity of the same novel food, and the mass of the food prior to and following its being provided to the model organisms was recorded.

Methods

A cordless electric drill was used to drill two holes into each side of eight plastic containers with a length of 15 cm, a width of 12 cm, and a height of 5 cm. The holes were 0.3175 cm in diameter, with approximately 3.81 cm between the holes on each side of the container. The purpose of drilling holes into the containers was to prevent the suffocation of the model organisms during experimentation. Each of the containers was labeled, numbers one to eight, for the purposes of keeping track of data. One hundred large *Blaptica dubia* were ordered, and 80 of the insects were randomly chosen for experimentation. The organisms were randomly assigned to each of the eight containers, with 10 insects in each container.

The *Blaptica dubia* underwent a week-long acclimation period, during which the containers were stored on a shelf in a dark cabinet. On the even days of the seven-day acclimation period, eight grams of the powdered "Dubia diet" were measured out in weight boats using a laboratory scale. Eight mL of tap water were measured in a 10 mL graduated cylinder and mixed with the 8 g of powdered food in a 50 mL beaker. The contents of the beaker were mixed with a stirring rod to form a coarse paste. The mixture was measured into amounts of 1.50 g, and this quantity of the food was applied to the center of each container. After a period of 24 hours, on the odd days of the seven-day acclimation period, the food remaining in each of the containers was removed with a scoopula and disposed of. The *Blaptica dubia* were only disturbed once every 24 hours during the acclimation period. Each time, they were temporarily transferred to another plastic container while their main container was wiped out and the feces removed. The holding container was rinsed and wiped out between exposures to each group of organisms. After each period of usage, the laboratory equipment was rinsed with tap water and dried with paper towels. For the purpose of safety, disposable rubber gloves were worn during each interaction with the *Blaptica dubia*, their feces, or the "Dubia diet." This pattern of 24-hour feedings on alternating days was continued over the course of the week-long acclimation period.

Following the seventh day of the acclimation period, the experimentation period began. Previously-frozen organic, skinless, cubed sweet potato was pureed in a food processor. This food was chosen to be the novel food because it was confirmed by a representative of Dubiaroaches.com that the *Blaptica dubia* were not exposed to sweet potato at their facility. As with the acclimation period, the food was measured into amounts of 1.50 g in a weigh boat and laboratory scale, and this quantity of the food was applied to the center of each of the eight containers. After a period of 24 hours, the remaining food was removed from the containers, massed, and disposed of. The mass of the remaining food in each container was recorded and added to the data table. Gloves were worn throughout this stage of experimentation, and similar procedures of removing excrement were applied.

Upon the removal of the food, the experimental groups, which were randomly selected to include the *Blaptica dubia* in the even-numbered containers, were injected with 5 µL of 0.24 M LiCl solution. The solution was formed by measuring one gram of lithium chloride with an analytic scale and dissolving the LiCl in a 100 mL solution with distilled water. The solution was formed in a 100 mL volumetric flask. This concentration of LiCl was based upon the methodology of Lee and Bernays, who studied associative learning in the grasshopper *Schistocerca americana*[4]. The researchers in the study injected their model organisms with 5 µL of a solution made from 50 µg of LiCl, and this ratio was used to calculate the molarity of the LiCl solution. A 25 µL gastight syringe was used to inject the *Blaptica dubia*. Disposable rubber gloves were worn consistently throughout the injection process. The syringe was primed according to the company instructions by drawing and dispensing samples of the LiCl solution to eliminate air bubbles that would cause inaccuracies. Five µL of the solution were drawn into the syringe, and with the *Blaptica dubia* immobilized in one hand, the microliter syringe punctured the cutaneous membrane adjacent to the fourth tergum from the posterior end of the insects at a 0° to 30° angle (Figure A1), following the procedure from a study involving the injection of Madagascar hissing cockroaches, *Gromphadorhina portentosa*[3]. This method of injection was used with all organisms in each of the four experimental groups. Following the care instructions provided by Hamilton Company, the microliter syringe was cleaned by rinsing with distilled water followed by acetone. All other lab equipment was rinsed thoroughly and dried.

Twenty-four hours after the injection of the *Blaptica dubia* in the experimental groups, all of the model organisms were reintroduced to the novel food, following identical procedures to those used upon the first exposure. Such procedures are detailed in Figure 1. Following data collection, a paired t-test was used to test for significance between the results of the two exposures and to determine whether the lithium chloride truly initiated the formation of a taste aversion in the model organisms.

Results

Table 1 shows the raw data of the mass of food (g) consumed in each group, both in the first and second exposure. It should be noted that groups two, six, and seven suffered a casualty of one insect each. The roaches from groups six and seven died during the acclimation period, prior to the collection of data, while the roach from group two passed away between exposures. For this reason, the ratio of consumption (grams/insect) was calculated as a method of comparing data across groups, despite discrepancies in the number of insects.

The ratio of grams of food per insect (Table 1) was used and interpreted to calculate the means(M), ranges, and standard deviations (SD) of each of the four data sets (Table 2). In both Exposure 1 (M=0.042, SD=0.007) and Exposure 2 (M=0.042, SD=0.006), the experimental group consumed a lower rate of the novel food than the Control group, which had a mean of 0.051 (SD=0.003) in Exposure 1 and a mean of 0.052 (SD=0.004) in Exposure 2. Additionally, the range of the data in the experimental group was greater in both exposures than that of the control group.

Figure 2 depicts the grams of novel food consumed per insect in each group, with the even-numbered groups being a part of the experimental group, and the odd-numbered groups being a part of the control group. The use of a bar graph allows for the observation of overall trends in the data between exposures. In this regard, very few trends existed, with some of the groups increasing across exposures (group one), some decreasing (group two), and others remaining constant in value (group three).

The two-tailed t-test outlined in Table 3 compared the ratio of grams/insect within the test group and control group, testing for significance across exposures, rather than groups. An alpha value of 0.05 was used to test for significance. The results of the paired t-test (Table 3) suggest that there were no significant differences between the mass of food consumed by insects in the experimental group, $t(4)=0.078$, $p=0.942$, or control group, $t(4)=-0.318$, $p=0.771$, from Exposure 1 to Exposure 2. The critical value for both the experimental and control groups was 3.182. Neither the control group, $t(4)=-0.318$, $p>0.05$, nor the experimental group, $t(4)=0.078$, $p>0.05$, showed a significantly similar difference to suggest that the lithium chloride injection elicited a taste aversion in the test group, and thus, the decision to fail to reject the null hypothesis was made.

Discussion

The purpose of this project was to determine whether LiCl evokes a food aversion in *Blaptica dubia* and to establish whether or not such organisms possess the complexities required to demonstrate taste aversion. The results of this study were hoped to discover the lowest threshold of invertebrates with the capacity for taste aversion. The hypothesis, that the amount of food consumed by insects in the experimental group upon the second exposure would decrease due to the formation of a taste aversion between lithium chloride and the novel food, was not supported. There is not sufficient evidence to suggest that there is a difference between the mass of the food consumed by the *Blaptica dubia* exposed to the lithium chloride, $t(4)=0.078$, $p=0.942$, and those not exposed to the LiCl, $t(4)=-0.318$, $p=0.771$.

The species *Blaptica dubia*, commonly known as the dubia roach, is an uncommon model organism, and it is primarily used as a feeder species for domestic amphibians and reptiles. As such, little research exists relating specifically to the species′ involvement in taste aversion studies. Dubia roaches were selected as model organisms in this study due to the lack of research regarding the development of CTAs in many species of invertebrates. The results of taste aversion studies in invertebrates vary based upon the model organism, and the goal of this study was to make progress in determining the lowest threshold of invertebrates that are able to display taste aversions. A large aspect of the significance of dubia roaches is the evolutionary history of their order, Blattodea. It is believed that close ancestors of Blattodea evolved during the early Cretaceous period, and their continued presence in the modern age suggests that they possess certain characteristics that are conducive to survival[5]. It was thought that the quality of taste aversion, which is extensively researched in vertebrates and known to allow organisms to recognize and avoid toxic substances, could have been one of the characteristics that allowed for the survival of the order Blattodea.

The paired t-test displayed in Table 3 suggests that there were no statistically significant differences between the consumption of the novel food in the experimental groups and control groups, suggesting that dubia roaches are below the threshold of invertebrates with the capacity to develop taste aversion. This finding likely means that the injection of the lithium chloride solution had little to no effect on the eating behavior of the *Blaptica dubia*. This conclusion contrasts with that of Lee and Bernays, who found statistically significant results ($p<0.025$) to suggest that lithium chloride causes taste aversion in grasshoppers, as exhibited by a rejection of a novel food following treatment[4]. Like Lee and Bernays, authors Arzuffi et al. found significant evidence to suggest that lithium chloride induces taste aversions in crawfish ($p<0.001$) by observing a decrease in consumed novel food and an increase in familiar food following exposure to treatment[7]. Although the variant findings from this study could mean that *Blaptica dubia* are incapable of forming CTAs, it is likely that errors in this experiment are to blame for the inconclusive results.

One factor that could have improved the reliability of the data and results is monitoring and keeping constant the temperature of the environment in which the roaches were kept. In his dissertation regarding the biology of *Blaptica dubia*, author Hao Wu mentions the crucial role of temperature in the growth and development of exothermic organisms[8]. Through his experimentation, the author finds that the normal growth and development of *Blaptica dubia* occurs between 20 °C and 30 °C. Growth was observed through the shedding of multiple exoskeletons over the course of experimentation. Temperature remains a point of uncertainty in this current study, as it was neither controlled nor monitored. It is possible that a variation in temperature over the course of experimentation impacted either the feeding behavior of the model organisms or the mortality of those organisms that were lost over the course of the study.

Table 2 shows that the mean, range, and standard deviation for both groups in Exposure 1, at which point the organisms had yet to experience the treatment, were not consistent, which could mean that the groups were not an accurate representation of the population, despite random assignment to groups. These patterns remained in the data collected following the second exposure, with the test group data having a lesser mean but greater range and standard deviation than the control group. In a 2018 study of taste aversion in mice, author Yutaka Hishimura found that when male mice are exposed to unfamiliar males members of their species, the degree to which CTAs are demonstrated is diminished. The gender and ages of the *Blaptica dubia* in this study were not monitored, as the organisms were in larval stages and had not yet developed certain gender-identifying characteristics. Controlling the genders and ages of the model organisms would have likely improved the reliability of the experiment and resulting data.

Another potential source of error is the small sample size used in this experiment. Despite there having been 80 organisms in the study, only eight points of data were collected due to the division of the insects into groups of 10. If the organisms had not been divided into such groups, the quantity of food consumed by the individual organisms would have likely been nearly insignificant and immeasurable using the available laboratory scales. Division into groups may also present potentially confounding social variables. No curious interactions between insects were observed, however, it is possible that the data was skewed by an unequal division of cockroaches of different ages or genders, which, as Hashimura found, could lessen the effects of taste aversion[9].

One other potential source of error in this study was the dosage of lithium chloride in the injected solution. The concentration of the 0.24 M solution was calculated by finding the molarity of 5 µL of a solution formed with 50 µg of LiCl, as was the ratio in a 1990 study using grasshoppers[4]. Because *Blaptica dubia* are such uncommon test subjects for taste aversion studies, a baseline LiCl concentration for the species does not currently exist. Most taste aversion studies, including that of Lee and Bernays, are preceded by a pilot study to determine the ideal dosage of the sickening agent for the model organism. This study was not preceded by such an experiment, and as such, it is possible that the dosage applied in this study was not appropriate for inducing temporary sickness in *Blaptica dubia.* In a study by Arzuffi et al., the ratio of 50 mg/kg of body mass was chosen as the baseline for the quantity of LiCl injected into the Louisiana crawfish, *Procambarus clarkii*[7]. Because crawfish, while invertebrates, are not insects like *Blaptica dubia*, the method of calculating dosage based upon mass was not applied to this study. It is also believed that the minute masses of the insects would not have led to the most accurate of measurements. Chua et al. used the same ratio of 50 mg of the drug chloroquine/kg of body mass to the average body mass of *Gromphadorhina portentosa* in a study testing the feasibility of using Madagascar hissing cockroaches in pathogen research[3]. While Madagascar hissing cockroaches are related to dubia roaches, the injection used by Chua et al. was of a different chemical compound, and it is unknown whether or not the same dosage would apply to a LiCl solution. It is possible that the concentration of lithium chloride solution, adapted from the study by Lee and Bernays, was not applicable to *Blaptica dubia*, and that using a dosage based upon average mass would have been more accurate. This factor is likely to have impacted the results of this experiment, and is certainly a point upon which to learn for subsequent studies.

Future research on this topic could attempt to expand knowledge of the behavior and regular eating patterns of *Blaptica dubia*, as a way to build a foundation for this topic. Additionally, studies could explore the social lives of the dubia roach, to determine whether or not social interactions can affect the influence of learned taste-aversions. Scientists could go deeper with their studies of taste-aversion by determining the biological and neural bases for the phenomenon. If this research were to occur, perhaps criteria for the capacity for taste aversions would be made clear.

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Tables and Figures

Figure 1. ***Experimental Design Diagram***

Title of the Experiment: The Effectiveness of Lithium Chloride in Eliciting a Taste Aversion Response in *Blaptica dubia*

Hypothesis: If the *Blaptica dubia* were injected with a LiCl solution following exposure to a novel food, then the amount of food consumed upon the second exposure would decrease due to the formation of an association between adverse symptoms of LiCl and the novel food.

Independent Variable: Injection of LiCl solution - μg (LiCl) and μL (water)

Levels of Independent Variable	LiCl solution (0 microliters) (control)	LiCl solution (5 microliters)
Number of Repeated Trials	40	40

Dependent Variable: Mass of food (g)

Control Group: *Blaptica dubia* not injected (simply presented with novel food for a second time)

Constants: Amount of novel food, concentration/amount of lithium chloride, location of injection site, species/origin of roaches, temperature of habitats, time of day (feeding/injection), length of time between feedings, type of novel food, amount of novel food, container, etc.

Table 1. Grams of Novel Food Consumed by Blaptica dubia Injected With 5 μL LiCl Solution (2, 4, 6, 8) and 0 μL LiCl Solution (1, 3, 5, 7)

	Group #	# of insects	Mass of food provided (g)	Mass of food remaining (g)	Difference in mass (g)	Grams/insect
First exposure to novel food (11/16-11/17)	1	10	1.50	1.00	0.50	0.05
	2	10	1.50	0.98	0.52	0.05
	3	10	1.50	1.00	0.50	0.05
	4	10	1.50	1.11	0.39	0.04
	5	10	1.50	0.95	0.55	0.06
	6	9	1.50	1.15	0.35	0.04
	7	9	1.50	1.06	0.44	0.05
	8	10	1.50	1.11	0.39	0.04
	Group #	# of insects	Mass of food provided (g)	Mass of food remaining (g)	Difference in mass (g)	Grams/insect
Second exposure to novel food (11/18-11/19)	1	10	1.50	0.99	0.51	0.05
	2	9	1.50	1.11	0.39	0.04
	3	10	1.50	1.00	0.50	0.05
	4	10	1.50	1.16	0.34	0.03
	5	10	1.50	1.01	0.49	0.05
	6	9	1.50	1.11	0.39	0.04
	7	9	1.50	0.98	0.52	0.06
	8	10	1.50	1.03	0.47	0.05

Table 1 shows the mass of the novel food that was consumed in the first and second 24-hour periods in both insects exposed to a lithium chloride solution and those not exposed to the solution. Due to casualties in certain groups, the ratio of grams of food to insect was calculated as a unit rate for the purpose of comparison.

Table 2. *Descriptive Statistics Summary Table of Mass of Novel Food Consumed by Blaptica dubia Injected with 5 μL LiCl Solution (Test) and 0 μL LiCl Solution (Control)*

Statistic	Exposure 1		Exposure 2	
	Even (Test)	Odd (Control)	Even (Test)	Odd (Control)
M	0.042	0.051	0.042	0.052
Range	0.013	0.006	0.013	0.009
SD	0.007	0.003	0.006	0.004

Table 2 contains the mean, range, and standard deviation of the data in each of the four groups: Experimental Exposure 1, Experimental Exposure 2, Control Exposure 1, and Control Exposure 2. The mean of the experimental group was consistently less than that of the control group, as was the range, however, the control group had a smaller standard deviation, in both exposures.

Figure 2. *Bar Graph Comparing the Consumption Ratio of Grams of Novel Food/Insect in Each Group Between Exposure 1 and Exposure 2*

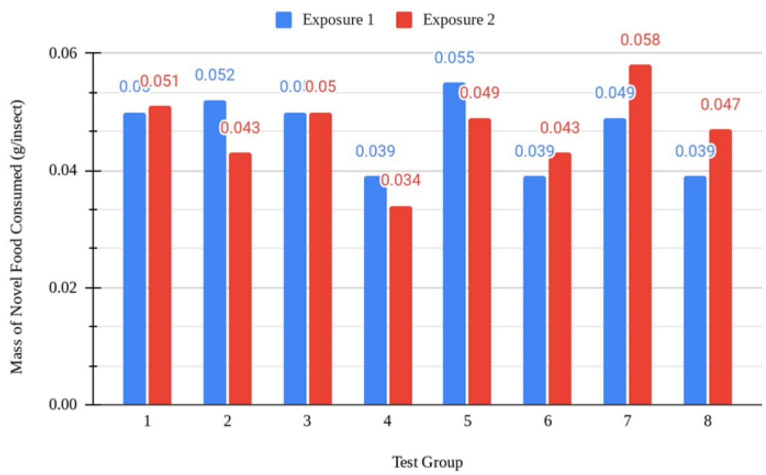

Table 3. *Inferential Statistics Paired t-test Comparing the Consumption Ratio of Grams of Novel Food/Insect Between Exposure 1 and Exposure 2 in the Test Group (LiCl) and Control Group (No LiCl)*

	n	*df*	*t*	*p*	Decision
Test	4	3	0.078	0.942	Fail to reject H0
Control	4	3	-0.318	0.771	Fail to reject H0

Table 3 shows the t-statistic and p-value found for the paired t-test, analyzing the significance of changes in the mass of food consumed by the *Blaptica dubia*, in both groups, over the course of the experiment. The alpha value used was 0.05, and neither the experimental, nor the control group was found to hold statistically significant findings.

Appendix

Figure A1. *Diagram detailing the injection site and pertinent anatomy of Blaptica dubia*

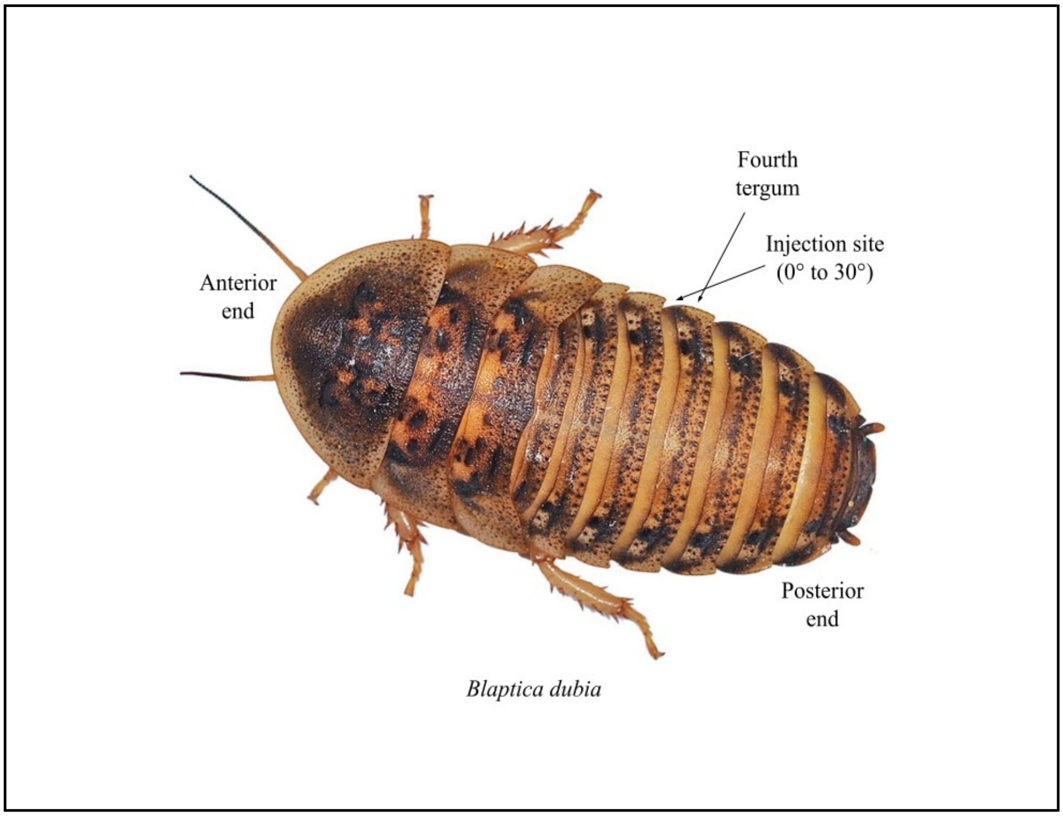

Figure A1 shows the specific site of injection used in this study. The LiCl solution was injected with a microliter syringe, which punctured the cutaneous membrane, or skin, adjacent to the fourth tergum from the posterior end of the *Blaptica dubia*. Tergum are hard plates that cover the abdomen of an insect. The syringe was inserted just underneath the tergum, puncturing the skin at a 0° to 30° angle, to avoid impaling the insect. These procedures were similar to those in a study performed by Chua et al.[3] Image adapted from *Emerald Isle Reptiles*[10].